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Cardiac Ionic Currents and Acute Ischemia: From Channels to Arrhythmias

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Carmeliet, Edward. Cardiac Ionic Currents and Acute Ischemia: From Channels to Arrhythmias. *Physiol. Rev.* 79: 917–1017, 1999.—The aim of this review is to provide basic information on the electrophysiological changes during acute ischemia and reperfusion from the level of ion channels up to the level of multicellular preparations. After an introduction, section II provides a general description of the ion channels and electrogenic transporters present in the heart, more specifically in the plasma membrane, in intracellular organelles of the sarcoplasmic reticulum and mitochondria, and in the gap junctions. The description is restricted to activation and permeation characterisitics, while modulation is incorporated in section III. This section (ischemic syndromes) describes the biochemical (lipids, radicals, hormones, neurotransmitters, metabolites) and ion concentration changes, the mechanisms involved, and the effect on channels and cells. Section IV (electrical changes and arrhythmias) is subdivided in two parts, with first a description of the electrical changes at the cellular and multicellular level, followed by an analysis of arrhythmias during ischemia and reperfusion. The last short section suggests possible developments in the study of ischemia-related phenomena.

I. INTRODUCTION

A. Aim and General Outline

The aim of the present review is to provide a description of ionic channels and electrogenic transporters present in the heart (sect. II), to describe the biochemical and ion concentration changes during acute ischemia and early reperfusion and the effects on channels (sect. III), to translate these findings in terms of modulation of electrical properties at the cellular and multicellular level and to analyze their role in the genesis of acute cardiac arrhythmias (sect. IV).

The reader mainly interested in the electrophysiological changes during ischemia and arrhythmias may directly start with section III and return later, when necessary for a better understanding of certain processes and notions, to section II. The reader with a main interest in the biophysics of ion channels is directed first to section II.

Section II provides a description of the activation-

inactivation kinetics and the permeation processes of channels and carriers; their role in the genesis of electrical activity is briefly discussed in this section. Section II does not include information on modulation or pharmacology. It is subdivided in sections describing channels and transporters in the plasma membrane, in the gap junction, and in intracellular organelles (sarcoplasmic reticulum and mitochondrion). Although information about eventual changes during ischemia is lacking for a number of channels and transporters, it is hoped that a systematic description and analysis will be helpful to understand more fully the actual state of knowledge, to put this information into perspective, and eventually to help to plan adequate experiments.

Section III describes how channels and transporters are modulated. The modulatory processes are not described for each channel separately but are grouped into "syndromes" related to ischemia, such as changes in ion concentration ($[K^+]_o$, $[Na^+]_i$, $[Ca^{2+}]_i$, and $[Mg^{2+}]_i$, where the subscripts o and i refer to extracellular and intracellular, respectively); disturbance of lipids resulting into the accumulation of long-chain acylcarnitines, lysophosphoglycerides, fatty acids, and arachidonic acid; production of radicals; secretion of neurotransmitters, hormones, and metabolites, with concomitant stimulation of adrenergic, purinergic, and muscarinic receptors; and the genesis of stretch. The analysis of each syndrome includes a description of the changes, the mechanisms involved, the effect on channels and transporters, and the final outcome at the electrophysiological and arrhythmia levels.

Section IV contains two major subdivisions: the electrophysiological changes at the cellular and multicellular level and the genesis of arrhythmias. Section IV*A* contains a description of the changes in resting and action potential, in excitability, refractoriness, and conduction and is followed by a discussion of possible mechanisms. Section IV*B* starts with an analysis of the general processes involved in the genesis of arrhythmias, followed by a description of the type of arrhythmias encountered during ischemia and reperfusion, and finally a discussion of possible mechanisms.

B. Ischemia Models

Cardiac ischemia is characterized by a deficient energetic input as well as a deficient waste removal. The result is failure of contraction, deterioration of electrical behavior, and eventual death of the cell. At the organism level, the end point may be lethal arrhythmias or mechanical pump failure. To study ischemia, different experimental models have been used. Coronary artery ligation or obstruction by a local thrombus mimics closely the clinical settings of myocardial infarction but does not allow a direct analysis of the changes in the ionic currents involved. For this purpose, voltage-clamp measurements have been applied to multicellular preparations and single cells subjected to hypoxia, uncouplers of the mitochondrial oxidative chain, inhibitors of glycolysis, superfusion with a solution containing a high concentration of K^+ and H^+, and deficient in glucose. In other models, the complex nature of the ischemic process has been dissected in different facets of specific biochemical changes that occur during ischemia: amphiphiles, radicals, catecholamines, adenosine, ACh, and stretch.

C. General Biochemical Changes During Ischemia and Reperfusion

Under aerobic conditions, NADH and $FADH_2$, formed during glycolysis and in the citric acid cycle, transfer their electrons to O_2 through the electron transport chain. This provides the energy to build up the chemiosmotic gradient that drives the synthesis of ATP. Oxidative phosphorylation is coupled to the demands of the cell. A feedback is generated by the breakdown products of ATP (772) and the rise in mitochondrial Ca^{2+} that modulates the activity of the mitochondrial dehydrogenases (692) and of the ATP synthase (199).

The turnover rate of high-energy phosphates is $30–40$ mol · g wet wt^{-1} · min^{-1}, while the storage is only 15 mol/g wet wt. This means that the time limit for exhaustion is short and only 15–30 s (339). When oxygen falls below a critical level in the cytoplasm, the electron transport and the process of H^+ ejection in the mitochondrion stops. The energy stored in the proton electrochemical gradient becomes insufficient to synthesize ATP in appropriate quantity. Some of the energy in ATP may be used to maintain the mitochodrion membrane potential (E_m) and to inhibit irreversible reactions leading to cell death (772). As a consequence, [ATP] may be expected to fall (Fig. 1) and [ADP] to increase. The cell, however, has efficient means to maintain the ATP level: *1*) energy demand falls very rapidly during the first 30 s of ischemia as a consequence of contraction failure, *2*) an important amount of phosphocreatine (PCr) is continuously used to restore ATP from ADP with concomitant increase in P_i, and *3*) anaerobic glycolysis starts and intensifies.

The fall in the ATP/ADP stimulates the glycolytic pathway by activation of three important enzymes: *1*) hexokinase responsible for the formation of glucose-6-phosphate; *2*) phosphofructokinase, responsible for the transformation of fructose-6-phosphate to fructose-1,6-biphosphate; and *3*) pyruvate kinase for the formation of pyruvate. The reactions leading to pyruvate are stimulated but glucose metabolism stops at the pyruvate-lactate

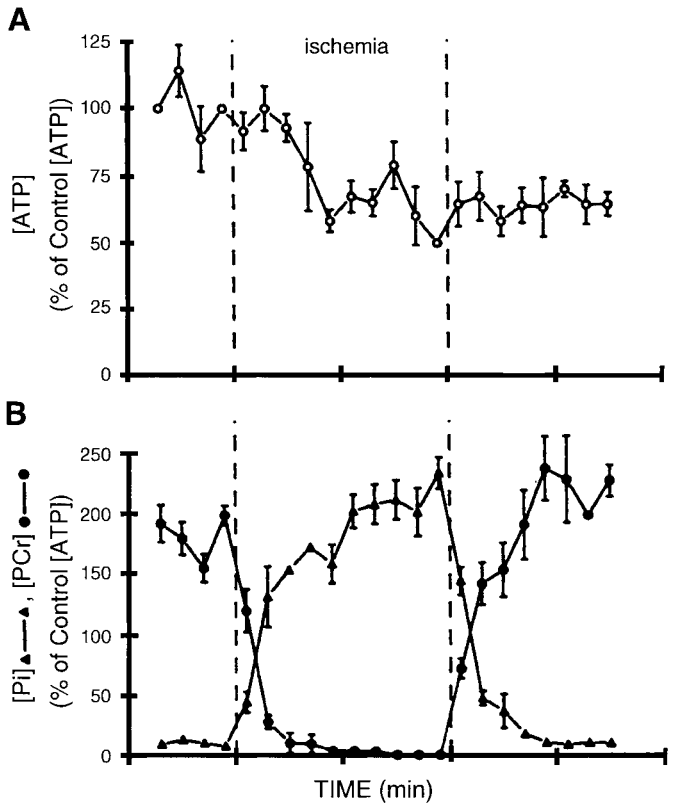


FIG. 1. Metabolic consequences of ischemia on intramitochondrial levels of ATP, P_i, and phosphocreatine (PCr) as percent of control [ATP] obtained from ^{31}P-NMR spectra in perfused ferret hearts. [From Marban et al. (640). Copyright 1990 American Heart Association.]

stage, due to inhibition of pyruvate dehydrogenase. Pyruvate is transformed in L-lactate under simultaneous oxidation of NADH to NAD^+. At the same time, phosphorylase *a* is transformed in its active form, facilitated by accompanying increases in AMP, P_i, Ca^{2+}, and cAMP and increases the breakdown of glycogen. High glycogen content of cells postpones the development of contracture during ischemia (190). Finally, glucose transport through the cell membrane is stimulated, secondary to a translocation of glucose transporters from an intracellular pool to the plasma membrane (1155) and increases the flow down to pyruvate. Glycolytic enzymes are especially dense in the subsarcolemmal space; stimulation of anaerobic glycolysis at this level is important for the regulation of intracellular ion concentrations and channels (KATP) (1082).

Stimulation of the glycolytic pathway explains why [ATP] stays remarkably constant during the initial 10–15 min of ischemia. However, the free energy change upon hydrolysis of ATP falls immediately because of the rise in [ADP] (281). This fall has important consequences for a number of ATP-driven transporters such the Na^+-K^+-ATPase and the Ca^{2+}-ATPase. The anaerobic glycolytic process is self-inhibiting however. When acidosis becomes too pronounced, glycolysis in turn is inhibited, and ATP synthesis is further reduced.

Whenever the free energy of ATP hydrolysis exceeds the energy stored in the proton electrochemical gradient, net ATP hydrolysis may occur; the ATP synthase then acts as an ATP hydrolase, and the energy is used to update the electrical gradient in the mitochondrion (224) (Fig. 2). Although the arrest of electron flow is expected to cause depolarization of the mitochondrial membrane during anoxia, such depolarization only occurs after a delay (244). The delay corresponds to the time needed to block glycolysis to cause serious ATP deficiency and rise in $[Ca^{2+}]_i$. Blocking ATP hydrolysis by oligomycin slows the fall in [ATP] (808) and delays activation of the mitochondrial mega-channel. When [ATP] drops to levels <1 mM and at the same time the concentration of H^+, $[Ca^{2+}]_i$ (>1 μM), PO_4^{3-} (>10 mM), and long-chain acylcarnitines (LCAC) increase (772), the mitochondrial membrane becomes abnormally leaky through activation of the mega-channel or transition pore in the inner membrane of the mitochondrion. Efflux of ATP through the plasma membrane is an additional reason for a fall of intracellular ATP. In the extracellular medium, this ATP is further broken down to adenosine and may activate purinergic P_1 and P_2 receptors.

Block of oxidative metabolism and fall in ATP/ADP will have consequences on lipid metabolism, the generation of oxidative stress, the release of catecholamines, and ion concentrations.

Upon reperfusion after 5–30 min of ischemia, oxygen consumption rapidly recovers. The NADH/NAD^+ quickly decreases, although the level may remain higher than the control for some time. After brief (5–10 min) ischemic bouts, PCr concentration quickly returns to normal, but recovery of ATP is slow. After longer periods of ischemia, PCr concentration still recovers within 5 min, but ATP, which may have dropped to 50%, stays at this low level for 30 min or more (641).

The preischemic pattern of substrate utilization is restored, i.e., oxidation of fatty acids (FA), is the main contributor to ATP synthesis; the level of glycolysis, however, remains elevated in the early period of reperfusion and is important for ATP generation. This situation may explain why block of glycolysis at this time is disadvantageous and results in aggravation of Ca^{2+} overload and release of intracellular enzymes. Such a block occurs when FA are present in high concentration (621). A high rate of FA oxidation generates NADH which inhibits pyruvate dehydrogenase. Glycolysis which stops at the pyruvate level is accompanied by an overproduction of protons and may negatively affect the recovery of $[Na^+]_i$ and $[Ca^{2+}]_i$ (612). High levels of lactate are deleterious for recovery.

The high level of oxygen consumption early after reperfusion stays in contrast to the continuing deficiency

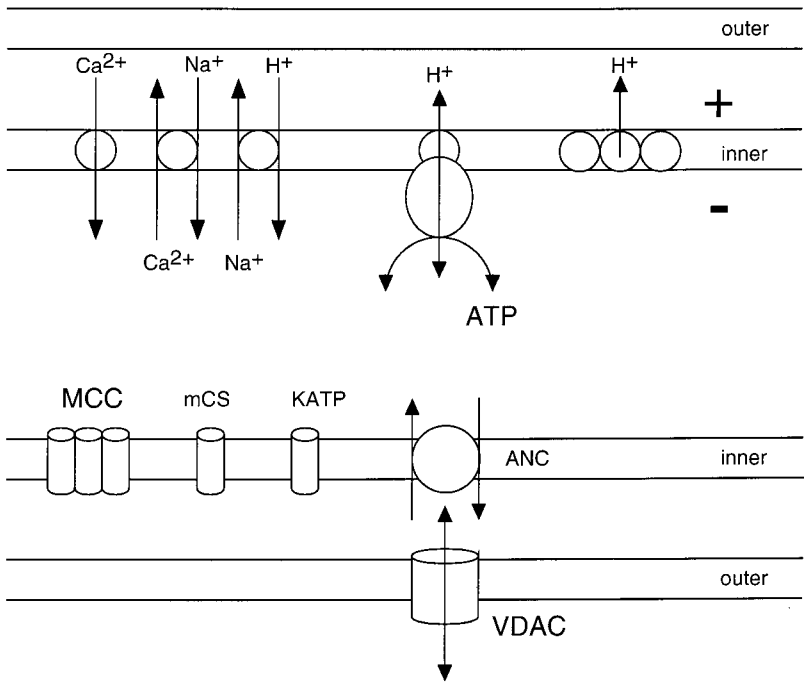


FIG. 2. Ionic channels and carriers in outer and inner mitochondrial membranes. Under aerobic conditions, electron-carrying chain ejects protons and generates an important electrical gradient over inner membrane. Proton inflow through synthase provides necessary energy for ATP synthesis. In anaerobic conditions, mitochondrion should depolarize; synthase however may now act as an ATPase and guarantee persistence of electrical as well as proton gradient. Ca^{2+} homeostasis in mitochondrion is dependent on this electrical and proton gradient. MCC, multiple conductance channel, mega-channel, or permeability transition pore; mCS, mitochondrial cento/picosiemens channel; KATP, ATP-sensitive K^+ channel; ANC, adenine nucleotide carrier; VDAC, voltage-dependent anion channel.

of the contractile machinery or "stunning." This uncoupling between oxygen consumption and contractility is not due to impairment of the respiratory chain flux and insufficient ATP synthesis. Energy is sufficiently present but apparently not used. As a possible explanation for the high rate of oxygen consumption, the existence of futile cycles has been proposed, such as the cycling of Ca^{2+} between the cytoplasm and mitochondria (189). The absorption of Ca^{2+} by the mitochondria as well as its removal via the Na^+/Ca^{2+} exchanger depends on the energy flux in the electron chain. The ejection of protons generates the negative matrix potential that drives the absorption of Ca^{2+} and creates the proton gradient that is required for removing Na^+ from the mitochondrial matrix via the Na^+/H^+ exchanger and subsequently Ca^{2+} via the Na^+/Ca^{2+} exchanger. Adenosine 5′-triphosphate, which for its synthesis is also dependent on the proton gradient, is used by the plasma membrane Na^+-K^+ pump to keep cytosolic [Na^+] low (Fig. 2). Under normal conditions, this cycle only requires little energy, but in the case of Ca^{2+} overload, the expenditure may become excessive. The situation is aggravated by an eventual intermittent opening of the mega-channel activated by elevated matrix [Ca^{2+}] (91). The leak created causes breakdown of the mitochondrial electrical gradient. Activation of the mega-channel may propagate from mitochondrion to mitochondrion, creating a Ca^{2+} wave in the cytosol (449).

Reperfusion after more than 30 min leads still to an important but only transient recovery of oxygen consumption, while contractile function remains severely and persistently depressed. Intracellular enzymes and other substances are lost. On the microscopic level, cells appear necrotic, and later important fibrosis develops.

II. ION CHANNELS AND TRANSPORTERS

A. Ion Channels and Transporters in the Plasma Membrane

1. Na^+ channels

In most excitable cells, the Na^+ current is responsible for the upstroke and the conduction of the action potential. In heart, the density of the channel is low in sinoatrial node (SAN) and atrioventricular node (AVN) cells and highest in Purkinje cells. The Na^+ channel is voltage operated and shows activation and inactivation. It is specifically blocked by tetrodotoxin (TTX) (for a general review, see Ref. 291); modulation of channels is described in section III.

A) ACTIVATION AND INACTIVATION. When a cardiac cell is depolarized, an inward current is generated that rises rapidly and decreases afterward on a much slower time course. The rise is sigmoidal (567) or exponential (685). The time to peak shortens with depolarization (634). The decline in macroscopic current or inactivation is best described by a sum of two exponentials. The time constant of the first component becomes shorter with depolarization.

Peak current-voltage relation can be translated into a conductance-voltage relation from which an activation

curve can be constructed. Peak current is voltage dependent and increases for more negative holding potentials. The relation has been explained as a change in availability of the channel to become activated. Both relations, activation and inactivation, can be described by a Boltzmann distribution, with slopes of ~6 mV. Midpoints of voltages in multicellular preparations are −30 mV for the activation process and −85 mV for the inactivation process.

The time course and the steady-state properties of the current have been explained in terms of two voltage-dependent processes, activation and inactivation independent from each other (425). The opening and closing of the channel depends on the movement of charged gates, and the hypothesis thus predicts the existence of a gating current. In heart cells, such a gating current has been measured for the activation process (50, 350, 372, 486) but not for the inactivation process, as if inactivation is voltage independent. The activation gating current, however, becomes smaller during the development of inactivation. The gating charge becomes immobilized, suggesting some coupling between the two processes (372, 486). Depending on the type of cell, coupling between activation and inactivation is variable. Coupling is strong in neuroblastoma cells (9) in which activation shows a variable onset and inactivation follows rapidly. In cardiac cells, the coupling is weak; openings always occur at the beginning of the depolarizing pulse (activation is fast) and their duration is variable (inactivation is slower and variable) (69, 90, 567, 587, 856).

Recovery from inactivation is normally very fast (1–10 ms), with rates increasing upon hyperpolarization. After long depolarizations, recovery can become very slow (order of seconds). The process has been called slow inactivation (125, 160, 825, 876). The existence of this phenomenon may be important to understand changes occurring during and after ischemia, where the cells are subjected to long depolarizations.

Is there more than one type of Na^+ current? In heart cells, decay of the Na^+ current is normally very rapid. In a limited range of potentials, where activation and inactivation overlap, a small noninactivating current or window current can be recorded, which is due to the cycling of channels between the rested, activated, and inactivated state (27, 325, 755). This is not due to any abnormal behavior of the channel, but in pathological conditions, the overlapping may increase, resulting in an enhancement of the current.

A slowly inactivating Na^+ current, which represents a small percentage of the total Na^+ current, can be recorded in rabbit (125) and canine (325) Purkinje fibers, in rat ventricular cells (826), and in expressed human Na^+ channels (801) over a broad range of potentials. In the rat, it becomes more pronounced during hypoxia (489) (Fig. 3). Part of it does not inactivate completely. Compared with the fast Na^+ current component, activation is shifted

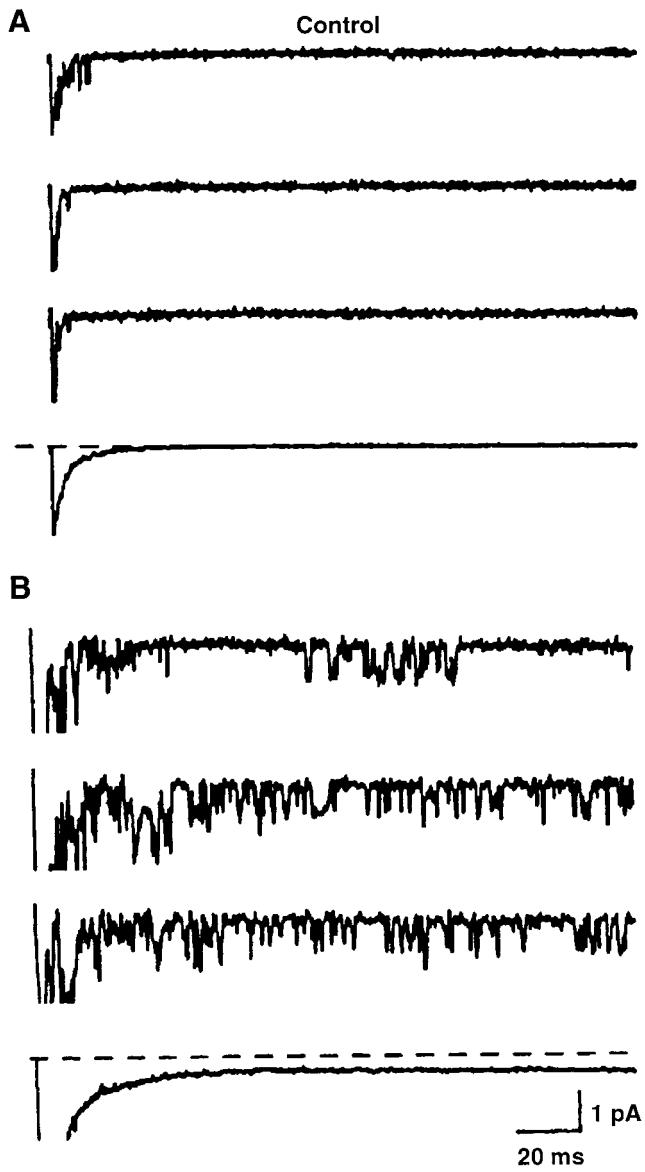


FIG. 3. Hypoxia increases persistent Na^+ channel activity. Voltage steps from −120 to −50 mV were applied to a cell-attached patch from a rat ventricular myocyte. *A*: control. *B*: during hypoxia, different cells. *Bottom trace* in each series is average current of 50 traces. [Adapted from Ju et al. (489).]

in the hyperpolarized direction. This may cause more pronounced overlapping of activation and inactivation, generating in this way a constant steady-state component. The current deactivates immediately upon hyperpolarization. At the single-channel level, it is characterized by a bursting behavior, i.e., clusters of repetitive short openings (5 ms) sometimes alternating with long openings (order of 200 ms) (1187). An activity characterized by short openings (<0.5 ms), becoming shorter with depolarization, has been described as "background" current because it carries current at the resting potential (1187). The current undergoes inactivation, however, and is very

selective for Na^+. According to Böhle and Benndorf (90), one and the same channel can show many modes of gating behavior; this channel may thus not be different from the slowly inactivating one. The slowly inactivating current has been described as being more sensitive to block by TTX (174), a finding which has been advanced in favor of the existence of a different isoform; the result, however, can also be explained by the slow kinetics of the TTX block (126).

After exposure of membrane patches to lysophosphatidylcholine (108, 1002), a persistent current can be recorded over a voltage range of −150 to 0 mV. It is probably due to a modulation of the fast component. The current does not deactivate upon hyperpolarization and is substantial at the resting potential. It may play an important role as an inward leak current, causing K^+ loss during ischemia.

A Na^+-dependent, TTX-insensitive background current is induced by high concentrations of ACh in guinea pig ventricular myocytes (659). The current reverses at −25 mV in normal Tyrode solution, and its single-channel conductance has been estimated by noise analysis to be ~2 pS (897). Because of its different pharmacological and single-channel characteristics, it can be regarded as a different isoform.

B) ION PERMEATION. In the presence of 150 mM $[Na^+]_o$, single-channel conductance is 20–25 pS; it is dependent on the concentration of external Na^+ with a dissociation constant (K_d) of 300–400 mM (885) and shows different substates (554, 727, 856). The channel is permeable to Na^+ and Li^+ and much less to K^+ (9.5%) and Cs^+ (2%) or tetramethylammonium (<1%) (885). A small Ca^{2+} permeability exists (3) and may increase in the presence of ouabain and upon β-receptor activation (840). The current-voltage relation is linear in the absence of bivalent ions; deviation from this linear behavior in normal bathing solutions is due to a voltage-dependent block by $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$; electrical distance is 0.3–0.4 (884). The block by $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ is characterized by a reduction in apparent single-channel conductance, suggesting a very fast block. Other bivalent ions efficiently but more slowly block the current: $Cd^{2+} > Mn^{2+} > Co^{2+} > Ca^{2+} > Mg^{2+} > Ba^{2+}$. Of importance to note is the highly sensitive block by Zn^{2+} and Cd^{2+} (297, 1042); these ions are much less effective in neuronal and skeletal muscle cells, whereas the opposite sensitivity exists for TTX. Internal $[Mg^{2+}]$ blocks outward current through the channel (786). The block is slightly voltage dependent with an electrical distance of 0.18 from inside.

At the molecular level, the channel consists of an α-subunit (260 kDa) and two β-subunits (36 and 33 kDa each) (291). The α-subunit, which is sufficient for channel activity, is composed of six transmembrane segments repeated four times in a tetrameric structure. The voltage sensor for activation has been related to the S4 segment. The short intracellular segment between domains III and IV has been identified as the fast inactivation gate. Spontaneous deletion of the KPQ segment in the junctional part gives rise to the LQT3 syndrome, characterized by a slowing of the inactivation process and generation of long action potentials. The absence or weak voltage dependency of the inactivation process is consistent with the location of this inactivation gate outside the electrical field of the membrane. Slow inactivation involves conformational changes in the external pore (C-type inactivation) (1058). The presence of sialic acid at the external surface shifts the activation and inactivation in the negative direction (64). Interaction with the cytoskeleton modulates the inactivation and activation voltage dependence; F-actin disruption and microtubule stabilization accelerate the shift in the negative direction that occurs during whole cell recording (636). The two β-subunits exert a modulatory role, speed up inactivation, and decrease block by local anesthetics (633).

The permeation process is dependent on the hydrophilic part of the α-subunit between transmembrane segments 5 and 6. The selectivity for Na^+ is due to specific amino acids in this region, and mutation of only two critical amino acid residues is sufficient to confer permeability properties similar to the Ca^{2+} channel. A cysteine in domain I is responsible for the high affinity of the channel for Zn^{2+} and Cd^{2+} and low sensitivity to TTX of the cardiac isoform.

2. Ca^{2+} channels

Different types of Ca^{2+}-permeable channels have been described in the plasma membrane of heart cells: the L- and T-type channels, both voltage activated, and a background channel (see Ref. 673). They can be differentiated on the basis of their electrophysiological and pharmacological characteristics. The density of the T- and L-type channels differs in different sections of the heart. The ratio of T-type over L-type channel is highest in Purkinje and sinoatrial cells where it approaches the value of 0.2–0.6, and it is less in atrial and ventricular cells where the ratio only attains 0.015–0.025 (48).

A) BACKGROUND CA^{2+} CHANNELS. Calcium-permeable channels are seen following incorporation of plasma membrane protein fractions in bilayers (806). No voltage steps are required for activation, and spontaneous single-channel activity with long openings of >100 ms can be recorded at negative E_m values of −90 mV. The conductance is 7 pS in isotonic Ba^{2+}; the channels are not blocked by dihydropyridines (DHP) and show no rundown. Their selectivity is not pronounced with a permeability ratio P_{Ba}/P_{Cs} of 10.

A similar channel but with higher conductance (22, 45, and 78 pS in Ba^{2+}) has been described in neonatal rat hearts (187). It is not blocked by Cd^{2+} or nifedipine;

instead, activity is rather increased in the presence of DHP but suppressed by protamine. In rat ventricular myocytes, the channel is induced by exposure of the inside-out patches to phenothiazines (593). Activity is also increased by exposure to oxygen free radicals and metabolic inhibition (1056). The channel may be responsible for the Na^+-independent Ca^{2+} entry pathway described for rat trabeculae (583).

B) L-TYPE CA^{2+} CHANNEL. Calcium influx through the L-type Ca^{2+} channel is responsible for the upstroke of the action potential in the SAN and AVN and plays an important role in determining the plateau and eventual spike-dome appearance of the action potential in other cardiac cells. It is further responsible for the coupling between excitation and contraction, induces release of Ca^{2+} from the sarcoplasmic reticulum, and regulates intracellular Ca^{2+} load. In this way it determines activity of a number of mitochondrial and cytoplasmatic Ca^{2+}-sensitive enzymes.

I) Kinetics. A) Activation and inactivation. Threshold for activation is around −25 mV, and half-maximum activation is attained at about −15 mV for most cells (see Ref. 673) and at more positive potentials (−3 mV) in the AV node (373). The rise in current follows a sigmoidal time course, suggesting a multistep process as the underlying mechanism. Activation is preceded by a gating current (50, 352, 486, 898). The density of the channels derived from the gating current is much larger than the ionic current density, suggesting that some of the channels although gating are not carrying current.

At the single-channel level, three modes of activity have been distinguished (137, 403). In *mode 1*, the channel shows repetitive short (<1 ms) openings and closures (0.2 and 2 ms), forming a burst of activity separated from other bursts by longer closures. A number of consecutive bursts may be grouped in a cluster. A variable waiting time precedes the openings; it decreases at more depolarized levels and corresponds to the faster activation and shorter time to peak values of the Ca^{2+} current. *Mode 2* occurs in the presence of DHP agonists (403) or after β-receptor stimulation (137, 767) and is characterized by much longer open times. *Mode 3* is characterized by the complete absence of openings or presence of only rare short openings. The frequency of this latter mode increases with preceding depolarizations and corresponds to the occurrence of steady-state inactivation.

The L-type Ca^{2+} current inactivates in two ways: a voltage-dependent and a current-dependent way. The existence of two types of inactivation explains the complex time course of current decay and the presence of a dip in the inactivation curve. Half-maximum steady-state inactivation occurs at −20 to −30 mV. The curve shows a minimum at ~0 mV and increases again at more positive potentials. When intracellular Ca^{2+} is well buffered, this turning up is absent and the decay of the current during a pulse is much slower. These observations have led to the conclusion that inactivation is dependent on voltage as well as on Ca^{2+} influx. The latter or Ca^{2+}-induced inactivation is the faster process, whereas the voltage-induced inactivation is rather slow. Activation and inactivation show a remarkable overlapping (window current) (414, 900).

B) Intracellular Ca^{2+} or current-dependent inactivation. Evidence for intracellular Ca^{2+}-dependent inactivation at the whole cell level is based on the change in time course of current decay with changes in $[Ca^{2+}]_i$ (see Ref. 673) (Fig. 4, *A* and *B*). The decay is faster the larger the Ca^{2+} current, and it is slowed in the presence of intracellular Ca^{2+} buffers. At the single-channel level (cell-attached patches), Ca^{2+} permeation through the channel reduces the open probability of subsequent reopenings of the channel and shifts the gating mode toward a mode with long-lived closed states (454). In excised patches, with Ba^{2+} as the charge carrier, steady-state elevation of Ca^{2+} in the range of micromolar concentration or flash photolysis of Ca^{2+} reduces the open probability of the Ca^{2+} channels. It is especially the Ca^{2+} originating from the sarcoplasmic reticulum (SR) that is responsible for the inactivation process during the first 50 ms of depolarization (949, 1154); at later times also Ca^{2+} permeating through the Ca^{2+} channel contributes to the inactivation (949). The increase in cytosolic Ca^{2+} by the release from the SR is indeed 10-fold greater than the Ca^{2+} entering the cell via the L-type Ca^{2+} channel (915). In favor of this explanation is the observation that inactivation is much slower after depletion of the SR by caffeine or in the presence of ryanodine. It is important to note that $[Ca^{2+}]$ seen by the channel may importantly deviate from the cytosolic $[Ca^{2+}]$ as measured by fluorescence techniques. Especially during the first 50 ms of a depolarizing pulse the concentration seen by the channel may be much higher. Such a difference may explain why the relative inhibition of the current estimated from the Ca^{2+} transient is greater during this initial period than later (611).

The existence of an intact cytoskeleton is important in determining the local $[Ca^{2+}]$. The cytoskeleton normally keeps the channels separated from each other and limits the rise in local $[Ca^{2+}]$. In ischemia, the cytoskeleton may become disturbed. Disruption of the cytoskeleton structure by colchicine or cytochalasin, with consequent clustering of the channels, favors inactivation, whereas substances such as taxol and phalloidin that stabilize the skeleton remove inactivation and improve reopening (taxol and colchicine act on microtubules; phalloidin on F-actin) (311).

Different mechanisms have been proposed to explain $[Ca^{2+}]_i$-dependent inactivation (719). *1*) A fall in driving force is improbable. In cell-attached patches, single-channel conductance does not change, whereas open probability is markedly reduced. *2*) Dephosphorylation by phosphatase (e.g., calcineurin) and proteolysis by Ca^{2+}-

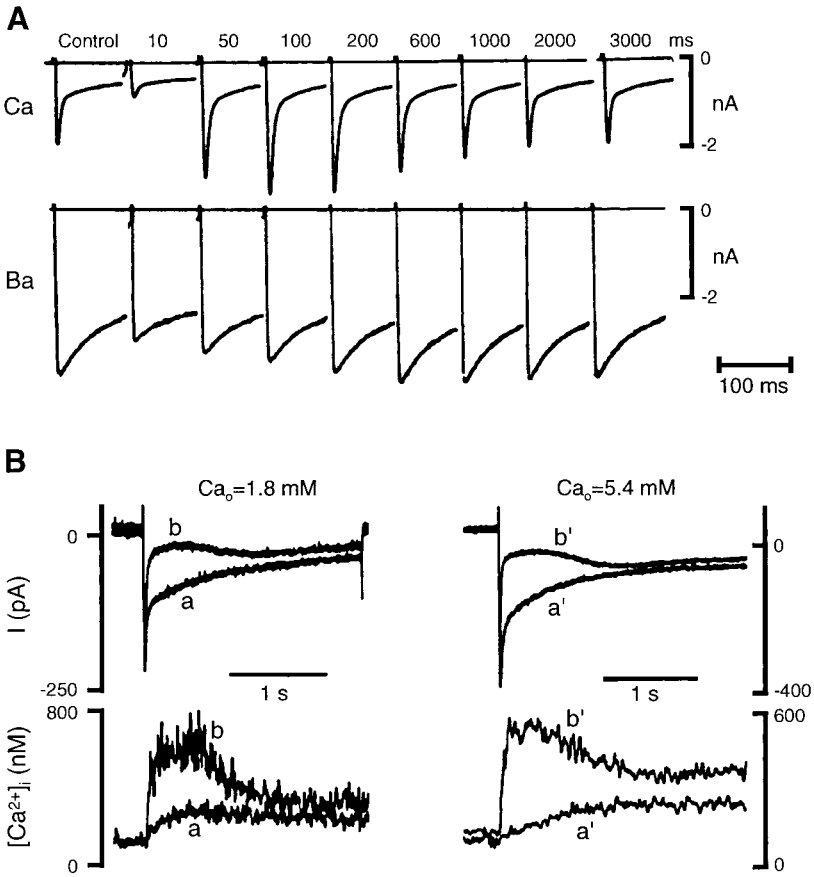


FIG. 4. *A*: existence of facilitation during recovery of Ca^{2+} current from previous inactivation in dog ventricular myocytes. Paired depolarizing pulses of 100-ms duration were applied from a holding potential of −80 mV to −10 mV with a delay variable between 50 and 3,000 ms. Ca^{2+} current recovers rapidly within 30 ms to its control value; afterward, current transiently becomes larger. Facilitatory effect requires presence of Ca^{2+} influx and is absent when external solution contains Ba^{2+}. [Adapted from Tseng (991).] *B*: Ca^{2+}-induced inactivation. Ca^{2+} currents (*top traces*) and Ca^{2+} transients (*bottom traces*) in guinea pig ventricular myocytes in presence of 2 different external Ca^{2+} concentrations (Ca_o). Superimposed tracings for control (*a*) and after Ca^{2+} loading (*b*) are shown. Note marked, rapid Ca^{2+}-induced inactivation in presence of Ca^{2+} overload, but also partial recovery during pulse. *I*, current; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration. [From Sipido et al. (911). Copyright 1995 American Heart Association.]

stimulated proteases (calpain) (351) are mechanisms that may be responsible for long-term changes in Ca^{2+} channel behavior but are too slow to explain the time course of changes during a single depolarization. *3*) Calcium binds to the channel protein and induces a change in configuration. In the cloned channel, a Ca^{2+} binding motif (an EF hand) exists at the COOH terminal of the α_{1C}-subunit; deletion eliminates Ca^{2+}-induced inactivation. The site is located near the inner mouth but outside the electrical field (205, 719). The reduction of Ca^{2+} current by $[Mg^{2+}]_i$ also has been explained by a direct binding to this site, reducing the number of functional channels (1132). Because Ca^{2+} binding is a fast process, it provides an explanation for the time course of inactivation and the observation that Ca^{2+} oscillations are accompanied by equivalent changes in current (911). Photolysis of Ca^{2+} results in a rapid inactivation, within 20 ms (351) to 75 ms (46); the process is not accompanied by a change in gating current, confirming that intracellular Ca^{2+}-induced inactivation and voltage-dependent inactivation are two distinct phenomena (273).

C) Voltage-dependent inactivation. The evidence for voltage-dependent inactivation is based on the following observations (see Ref. 673). *1*) Under conditions where intracellular Ca^{2+}-dependent inactivation is excluded (e.g., current carried by monovalent cations or current carried by Ba^{2+} or Sr^{2+}), the current decays with time, and the rate of decay is faster the higher the depolarization. *2*) Inactivation develops when prepulses are applied that do not result in Ca^{2+} inward current. *3*) Inactivation is present in channels incorporated in lipid bilayers with Ca^{2+} buffered (806). *4*) Inactivation is slowed by trypsin treatment, but the intracellular Ca^{2+}-dependent inactivation is not affected (862). No charge movement occurs on inactivation, but as inactivation of the current proceeds, the charge movement that accompanies activation becomes smaller (351, 898).

D) Recovery from inactivation: Ca^{2+}-induced facilitation. Upon hyperpolarization, the Ca^{2+} current recovers from inactivation induced by a previous depolarization. Because inactivation is voltage and intracellular Ca^{2+} dependent, it is logical to expect repriming also to depend on these two parameters. For the voltage-induced inactivation, the rate and the degree of recovery is greater the more hyperpolarized the membrane, with time constants in the order of 300 ms at −50 mV and 100 ms or shorter at −80 mV. A much slower component (seconds) is present after long depolarizations, indicating the occurrence of slow inactivation (94, 868). This slow inactivation may play a role in overdrive suppression (1074). Recovery from Ca^{2+}-induced inactivation as such is voltage independent (911, 912) but indirectly it is modulated by volt-

age, since the fall in $[Ca^{2+}]_i$ is dependent in part on the Na^+/Ca^{2+} exchange that is faster the more negative the E_m.

At negative holding potentials, recovery from inactivation may show an overshoot, i.e., the Ca^{2+} current transiently becomes larger than in steady state. The original finding of an overshoot was made in Purkinje fibers treated with digitalis (505) but can also be observed without Ca^{2+} overload (see references in Refs. 606, 770). The potentiated Ca^{2+} current is characterized by a larger peak and a slower time course of decay (991, 1159, 1193) (Fig. 4).

At the single-channel level, facilitation is characterized by an increase in open probability (P_o) with a larger proportion of long openings (413) (*mode 2*) and an increase in number of functional channels (1132). In all these approaches, it is clear that a moderate increase in Ca^{2+} is required (991, 1159, 1193); the overshoot in recovery is inhibited by rising intracellular Ca^{2+} buffering, or by ryanodine or using Ba^{2+} as the current carrier. Excessive rises in Ca^{2+} lead to inhibition by Ca^{2+}-induced inactivation (351).

How elevated Ca^{2+} causes facilitation remains a matter of debate. Phosphorylation of the channel protein is a possibility. Flash photolysis induces facilitation with a delay, and the effect is counteracted by inhibitors of protein kinases (PK) (18, 1159). Other groups, however, did not find an effect of PK inhibitors (46, 1132), and facilitation still occurred with nonhydrolyzable ATP analogs (1132) or even improved (46). The conclusion of these authors is that a Ca^{2+}-nucleotide complex directly potentiates Ca^{2+} current (I_{Ca}) through a phosphorylation-independent mechanism. The existence of two phases in the recovery (decrease followed by an overshoot) has practical consequences in determining down- or upregulation of I_{Ca} as a function of frequency and diastolic E_m.

II) Permeation and selectivity. The channel is 500–1,000 times more permeable to bivalent ions such as Ca^{2+} and Ba^{2+} than to monovalent ions. The exclusion of monovalent ions depends on the presence of a minimum concentration of bivalent ions. In the absence of bivalent ions, the channel becomes highly permeable to monovalent ions (651). Although restricted, the permeability for K^+ is responsible for a substantial current during the action potential, the reason being that the concentration of intracellular K^+ is quite high compared with the nanomolar free Ca^{2+} concentration. The K^+ contribution also explains why the reversal potential (E_{rev}) of the Ca^{2+} current is much less positive than expected for the equilibrium potential for Ca^{2+}. The bivalent ion current through the channel increases with the concentration and shows saturation. Compared with the single-channel conductance in isotonic Ba^{2+} (8–10 pS and 15–25 pS, see Ref. 673), the single-channel conductance at the physiological concentration of 1 mMCa^{2+} was found to be surprisingly high at 7 pS (1162). The presence of negative charges at the pore mouth of the channel, which attract bivalent ions and increase their local concentration (331), is probably the reason for this behavior. In the presence of both Ca^{2+} and Ba^{2+}, the current across the channel is smaller than in the presence of either Ca^{2+} or Ba^{2+} alone (997). This kind of behavior has been called anomalous mole fraction behavior and suggests a multi-ion channel. Different ions thus interact in such a way that the flux of one species is hampered by the presence of the other species. Multi-ion occupancy also explains the high conductance and at the same time the high selectivity. High selectivity is conditioned by high affinity; high conductance by the presence of more than one ion in the channel and repulsion of one ion by the other. The multi-ion nature of the channel also explains the flickery block behavior of the channel in the presence of elevated proton concentration (784).

The channel can be blocked by a number of extracellular bi- and trivalent ions such as Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, and La^{3+} (997). The ions Cd^{2+}, Zn^{2+}, and La^{3+} block the channel in a voltage-dependent way with an apparent electrical distance of 0.15 for Cd^{2+} and Zn^{2+} and 0.60 for La^{3+}. Intracellular Mg^{2+} is needed to activate enzymes that phosphorylate the channel but may, on the other hand, reduce the current that has been augmented by isoproterenol or BAY K 8644 by a direct blocking action and by activating phosphatases (1090, 1132). The cAMP-dependent phosphorylation reduces the sensitivity to $[Mg^{2+}]_i$ block (1131).

The cardiac Ca^{2+} channel molecular structure consists of four subunits: two α-subunits, α_1 and α_2, a β-subunit, and a δ-subunit (a γ-subunit is exclusively expressed in skeletal muscle). The α-subunit is sufficient to express channel activity. It resembles the Na^+ channel with four times six transmembrane segments, a highly charged S4 segment that probably acts as the voltage sensor for activation, and an intracellular link between domain II and III responsible for inactivation. Trypsin treatment removes voltage-dependent inactivation but not internal Ca^{2+}-dependent inactivation (862). Binding of Ca^{2+} to the COOH terminal is a possible mechanism for Ca^{2+}-induced inactivation (205).

The highly conserved glutamate residues located in the pore region of all four repeats are involved in high-affinity bivalent binding (1146). A mutation from E to Q in domain III has shown that this group is the strongest determinant of Ca^{2+} binding (331).

The function of the α-subunit is markedly modulated by the β-subunit. Coexpression of the two results in a fourfold increase of peak current, which is not due to a change in single-channel conductance (331) but to a marked increase in density of functional channels (488, 718, 759). The gating current remains unchanged, but coupling between conductance and gating is improved (759).

The L-type Ca^{2+} channels is highly regulated; this aspect is analyzed in section III.

C) T-TYPE CA^{2+} CHANNEL. A Ca^{2+} current of short duration is activated at potentials more negative than the threshold for the L-type Ca^{2+} current (48, 726; see review in Ref. 1026). The current is well represented in SAN cells, atrial cells, Purkinje cells, and nodal cells. In the embryonic chick ventricle, it is the major Ca^{2+} current. The current is not found in human atrium (258, 606) or human ventricle (76). The channel has been proposed to play a role in pacemaking. It may also interfere with steroidgenesis, cell proliferation, and cardiac growth (400).

I) Activation, inactivation, and repriming. Threshold for activation is around −70 to −50 mV and maximum activation is seen at −30 to −10 mV (see Ref. 1026). Inactivation is rapid and complete, with time constants of 30 ms at −50 mV, becoming shorter at more depolarized levels. This behavior is opposite to the L-type channel, where inactivation is decelerated at positive potentials. Steady-state inactivation extends from −85 to −40 mV with half maximum around −60 mV and slope of 5.5 mV (14, 36, 412). An increase in $[Ca^{2+}]_i$ does not induce inactivation but rather facilitates the T-type current (13, 994). At the single-channel level, this increase in current is characterized by a shift to long openings (*mode 2* behavior).

Repriming is voltage dependent and becomes faster with hyperpolarization: 250 ms at −70 mV and 100 ms at −90 mV (145). It is slower the longer the preceding depolarization, suggesting the existence of slow inactivation (412).

II) Permeation. In 100 mM $[Ca^{2+}]$, the single-channel conductance is 8 pS, compared with 20 pS for the L type. Contrary to the behavior of the L-type channel, permeability for Ca^{2+} and Ba^{2+} ion is the same (48, 900). The channel is permeable to Sr^{2+}, blocked by Ni^{2+}, but much less sensitive to Cd^{2+}. Extracellular protons inhibit the channel with greater efficiency than the L-type current, whereas intracellular protons have no effect (1000). Extracellular Mg^{2+} reduce the current and shift the activation and inactivation curves in the positive direction (1113).

Recently two isoforms, the G and H isoform of the α-subunit, have been cloned (760). Sialic acid probably forms an important component of the extracellular part of the channel (1150).

3. K^+ channels

Because the equilibrium potential of K^+ is rather negative, all cardiac K^+ channels when activated will carry outward current, repolarize the membrane during the action potential, or stabilize the membrane at a hyperpolarized level. Among the many K^+ currents, distinction can be made between voltage-activated currents (I_{to}, I_{Kur}, I_{Kss}, I_{Kr}, and I_{Ks}), ligand-activated currents (I_{KACh}, I_{KATP}, I_{KNa}, and I_{KAA}), and a current (the inward rectifier I_{K1}) that apparently does not gate and can be called a background current. Under physiological circumstances the voltage-activated K^+ currents, I_{KACh} among the ligand-activated and I_{K1}, play an important role in shaping the normal action potential. Under ischemic conditions, ligand-activated currents, especially I_{KATP} and I_{KAA}, become primordial, whereas some of the "physiological" currents are inhibited. Voltage-activated K^+ currents show activation and inactivation upon depolarization; the rates of these two processes can vary from fast to ultraslow. Ligands can bind to receptors, which then activate the channel via a G protein, or can interact directly with an intracellular site of the channel.

The amino acid composition of most K^+ channels is known (201), and recently, the molecular structure of the pore has been elucidated from X-ray analysis of the crystallized molecule (234). The channels show a remarkable homology with the Na^+ and Ca^{2+} channels. However, whereas Na^+ and Ca^{2+} channels consist of tandemly linked four domains of six transmembrane segments that are connected in one long polypeptide, only one domain with six transmembrane segments is found for the K^+ channel. Segment 4 shows a high density of positive charges and acts as potential sensor. Two types of inactivation have been described in expressed channels (794): N-type inactivation in which the negatively charged NH_2 terminal acts as a ball and blocks the open channel and C-type inactivation (COOH terminal) in which conformational changes on the extracellular side close to the pore result in some kind of constriction. C-type inactivation is sensitive to drug binding and extracellular K^+. The ligand-activated and background channels have a simpler structure and contain only two transmembrane segments. Recently, a new family of K^+ channels with two pore segments in tandem and four transmembrane segments have been expressed. They act as background channels (530); some of them are activated by arachidonic acid and polyunsaturated fatty acids (280). A tetrameric structure for all K^+ channels is highly likely. The pore in K^+ channels is formed by a stretch of 19 amino acids in the link between S5 and S6 in the four repeats. The motif GYG (or of GFG) in the P-region is the signature of K^+ selectivity, but other residues also participate in determining K^+ selectivity.

A) K^+ OUTWARD CHANNELS WITH FAST ACTIVATION. Upon depolarization, three different K^+ currents are rapidly activated. They can be distinguished by their rate of inactivation, which is relatively fast for I_{to}, slow to ultraslow for I_{Kur}, and nonexistent for I_{Kss}, also called background current. On the latter current, no detailed information is available at the present time.

I) The fast transient outward current. The fast transient outward current (I_{to}) is a transient outward K^+ current that is rapidly activated and inactivated and

blocked by millimolar concentrations of 4-aminopyridine (4-AP). The criterion of 4-AP sensitivity is not exclusive. In some species, another K^+ current, the I_{Kur}, is also blocked even by micromolar concentrations of the drug (see below), and in the dog ventricle part of the I_{to} is insensitive to millimolar concentrations (607). It should be distinguished from another transient outward current carried by Cl^- and activated by $[Ca^{2+}]_i$; this current is also called I_{to2}. In this review it will be indicated as I_{ClCa}. I_{to} is partly responsible for the initial fast repolarization or phase 1 during the action potential. The density of I_{to} varies among species and in a particular species it varies in different parts of the heart (see Ref. 117). It is more expressed in the atrium and Purkinje fibers and in the ventricle more in the epicardial than endocardial fibers. The density of I_{to} in the heart increases after birth (259, 483, 639), although its presence is variable (191, 342), an observation which is possibly related to pathological downregulation.

A) Activation and inactivation. The current is activated upon depolarization. Values for time course and steady-state vary among species and experimental conditions. In the rabbit, time course is fast and monoexponential (269); in the ferret it is sigmoidal (118). Midpoint voltage values for steady-state activation vary between −10 mV in the rabbit (269) and +20 mV in the ferret (118).

After activation, the current decays. The time course of inactivation has been described as monoexponential or biexponential. Time constants again vary but are in the order of 25–75 ms and are voltage independent. Steady-state inactivation shows half-maximum potentials between −50 and −15 mV (see Ref. 117) (Fig. 5*A*). Recovery from inactivation is very sensitive to voltage, being faster the more hyperpolarized the membrane. It is also facilitated by increasing $[K^+]_o$ (284); this supports the hypothesis that inactivation is of the N type (750). For C-type inactivation, the process is slowed by extracellular K^+ acting as a "foot in the door."

Actual time constants for recovery vary with species. In most species, including humans (15, 284, 342), recovery is fast with time constants in the order of 20–60 ms at −80 mV; frequency dependence is small (Fig. 5*B*). In rabbit atrium and ventricle, in sheep and dog Purkinje fibers (see Ref. 117), and in human subendocardial fibers (708), recovery is slow to very slow (time constants of 1–6 s). In these latter preparations, the current is markedly reduced (272) and shortening of the action potential markedly less at elevated frequencies (512).

B) Permeation. On the basis of measurements of E_{rev} (25, 118, 158, 416, 707, 708, 1088), the I_{to} current is considered to be mainly carried by K^+, although it seems less selective than other K^+ currents, such as I_{K1}. At the single-channel level, the current-voltage relation is linear and single-channel conductance in 145 mM $[K^+]_o$ is on the order of 10–30 pS (63, 158, 717) with the exception of a much lower value of 3–4 pS for ferret ventricle (118). The conductance increases at elevated $[K^+]_o$, with a K_d of 200 μM (284).

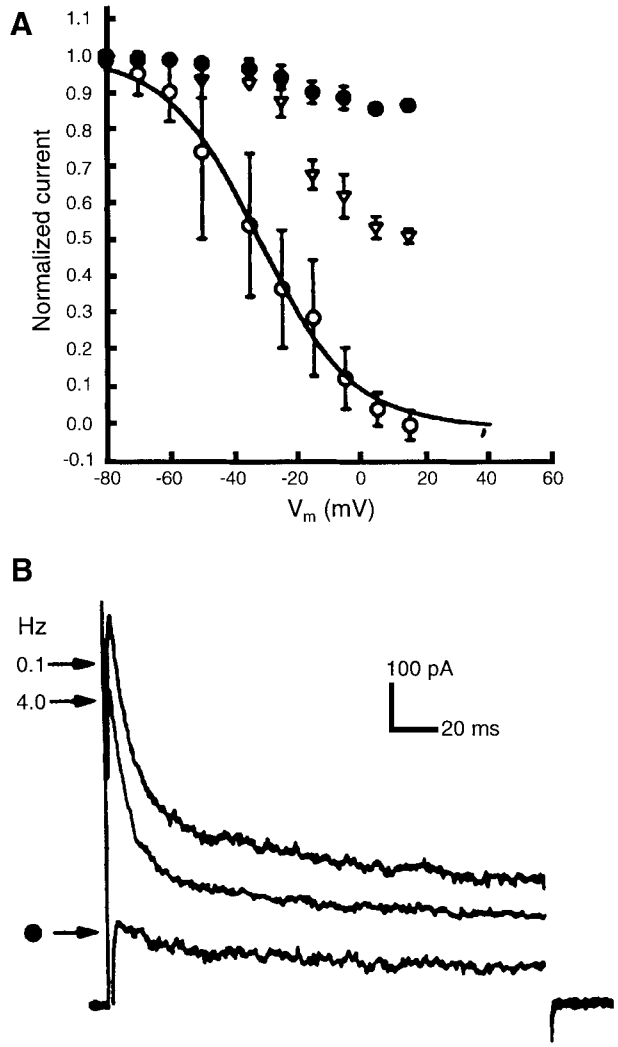


FIG. 5. Inactivation and frequency dependence of I_{to} and I_{Kur} in human atrial myocytes. *A*: identification of 2 outward currents based on their different time dependence of inactivation. For prepulses of 400 ms between −80 and −20 mV, peak currents (○) inactivated while late currents (●) were constant. For longer prepulses (2,500 ms) also, late current partially inactivated (▽). [From Firek and Giles (284). Copyright 1995 Elsevier Science.] *B*: rate dependence of I_{to} and I_{Kur}. Atrial myocyte was stimulated at 0.1 and 4 Hz (*top traces*); trace marked by solid circle is difference current and demonstrates that current affected is I_{Kur}. [From Fermini et al. (272).]

As molecular substrates for I_{to}, Kv4.2 (922) and Kv4.3 (229) have been proposed. On the basis of differences in voltage dependence, kinetics of inactivation and recovery, and block by 4-AP, Kv4.2 is the better candidate for I_{to} in the human, rat, and ferret, and Kv4.3 in the canine and human subendocardium. Kv1.4 may be the better choice for the sheep and the rabbit. In the rat, expression of the protein shifts from Kv1.4 to Kv4.3 after birth and during thyroid treatment (1094).

II) I_{Kur}. A rapidly activated K^+ current, with no or very slow inactivation is present in different heart preparations. The voltage dependency of the slow inactivation process and the sensitivity to 4-AP is species variable, and on this basis, it can be concluded that the current does not correspond to a unique channel. On the basis of the sensitivity to 4-AP, the currents can be subdivided into two groups. In the human atrium (15, 192, 388, 1065), dog atrium (1164), cultured rat neonatal ventricle (347), and mouse ventricle (287, 1182), I_{Kur} is exceptionally sensitive to 4-AP and completely blocked by concentrations of 50 μM or less. In rat atrium (97) and ventricle (25) and human ventricle (707), the current is insensitive to 4-AP. In many publications it is described as a noninactivating component of I_{to}.

A) Activation and inactivation. On depolarization to levels positive to −40 mV, an outward current remains after subtraction of a rapidly inactivating I_{to} in many species: rat atrium (97, 1021), rat ventricle (25, 347, 1083), human atrium (890, 1065) and human ventricle (707, 1087), rabbit ventricle (272), guinea pig ventricle (1161), and dog atrium (1164). It is rapidly activated and shows no or only very slow decay. The current inactivates however. Inactivation has been determined using long (tens of seconds) conditioning pulses. Midpoint inactivation potential is variable: −70 mV (25) and −90 mV (1083) in the rat and much less negative values of −9 and −20 mV in the human atrium (284, 890, 1065) (Fig. 5*A*). In accord with the existence of slow recovery from inactivation, the current is markedly reduced at elevated frequencies in the rat ventricle (25), rabbit ventricle (272)), and human atrium (272, 284, 890) (Fig. 5*B*). In these preparations, a rest current (I_{Kss}) remains, which seems different from I_{Kur}. It is reduced by α-receptor stimulation in rat atrium (1021) and by β-receptor stimulation in rat ventricle (849).

B) Permeation. I_{Kur} is assumed to be carried by K^+, but direct demonstration is mostly lacking. In human atrium, tail currents reverse at negative potentials, suggesting a predominant (388, 1065) but not exclusive (191) permeability to K^+. In favor of the K^+ nature is the observation of sensitivity to tetraethylammonium (TEA) in rat ventricle (25) and dog atrium (1164) and block by Ba^{2+} in guinea pig ventricle (33, 1161) (in humans the current is not sensitive to Ba^{2+}). Single-channel conductance in 5.4 mM $[K^+]_o$ is in the order of 14 pS for the guinea pig ventricle (1161) and 20 pS for the dog atrium (1164) and is sensitive to $[K^+]_o$. Fully activated current-voltage relations show outward rectification (388, 1065).

The Kv.1.5 protein is a possible molecular candidate for the I_{Kur} current in the human atrium (271, 923, 1065). It shows a high sensitivity to 4-AP (1067) and a limited, partial inactivation at positive potentials but is insensitive to TEA. The single-channel conductance is 17 pS. The protein is present in the rat and the human atrium and ventricle, as determined by immunolocalization; it is highly concentrated in the intercalated disks (669). In the dog, Kv3.1 has been proposed as a molecular candidate (1164).

B) K^+ CURRENTS WITH DELAYED ACTIVATION: DELAYED K^+ CURRENTS. On the basis of kinetics, rectification, sensitivity to blockers, and modulation by intracellular messengers, two delayed K^+ currents, I_{Kr} and I_{Ks}, can be distinguished (153, 838); I_{Kr} shows activation and inactivation, I_{Ks} only activation. Both are present in the human atrium (1066), human ventricle (77, 556, 605, 1033), guinea pig ventricle (838), guinea pig atrium (433), dog ventricle (324, 613) and atrium (1165), rabbit atrium (700) and ventricle (832), mouse neonatal ventricle (1055), and rat ventricle (141, 1115). Only I_{Kr} has been clearly described for the cat ventricle (290), the ferret ventricle (617), and rabbit SAN (1032) (466). I_{Ks} seems the only delayed current in the guinea pig SAN (22). Density of the two currents varies in different layers of the myocardial wall. In midmyocardium of the dog ventricle, expression of I_{Ks} is small (613); this explains the longer action potential in these cells. In the ferret ventricle, the ERG protein (Kr) is most abundant in the epicardial layers (99).

I) Rapid delayed K^+ *current*, I_{Kr}. A) Kinetics. I_{Kr} activates rapidly for depolarizations positive to −40 mV, with a midpoint voltage between −20 and −5 mV; this value is $[K^+]_o$ independent (889). Time constants of activation vary among species; in the guinea pig, they are in the order of 175 ms at −30 mV and shorten at more positive or more negative potentials to ~50 ms (838). In the rabbit ventricular cell, time constants for activation are longer, 500 ms at −40 mV to less than 100 ms at 0 mV (128). Compared with I_{Ks}, these time constants are shorter, a finding on which the distinction of the two currents has been based. Deactivation does not follow the same pattern. It is fast in the guinea pig but much slower in the rabbit (128) and in the dog (324); in these preparations, the time course is composed of at least two exponentials, of which the first one is on the order of 0.5 s and the second on the order of 5–10 s at −40 mV (129, 324).

At the single-channel level, the mean open time is on the order of 3 ms. Closed time distribution is biexponential with values of 0.6 and 22 ms (at −100 mV and 100 $[K^+]_o$) (889).

In whole cell recordings, the time course of the macroscopic current shows saturation with no indication of a secondary decrease. The tail currents on hyperpolarization, however, are preceded by a "hook," and the current temporarily increases before it declines in an exponential way (838, 889). The initial increase in outward current has been interpreted (889) as due to recovery from inactivation, a process supposed to be faster than the deactivation process. The hypothesis implies that the current during depolarization very rapidly undergoes inactivation, before there is any substantial activation (Fig. 6). It implies that steady-state inactivation extends over a voltage range that

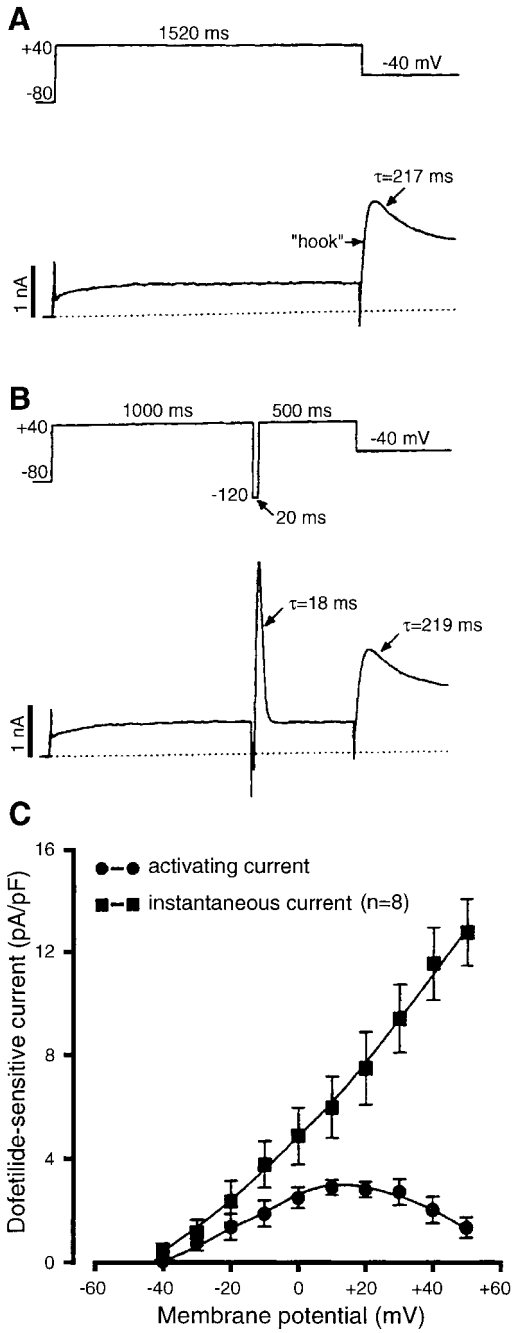


FIG. 6. Rapid inactivation determines inward rectification. Experiments were performed on HERG channels expressed in AT1 cells. In *A*, a depolarizing pulse to +40 mV reveals a small activating current, followed by a prominent tail at −40 mV. τ, Time constant. In *B*, protocol was identical except that a brief 20-ms hyperpolarizing pulse to −120 mV was interpolated in pulse to +40 mV. This hyperpolarizing pulse was followed by a large outward current that decayed rapidly. Result is interpreted as being due to rapid removal of inactivativation by hyperpolarizing pulse, followed by fast inactivation at +40 mV. In *C*, data from 8 experiments are displayed. Activating current (circles) (see also *A*) display inward rectification. Instantaneous currents, following a short hyperpolarizing pulse (see *B*), show a quasilinear, slightly outward going current-voltage relation (squares). [From Yang et al. (1141). Copyright 1997 American Heart Association.]

is quite positive. The consequence is that the current rectifies in the inward direction. Inactivation preceding activation has also been demonstrated in the expressed HERG channel (836, 932, 1141), which has been shown to be responsible for the I_{Kr} current. Fast recovery from inactivation is the reason for an increase in the number of openings of the Kr channel upon repolarization during early diastole in nodal cells (466, 1033).

Inactivation of the I_{Kr} channel is of the C type (836). The evidence is based on the following observations: *1*) truncation of the NH_2 terminal has no effect on the phenomenon; *2*) intracellular TEA has no effect, but external TEA reduces the current and slows inactivation; and *3*) an increase of $[K^+]_o$ slows inactivation in expressed channels and in AT-1 cells (1141). It is further of interest to mention that increases in $[Mg^2]_i$ or $[Ca^{2+}]_i$, which generate inward rectification in other K^+ channels, do not change rectification of I_{Kr} (836).

B) Permeation. Although preferentially permeable to K^+, the channel's K^+ selectivity is less pronounced than that of I_{K1}. Especially at lower $[K^+]_o$, the E_{rev} is quite positive to the equilibrium potential for K^+ (E_K). The conductance falls at lower $[K^+]_o$ (850, 889), a phenomenon explained as block by external Na^+ or more pronounced inward rectification. C-type inactivation and thus inward rectification is enhanced at low $[K^+]_o$ (1141).

In 150 mM $[K^+]_o$, the single-channel conductance is ~10 pS in SAN (466), AVN cells of the rabbit (889), human ventricular cells (1033), and guinea pig atrial cells (433). A value of <2 pS can be extrapolated for normal Tyrode solution.

External bivalent and trivalent cations block the channel. Especially sensitive is the block by Co^{2+} and La^{3+} (10 μM) (265, 837). External Cd^{2+} causes a positive shift of the activation curve (290) and a reduction in inward rectification, in this way increasing the current during a depolarizing pulse (749). The block by Ca^{2+} and Mg^{2+} is reduced by elevating $[K^+]_o$ but does not change inward rectification (421, 422). The HERG gene is responsible for the I_{Kr} protein expression (836, 932).

II) The slowly activated I_{Ks} current. A) Activation. The I_{Ks} current only shows activation and no inactivation. Activation occurs over a broad range of depolarizing potentials. In many experimental conditions it is difficult to obtain a clear-cut saturation. Half-maximum values vary considerably from −13 mV (666) to 26 mV (38). Kinetics are slow; the time course of the rise in current is sigmoidal, whereas the decay of the tails is monoexponential at voltages negative to −50 mV but biexponential at more positive potentials (605, 666). Deactivation is slow in the guinea pig but relatively fast in the dog and the rabbit (128, 324, 613). At the single-channel level, kinetics are complex with many open and closed times (38, 242).

B) Permeation. The channel is less selective than I_{Kr}. The E_{rev} is more positive than that for I_{K1} and changes

only by 49 mV for a 10-fold change in $[K^+]_o$ (666). The fully activated current-voltage relation approaches linearity, except for the current in frog atrial cells where inward rectification is present (243).

Single-channel conductance is relatively low with estimations of 5.4 pS in guinea pig ventricle (38), 3 pS in guinea pig atrium (433), and a greater value of 20 pS in frog atrial cells (242).

Extracellular K^+ has no direct effect on the conductance but affects the current through changes in the chemical gradient; thus in zero $[K^+]_o$, the current is greatly increased, especially in Na^+-free conditions (850). Cobalt (1 mM) (265) and La^{3+} (at 100 μM or higher) (38, 837) block the current. A rise in $[Na^+]_i$ or $[Ca^{2+}]_i$ enhances I_{Ks} (978, 728).

C) Molecular structure. Coexpression of the minK and the Kv.LQT1 generates a current with the characteristics of the cardiac I_{Ks} (39, 835). The KvLQT protein has the classical constitution of voltage-activated K^+ channels. The minK protein consists of only 129 or 130 amino acids and a single putative transmembrane domain, with the NH_2 terminal turned to the external side of the membrane (956, 1023). It plays an essential role in the function of Kv.LQT1 and can be considered a regulator protein.

c) THE INWARD RECTIFIER. The inward rectifier current (I_{K1}) is the current responsible for maintaining the negative resting potential in cardiac cells; it also plays an important role during the final rapid repolarization during an action potential (894). The density of the I_{K1} is highest in the Purkinje and ventricular system (445), less in atrium (396); in the SAN, the I_{K1} current is absent (459). A substantial increase of the current occurs during development from the neonatal to the adult stage (487, 1049).

I) Activation-deactivation: inward rectification. Is I_{K1} a background or voltage-activated current? The I_{K1} current, the first K^+ current to be characterized in cardiac cells, was considered initially to be a time-independent background current. Its pronounced inward rectification provided an explanation for the existence of a long plateau in the cardiac action potential (see Ref. 133). With the improvement of recording techniques, it became clear that I_{K1} showed time-dependent changes, which were analyzed as activation, deactivation, and inactivation (569). Upon hyperpolarization from a holding potential of -50 mV to -100 mV, a quasi-instantaneous current jump is followed by an exponential increase in inward current to a steady state (385, 569, 982). On depolarization, the reverse sequence is seen. This led to the hypothesis that the I_{K1} channel opens and closes by an intrinsic gating process not different from other voltage-operated channels. More recently, the time-dependent changes in the current or gating have been recognized to be generated by a time-dependent block-unblock by Mg^{2+} (656, 1009) and polyamines (622, 1093). Magnesium and putrescine ions are responsible for the very rapid phase, spermidine and spermine ions for the slower phase (622, 723) (Fig. 7). The difference in rate corresponds to the difference in positive charge (263, 1139). The block is voltage dependent, with an electrical distance of 0.3 for $[Mg^{2+}]_i$; polyamines seem to penetrate deeper in the pore (622, 723). With time at the depolarized level, the block shifts from fast Mg^{2+} to slow polyamine block. On hyperpolarization, this is seen as an increase in the slower phase of activation (463). This new concept of activation being an unblocking is in accord with the information obtained on the molecular structure of the inward rectifier family, in which a voltage sensor or S4 segment is lacking. The I_{K1} molecule consists of only

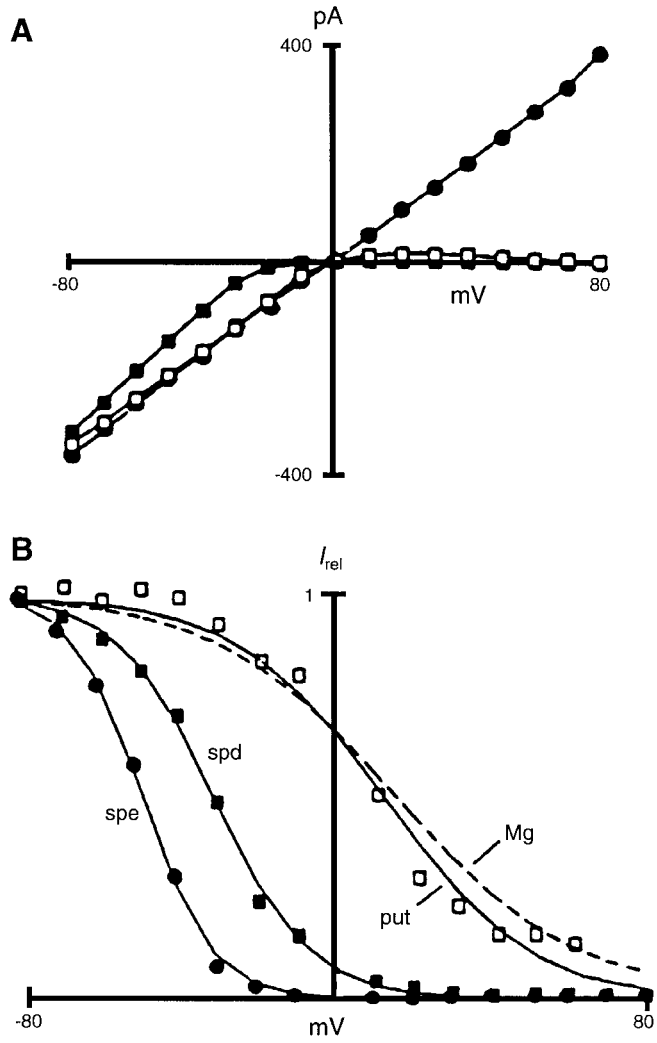


FIG. 7. Block by intracellular positvely charged substances determines inward rectification. Experiments performed on HRK1 channels expressed in *Xenopus* oocytes. *A*: current-voltage relations for 20-ms test pulses to different potentials, after patch isolation (●) and in presence of 25 μM (□) and 1 μM spermidine (■). *B*: normalized currents as a function of membrane potential in 500 μM spermine (spe), spermidinine (spd), and putrescine (put). Curves are fitted by Boltzmann functions with electrical distances of 2.64 for spe, 2.21 for spd, 1.69 for put, and 1.12 for Mg^{2+}. [From Lopatin et al. (622). Copyright 1994 Macmillan Magazines Ltd.]

two transmembrane segments with a H5 or pore sequence in between (563, 954). The I_{K1} channel can thus be considered a background channel, and a distinction between gating and permeation becomes less obvious. Whether on top of block-unblock there still exists an intrinsic gating mechanism is not fully resolved, and recent experiments on IRK1 channels expressed in oocytes have been explained in this way (893).

At the single-channel level, activation at hyperpolarized levels is correlated with a change from a lower to a higher conductance substate eventually to the fully open state and a prolongation of the open time: 10 ms around E_K and 100 ms at 60 mV negative to the E_{rev} (569, 652, 756, 990).

Activation or unblocking from Mg^{2+} or polyamines depends on $[K^+]_o$, $[K^+]_i$, and the time spent in the depolarized state. In K^+-free medium, no I_{K1} current can be recorded (see references in Ref. 123); the channel seems to remain blocked. The process has been called "K^+ activation" (123, 155).

Also, intracellular K^+ interferes with activation: the higher $[K^+]_i$, the faster the current rise during activation (735). The K^+ gradient or E_K furthermore determines the position of the apparent activation curve on the voltage axis (167).

At hyperpolarized levels, the current after being "activated" frequently undergoes a secondary decrease or inactivation, which is due to block by external ions such as Na^+, Mg^{2+}, and Ca^{2+} (86, 386). At the single-channel level, the inactivation corresponds to a fall in open probability (831).

II) Permeation and selectivity. The I_{K1} channel is very selective for K^+ (498, 756, 830). Reported values for single-channel conductance differ appreciably and show a cluster at ~22–28 pS (655, 671, 831) and another at ~45 pS (145 mM $[K^+]_o$) (305, 468, 498, 569). In expressed channels, the variabitlity is even larger with values varying between 9 and 20 pS (1092). No explanation for this difference is available.

In guinea pig ventricular myocytes, the channel shows a fully open conductance of 22 pS and three substates of 7-pS difference (652, 655) or a fully open state of 28 pS and four substates (671). In the analysis of Matsuda (655), the channel is supposed to form a three-barreled structure; it provides an explanation for the occurrence of three substates.

The conductance largely depends on $[K^+]_o$; in quantitative terms, the conductance increases with $[K^+]_o$ raised to a power value varying between 0.21 and 0.62 (756, 831).

Extracellular and intracellular Cs^+ and Rb^+ can be regarded as blockers of the channel. The block is voltage dependent with an electrical distance of 0.6 and 0.14, respectively. The block by Cs^+ results in a highly flickering mode of activity (498, 830) and in 20% the channel shift to a lower substate (653, 655). External Ba^{2+} is a very efficient voltage-dependent blocker; it shortens open time and decreases the number of openings (51, 498, 830).

The effect of protons is discussed in section II*A2*.

D) LIGAND-ACTIVATED K^+ CURRENTS. *I) ACh-induced K^+ current.* Slowing of the heart beat is caused by activation of a specific K^+ channel, the ACh-induced K^+ current (I_{KACh}), in the SAN (see review in Ref. 1095). In lower vertebrates and birds, the current is present in atrial as well as ventricular cells (381). In the mammalian species, the current is expressed in atrial cells, AV node cells, and Purkinje cells; in ventricular cells, the channel is not present in all species but has been described for the ferret (95), the rat (676, 1119), the dog (1142), and the human (558).

A) Activation. Activation of I_{KACh} occurs upon binding of ACh to the M_2 muscarinic receptor. The receptor is directly coupled to the K^+ channel via a guanine nucleotide-binding protein or G protein, characteristically inhibited by pertussis toxin. Activation of the channel is due to binding of the $\beta\gamma$-subunits to the channel as demonstrated in isolated native (1126) and cloned channels (800). G$\beta\gamma$ binds to the COOH terminal of the channel (456).

Activation of the G protein connected to the K^+ channel is also possible through stimulation of other receptors: adenosine (P_1 receptors) (54, 926), external ATP (P_2 receptors) (665), somatostatin (602), calcitonin gene-related peptide (522), endothelin (523), a serum albumin-associated factor of phospholipid nature (105), and sphingosine-1-phosphate (1019).

The presence of an agonist, however, is not absolutely necessary, and background openings of the channel have been observed in different situations. Such activity has been related to the presence of ATP, of increased $[Na^+]_i$, or of leukotrienes. Leukotrienes act on the G protein and stimulate the exchange of GDP for GTP (570). For activation by ATP, different mechanisms have been proposed; local GTP formation from GDP through activation of nucleotide diphosphate kinase (NDPK) (395, 492), an unknown effect of NDPK (1122), phosphorylation (531, 930), which makes the channel sensitive to $[Na^+]_i$ (943), or generation of phosphatiditylinositol 4,5-bisphosphate (PIP_2) by ATP-dependent lipid kinases (440).

B) Permeation and rectification. The channel is very selective for K^+. The conductance is highly sensitive to extracellular K^+ concentration. In symmetric conditions (150 mM $[K^+]$), the conductance is 40–44 pS. The open times are very short (1–3 ms), occurring in bursts (829, 924). Open probability in human atria is very variable, from 0.03 to 0.3 in the presence of 10^{-5} M ACh (396).

At the whole cell level, the current is characterized by inward rectification. The current activates on hyperpolarization and deactivates incompletely on depolarization (731). The phenomenon resembles the time-depen-

dent changes in the I_{K1} current, although they are of a slower nature and smaller in amplitude. Similar to the I_{K1} current, rectification is explained by block of the open channel by intracellular Mg^{2+} and polyamines (1127). The I_{KACh} is blocked by classical K^+ channel blockers such as Cs^+ and Ba^{2+} (130, 698, 1170).

Fade or desensitization is the process by which the current through the channel decreases, although the agonist concentration remains constant. Upon washout of the agonist, the response to a second exposure remains temporarily depressed (131). In the heart, fade is more pronounced for the KACh channel and less for other channels coupled to M_2 receptors (I_{Ca}, I_f, and I_{Ks}) (95, 131, 430).

For relatively short exposures of ACh, fade occurs in two phases: a rapid phase (up to 30 s) (524, 571) occurs at the channel or G protein level followed by a slower one (up to 3 min) in which the receptor is involved (1061, 1171). At the single-channel level (524), the fast phase is characterized by a shortening of the open time from 4.3 to 1.1 ms; it is probably due to dephosphorylation of the channel or of the G protein (903) or inhibition of the ATP effect (531); the desensitization is heterologous and is not restricted to a single type of receptor. The slower phase is characterized by a reduction in the frequency of opening (524) and is probably related to a phosphorylation of the muscarinic receptor by a receptor kinase; this kind of desensitization is thus homologous (1171). In accordance with this explanation, the fast phase still occurs when the receptor is short-circuited by acting directly on the G protein via guanosine 5′-*O*-(3-thiotriphosphate) (430) or arachidonic acid metabolites (860), but the slow phase is absent.

A third phase of fade occurs when cells have been exposed to the agonist for many hours. The underlying process in that case seems to be internalization (106).

C) Molecular structure. From a structural point of view, a combination of two inward rectifier channels is proposed to be responsible for I_{KACh}: the Kir3.1 and Kir3.4 (142, 563). Coexpression of the two proteins in combination with the M_2 receptor elicits an activity that highly resembles KACh (562).

II) The ATP-inhibited K^+ channel: KATP. The KATP channel, first described in heart cells (729), is present in many other cell types (465). In heart, it is expressed in ventricular, atrial, as well as nodal cells from different species, including the human heart (32). In heart cells it seems to exert a protective role during an ischemic insult. By shortening of the action potential, generation of inexcitability and shifting the E_m closer to the equilibrium potential for K^+, excessive loss of K^+ is avoided. Activation of the channel also seems responsible for preconditioning or protection against a second insult.

A) Activation. In inside-out patches, a K^+ channel with a high conductance is activated when the cytoplasmic [ATP] is decreased below a critical concentration. ATP normally decreases the P_o. The K_d determined in inside-out patches is ~0.1 mM (540, 725, 729). This value should be considered a mean value. For individual channels, the K_d may differ by as much as three orders of magnitude and ranges from 9 to 580 μM (279). Hill coefficients vary between 1.0 and 5.0. Anionic ATP as well as MgATP are able to inhibit the cardiac channel. The sensitivity to ATP is higher in the presence of free Mg^{2+} (278). Kinetics of the single-channel activity are complex with multiexponential distributions for open and closed times and bursts (788). The burst duration is inversely related to [ATP], and intraburst kinetics depend on Mg^{2+} concentration (1186).

The K_d value is much lower than what is considered the normal [ATP] of 5 mM or more. How is it then possible to activate the channel? Two considerations should be made: the local [ATP] seen by the channel can be lower than the bulk concentration, and the sensitivity to block by ATP is variable.

1) The subsarcolemmal [ATP] that is seen by the channel is much lower than the bulk value when the Na^+-K^+-ATPase (781, 998) or the adenylate cyclase (435, 858) is activated, or when anaerobic glycolysis is blocked (1081).

2) The sensitivity to block by ATP can be reduced by acidosis, dinucleotides, an increase in lactate or decrease in taurine, congestive heart failure, an increase in $[Mg^{2+}]_i$ or $[Ca^{2+}]_i$ and polycations, receptor activation, changes in the cytoskeleton, and, most important, changes in PIP_2 concentration at the intracellular side of the membrane.

Intracellular acidosis, although reducing single-channel conductance (266), increases P_o as long as ATP is not totally depleted (193, 590). The concentration-response curve is shifted to higher [ATP] (560). The presence of Mg^{2+} is required and suggests a change in phosphorylation of the channel (193). When acidosis is too pronounced and the pH falls below 6.0, P_o is markedly reduced (560).

Lactate (366, 516) has an activating effect, whereas taurine (364) and Cl^- (215) reduce activity. The inhibitory effect of Cl^- may be due to protein destabilization.

Dinucleotide and MgADP shift the inhibitory curve to higher [ATP]; the mechanism is not of a competitive nature, since both substances bind at different sites (494, 729, 970). Adenosine 5′-diphosphate can also reactivate the channel after rundown due to the absence of ATP or presence of high $[Ca^{2+}]_i$. Dinucleotides thus exert effects similar to K^+ channel openers.

Higher concentrations of ATP are needed to block the channel when the concentration of Mg^{2+} or Ca^{2+} and polycations such as protamine, polylysine, and poly-arginine are small. These cations are supposed to neutralize negative charges at the mouth of the channel; in their

absence, access of ATP to its binding site is antagonized (212).

Activation is further facilitated by phosphorylation and G protein interaction. Protein kinase C-induced phosphorylation (439, 610) occurs after stimulation of α_1, P_1, P_2, M_1, and M_2 receptors (608, 619, 1062). A direct coupling to the channel via a G protein is present for P_1 and M_2 receptors (469, 532, 540, 969).

The functional availability of the channel is dependent on the presence of an intact cytoskeleton. Actin filament-depolymerizing agents (cytochalasin and desoxyribonuclease I) accelerate rundown in excised patches (968), whereas actin stabilizers (phalloidin) or PIP_2 (inhibitor of F-actin severing protein) inhibits rundown (306). Disruption of actin filaments also impairs the sulfonylurea inhibition of the channel (98, 1151).

Recently, a dramatic decrease in sensitivity to ATP of Kir6.2 expressed channels has been described after addition of micromolar concentrations of PIP_2. The hypothesis is formulated that the COOH terminal of the inward rectifier binds to PIP_2 in the membrane and keeps the channel activated unless [ATP] is raised to the millimolar range (47, 904).

B) Permeation, conductance, and rectification. The ATP-dependent channel shows a high conductance of 80 pS in symmetrical $[K^+]$ conditions (494, 729). It is very selective for K^+, with a Na^+ permeability of only 0.01 relative to K^+. The conductance depends on $[K^+]_o$ (724). In physiological $[K^+]_o$, the conductance falls to 25 pS (277).

In the presence of high $[K^+]_o$, the channel shows inward rectification. At normal physiological $[K^+]_o$, however, the current shows no rectification or even outward rectification up to 0 mV (722, 934) (Fig. 8*A*). Outward rectification is also characteristic for the current generated during metabolic inhibition (462), hypoxia (61), and in the presence of potassium channel openers. Interpretation of rectification in those cases should take into account the simultaneous inhibition of the I_{K1} current. At positive voltages, a weak inward rectification is seen, which is caused by Mg^{2+} block and not by spermidine or spermine ions (1127).

External cations like Ca^{2+}, Sr^{2+}, Ba^{2+}, Na^+, and H^+ block the channel, but efficiency is small. Zn^{2+}, Cd^{2+}, and Co^{2+} with K_d values of, respectively, 0.46, 28, and 200 μM are the most efficient (573). The block is voltage independent and reduced in ATP depletion. The channel is blocked by intracellular Cs^+ (490).

C) Molecular structure. The cardiac channel is of heteromultimeric nature (455), composed of a protein from the "inward rectifying" family, the Kir6.2 and the sulfonylurea receptor (SUR-2A), a protein from the ATP-binding cassette superfamily (4); to this family also belong the cystic fibrosis transmembrane conductance regulator (CFTR; see sect. II*A4*), the P-glycoproteins, and the multiple drug resistance receptor (MDR).

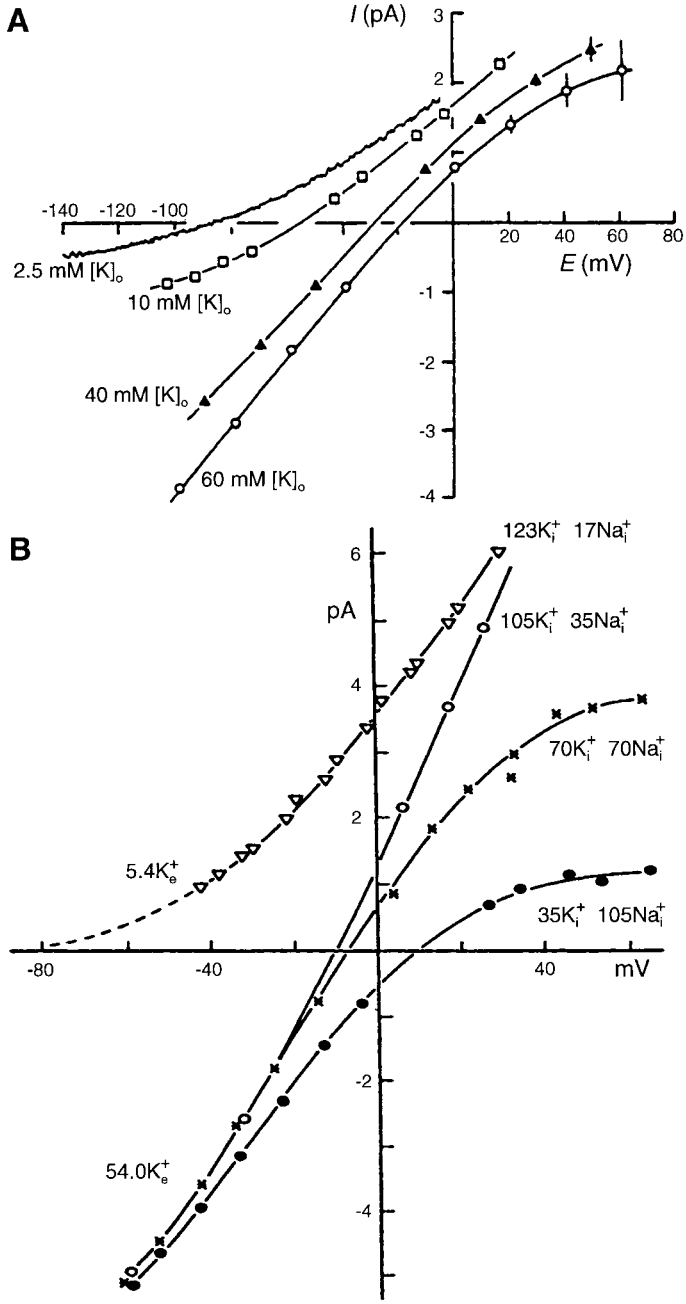


FIG. 8. Goldman-type behavior of ATP-sensitive K^+ current (I_{KATP}) and Na^+-activated K^+ current (I_{KNa}). *A*: current-voltage curves for individual ATP-sensitive K^+ channels from frog skeletal muscle at different $[K^+]_o$. Inside-out patches were used. $[K^+]_i$ was 120 mM throughout. [From Spruce et al. (934) with permission.] *B*: single-channel current-voltage relationships of I_{KNa} in inside-out patches of guinea pig ventricular myocyte. Experimental conditions for $[K^+]_i$, $[K^+]_o$, and $[Na^+]_i$ are given on figure. Relation shows inward rectification when $[K^+]_o$ is greater than $[K^+]_i$ and outward rectification for opposite condition. [From Luk and Carmeliet (625). Copyright 1990 Springer-Verlag.]

The cardiac type is rather insensitive to spermine and spermidine ions (1127), and its block by Mg^{2+} is weak.

The K_d is 2.0 mM at 0 mV and 0.29 mM at −40 mV; the Hill coefficient is 1. The SUR molecules are characterized by multiple membrane-spanning domains and two nucleotide-binding domains (NBD). They act as receptors for sulfonylurea drugs and K^+ channel openers. Activation by MgADP also occurs at the SUR, probably at one of the NBD. Both ATP (338) and GTP (989), on the other hand, inhibit the channel by binding to the Kir6.2 subunit. The expression of SUR-1 facilitates the incorporation of Kir6.2 in the plasma membrane of HEK cells (484).

III) The Na^+-activated K^+ channel. The Na^+-activated K^+ channel belongs to the ligand-activated channels and is activated by $[Na^+]_i$. It was first described in cardiac cells (497).

A) Activation by $[Na^+]_i$. The channel is selectively activated by $[Na^+]_i$; other substitutes such as Li^+ are inefficient (497, 624). Relatively high concentrations of $[Na^+]_i$, however, are required (K_d 66 mM) (497). A similar sensitivity has also been found by other authors (625, 804). The sensitivity to $[Na^+]_i$ is variable from one patch to another and in the same patch for different channels. The P_o is independent of voltage between −100 and +60 mV and only declines for very negative potentials (834). The P_o is greater in cell-attached than in inside-out patches (804); the response curve for $[Na^+]_i$ is shifted to lower concentrations, and maximum activity is increased (804).

The channel's activity is characterized by two open states with time constants in the order of 0.5 and 10 ms and three closed states with time constants in the order of 0.5, 2, and 20 ms (497, 624). Sometimes the channel may enter a quiescent state for many seconds.

B) Permeation. The KNa channel shows a very high conductance (497, 625, 834, 1068) with values up to 200 pS. Its actual value depends on the ratio of intracellular to extracellular $[K^+]$, on the voltage, and on the presence of other ions, such as H^+, Na^+, and Mg^{2+}. Permeation shows Goldman-type behavior with a current-voltage relation that is linear, inward rectifying, or outward rectifying depending on the ratio of intracellular to extracellular $[K^+]$ (625) (Fig. 8*B*). Under physiological conditions, rectification is outward and predicts the channel to carry large currents during the action potential.

The channel shows many (up to 12) substates (834, 1068). An analysis of block by Mg^{2+} and Na^+ is not in favor of a multibarrel structure of the channel (1068).

Selectivity of the KNa channel for K^+ is high. The change of the E_{rev} with different K^+ concentrations follows the predicted values of the Nernst equation (497, 625). Substitution of K^+ by Rb^+ or Cs^+ results in block of the channel (625); P_{Na}/P_K has been estimated to be 0.02 (497). Increases in $[Mg^{2+}]_i$ and $[Na^+]_i$ (1068) cause the channel to shift to a lower conductance state, especially at positive potentials (625).

IV) K^+ channel activated by fatty acids and amphiphiles. Arachidonic acid, unsaturated fatty acids, and phospholipids activate K^+-selective channels in neonatal rat atrial cells (527) and in adult rat atrial and ventricular cells (528) (Fig. 9).

A) Activation. Activation occurs upon addition of fatty acids to either side of the membrane but is more efficient if applied from the cytosolic side. Available evidence suggests that unsaturated fatty acids with two double bonds are required for efficient activation. The channel shows a slight voltage dependence with higher activity at depolarized levels. Arachidonic acid activates the channel directly and not via one of its metabolites, since activation occurs in the presence of inhibitors of cyclooxygenase, lipoxygenase, or epoxygenase. The activity of the fatty acid-induced channel is increased by stretch (525), but stretch as such can also activate the channel, and this effect is not due to stretch-induced release of fatty acids. Once activated by fatty acids, activity can be increased by low pH (525) (Fig. 9). A channel with slightly different rectification characteristics is activated by phospholipids (1050).

B) Permeation. The channel is rather selective for K^+. The channel activated by arachidonic acid shows outward rectification; the channel activated by phospholipids has a linear current-voltage relation (527, 1050). Outward rectification is due to a greater single-channel conductance and a higher P_o at depolarized levels. The single-channel conductance, 94 pS in 140 mM symmetrical conditions, is greater than that of the KATP channel. The channel is thus different from KATP; activity of the latter is actually inhibited by fatty acids. Openings of the channel activated by fatty acids (KFA) occur in bursts. The current is blocked by 1 mM Ba^{2+} but not by TEA, 4-AP, quinidine, or apamine.

As possible molecular substrate a two-pore-forming K^+ channel can be proposed. The channel expressed in neuronal tissue is activated by arachidonic acid (280); other representatitives of this family, however, are insensitive to arachidonic acid (530).

4. Cl^- channels

Four different types of Cl^- channels have been decribed in cardiac cells (444): a channel activated by PKA-dependent phosphorylation, which is probably identical to the channel activated by PKC, a $[Ca^{2+}]_i$-activated channel, a $[ATP]_o$-activated channel, and a stretch- or swelling-induced channel. All channels, except the PKA-induced Cl^- channel, are blocked by disulfonic stilbene compounds (DIDS and SITS); the PKA-activated channel is blocked by 4,4′-dinitrostilbene-2,2′-disulfonic acid (DNDS). They show pronounced outward rectification in symmetrical Cl^- conditions; this rectification is much less present for the PKA-induced current. Two channels have

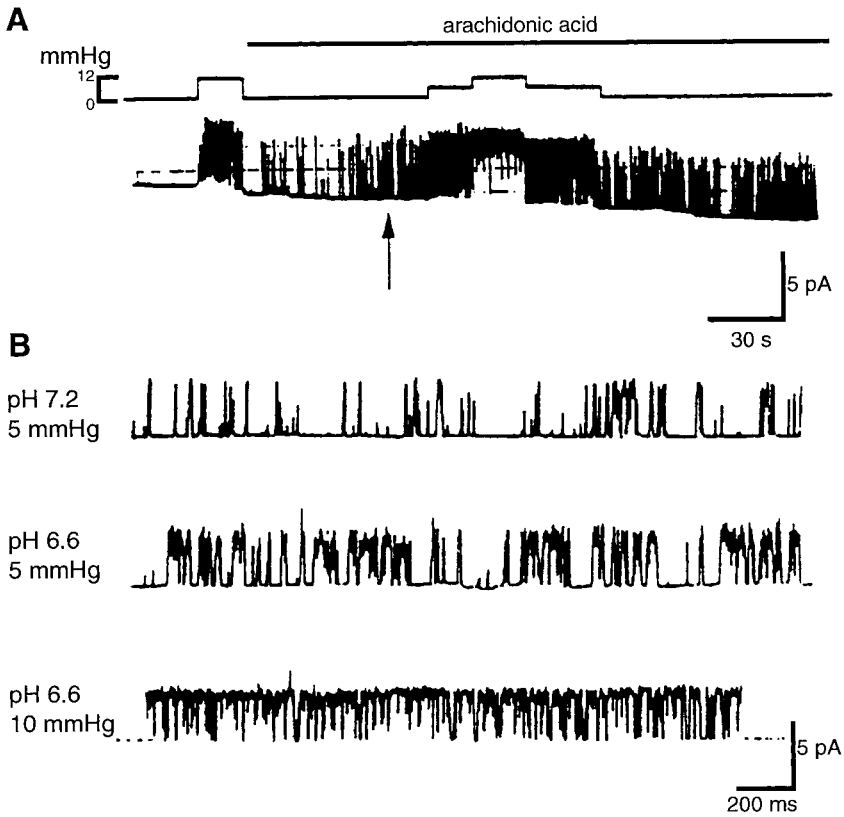


FIG. 9. *A*: single-channel activity of a K^+ channel in inside-out patches of a rat ventricular myocyte (membrane potential +40 mV) in symmetrical 140 mM K^+ conditions. Negative pressure applied to pipette and arachidonic acid (10 μM) added to cytoplasmic side activated same channel. *B*: increased sensitivity of K^+ channel to pressure by intracellular acidosis. Inside-out patch was used. Channel was first activated by negative pressure applied to pipette; activity was further enhanced by lowering pH of cytosolic solution. [Adapted from Kim (525).]

been cloned and expressed: the CFTR channel, which is responsible for the PKA-stimulated channel (378, 596, 710), and ClC-3, which accounts for the swelling-induced current (241).

Because the E_{Cl} is positive to the resting potential, activation of Cl^- current causes depolarization of the resting potential but accelerates repolarization early during the action potential (237). The physiological role of Cl^- currents, however, cannot strictly be delineated, the reason being that the condition to activate the currents may only be present in physiopathological conditions: secretion of catecholamines for the I_{ClPKA}, cell swelling by osmotic forces for $I_{Clswell}$, secretion of ATP in the extracellular medium for I_{ClATP}. Even the I_{ClCa} that is activated by a physiological processes, i.e., the release of Ca^{2+} from the SR, does not seem to be expressed in all cells (absent in human atrial cells, Ref. 604). The density and expression of the currents is very variable; in general, I_{ClPKA} is better represented in ventricular cells and $I_{Clswell}$ in atrial cells.

A) PKA- AND PKC-DEPENDENT Cl^- CHANNEL. The PKA-activated Cl^- current (for review, see Ref. 308) is typically present in the ventricle, less in the atrium; it has been described for the ventricle of the guinea pig (34, 250, 660, 1012), rabbit (especially in the subepicardium, Ref. 383), and cat (1178) and in the human atrium and simian ventricle (1071). It is not present in the rabbit SAN or atrium, the dog atrium and ventricle, or the mouse and rat ventricle (437).

I) Activation. The PKA-dependent channel is typically activated after β-receptor stimulation (308). The biochemical pathway involves phosphorylation of the regulatory domain of the CFTR protein. This protein consists of two membrane domains each of six transmembrane segments, two NBD and one regulatory domain, all cytoplasmatic. β-Receptors and histamine receptors (383, 447) are positively coupled to PKA; negative coupling occurs via M_2 (964), endothelin A (475), and ANG II receptors (733). The nitric oxide (NO) synthase pathway does not seem to be involved (1168). Activation via cAMP is facilitated by simultaneous inhibition of tyrosine kinase (901) and previous exposure to ACh (1167).

Phosphorylation results in a change from silent to active channels (248) and modulates the kinetic behavior (446, 448). The regulatory domain of the channel can be partially or highly phosphorylated, each of these steps corresponding to two types of activity. In a first phosphorylated stage, the channel binds ATP at NBD1, followed by hydrolysis (this step can be reversed by okadaic acid-sensitive phosphatase). With P_i leaving the protein and ADP remaining on the NBD1 site, the channel is activated and shows brief openings; the channel closes when ADP is released from the channel. A second phosphorylation (reverse reaction is okadaic acid insensitive) makes it

possible for the molecule to bind a second ATP molecule at site NDB2. Binding of ATP to this second site and its hydrolysis inhibits the release of ADP from *site 1* and stabilizes the channel in the open state. The activity is characterized by long openings (1 s) and closures of 0.2 and 10 s (250). As long as the hydrolysis products stay on the protein, the channel is locked in the open state with a P_o close to 1.0. Egress of the hydrolysis products suppresses the stabilization and causes channel closure. Inhibition of phosphatase 1 and 2A by calyculin A increases markedly activation of the channel by forskolin (1138).

II) Permeation and conductance. The single-channel conctance is between 7 and 14 pS (710). In asymmetric [Cl^-], the current-voltage relation is outwardly rectifying for the whole cell as well as for the single channel. Current can be carried by other anions, such as ATP, but the conductance is small (871). In ischemia, it may be responsible for the release of ATP from the cell. The permeability sequence for small anions is $Br^- > Cl^- > I^-$ (710, 744, 1012). The channel is slightly permeable to cations with a P_{Cl}/P_{Na} of 10–20 (149).

The channel is blocked by 9-aminoacridine (9-AC) and DNDS (34, 382, 693) as well as glibenclamide (981, 1133) but insensitive to tamoxifen and DIDS and SITS (382).

III) Are PKA- and PKC-induced currents different currents? Activation of PKC opens a Cl^- channel in guinea pig (168) and feline ventricular myocytes (1178), which shows single-channel conductance (9 pS) and kinetics identical to the PKA-activated channel (168, 1178). The currents are not additive and are blocked by the same substances, i.e., 9-AC and DNDS but not by DIDS. The question remains whether the permeability sequence is different (1052). The regulatory domain of the cloned molecule contains consensus phosphorylation sites for both kinases. It is interesting to note that in some preparations in which PKA is ineffective in opening a Cl^- channel, activation occurs via external ATP and PKC (500, 597, 664). In guinea pig ventricular myocytes, PKC activation alone did not activate the channel, but it potentiated the channel supramaximally stimulated by isoproterenol (683). Activation of PKC may also be the mechanism underlying the occurrence of a Cl^- current after stimulation of AT_1 receptors by ANG II (693), since intracellular Ca^{2+} is required to elicit the current.

B) SWELLING-INDUCED OR STRETCH-ACTIVATED CL^- CHANNEL. A volume-activated Cl^- channel is present in atrial cells of the rabbit (357), guinea pig (902, 1012), dog (929, 992), and human (745, 827) and in cultured cells of the chick embryo (1176) and possibly in neonatal rat cells (186).

I) Activation. Activation occurs by exposure of cells to hypotonic solutions, by pressure through the pipette, or after incorporation of an anionic amphipath in the outer leaflet of the membrane that increases the membrane curvature (992). Although stretch may be accompanied by changes in the cytoskeleton, activation does not require the integrity of the cytoskeleton. Application of colchicine, which disrupts microtubules, or cytochalasin, which disrupts F-actin, did not change activation by swelling. Activation is not immediate but takes minutes to develop (929). A phosphorylation by tyrosine kinase has been proposed as the underlying process (745, 974), since the process is blocked by inhibition of this enzyme. It is known that cell stretch can lead to activation of tyrosine phosphorylation (820). Under isotonic conditions, the channel is active in a small percentage of cells (240). Activation does not require stimulation of PKA or a rise in $[Ca^{2+}]_i$ (357, 992). Once activated by volume, forskolin amplifies the current (745), probably by a direct effect of cAMP. Protein kinase C stimulation does not activate but rather inhibits the current (238, 241). Some of these channels are voltage sensitive and show inactivation on depolarization (rat, Ref. 186; guinea pig ventricle, Ref. 902).

II) Permeation. The volume-activated channel is outward rectifying with a conductance of 28 pS at the E_{rev} and 50–60 pS at positive potentials. The P_o is elevated (0.8); DIDS, SITS, and tamoxifen block the channel (239, 240, 1012). The channel is permeable to anions with a permeability sequence of $I^- > NO_3^- > Br^- > Cl^-$ > aspartate (1012). Taurine and inositol efflux occurs upon cell swelling probably via this pathway (473). In the ClC-3 clone, one amino acid mutation (N579K) is sufficient to change the permeability preference from I^- to Cl^- (241). In neonatal rat, a channel has been described (186) with a very large channel conductance of 400 pS and a P_{Cl}/P_{Na} of 25; this channel may be different from the other described above.

C) INTRACELLULAR CA^{2+}-ACTIVATED CL^- CURRENT. A transient and intracellular Ca^{2+}-dependent outward current, carried by Cl^-, has been demonstrated in rabbit myocytes (1192) and is called i_{to2}. The current exists in rabbit Purkinje cells (910), rabbit atrium (604), canine ventricular (1191) and canine atrial cells (1165), ferret ventricular myocytes (985), and cultured chick heart cells (618). It has not been found in the human atrium (604).

I) Activation. During the cardiac action potential, a Cl^- current is activated by Ca^{2+} released from the SR, subsequent to entry of Ca^{2+} via L-type Ca^{2+} channel. Evidence supporting this hypothesis is based on the observation that the current is prevented by block of the L-type Ca^{2+} channel, block of the SR Ca^{2+} release channel, and intensive buffering of intracellular Ca^{2+}, whereas stimulation of Ca^{2+} influx by isoproterenol has the opposite effect. Exposure to caffeine, which releases Ca^{2+} from a preloaded SR store, elicits a current even at negative E_m values, suggesting that the essential phenomenon leading to activation is the increase in local [Ca^{2+}] and not the depolarization (912). Activation and inactivation curves have been described (513); they should not be regarded as the expression of voltage dependence of the

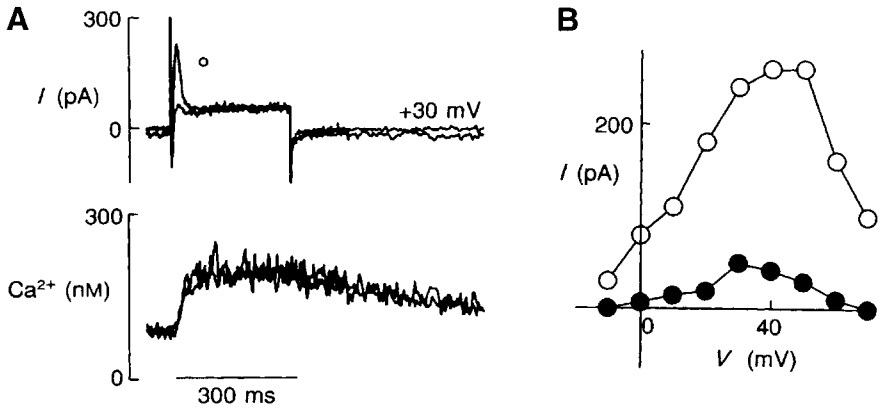


FIG. 10. Ca^{2+}-activated Cl^- current in rabbit single Purkinje cells. *A*: examples of membrane currents (*top traces*) and Ca^{2+} transients (*bottom traces*). Current indicated by open circle is control; other is in presence of 100 μM DIDS. Current is time dependent and much shorter than Ca^{2+} transient. DIDS blocks transient current but does not change Ca^{2+} transient. *B*: current-voltage relationships for Cl^- current obtained as difference between peak current and current at end of pulse, under control conditions (○) and in presence of 100 μM DIDS (●). [From Sipido et al. (910).]

Cl^- channel but generated mainly by the voltage dependence of the Ca^{2+} current.

In Purkinje cells of the rabbit (910) and in ferret ventricular myocytes (985), the Cl^- current is much shorter than the intracellular Ca^{2+} transient and already declines before the Ca^{2+} transient reaches its peak value (Fig. 10). The rapid fall in current is not due to some type of accomodation or "inactivation." Steady activation with no time-dependent decline in activity is obtained in canine permeabilized ventricular myocytes exposed to variable [Ca^{2+}] (513); in inside-out patches, single-channel currents did not show any decrease with time (169). A possible explanation would be that the local subsarcolemmal Ca^{2+} transient seen by the channel is much shorter than the cytoplasmic Ca^{2+} transient. Subsarcolemmal [Ca^{2+}] as seen by the Na^+/Ca^{2+} exchanger, however, does follow the time course of the intracellular Ca^{2+} transient (780). At the present time, the simplest explanation is to assume that activation of the channel requires an elevated [Ca^{2+}] (high threshold), such that the time course of the current is only determined by the short peak of the transient. In Ca^{2+} overload, the Cl^- current is prolonged and shows much slower kinetics, although the duration remains shorter than the intracellular Ca^{2+} transient (747). It is important to note that the current can be activated at negative potentials (912, 1191) and thus may play a role in the genesis of delayed afterdepolarizations and arrhythmias.

II) Permeation. A permeability study (513) shows the following sequence: $SCN^- > I^- > Br^- > Cl^-$; the channel is, however, impermeable to large organic anions such as aspartate (used as substitutes to change the equilibrium potential). The current-voltage relation has been described as outward rectifying (1192) or linear in symmetric conditions (910). In canine ventricular cells (169), the single channel has a small conductance of 1.5–2 pS and shows long openings. The apparent K_d is rather high, 150 μM; it should be mentioned, however, that [Ca^{2+}] seen by the channel can be very different from the bulk concentration. The current is blocked by SITS and DIDS (169, 910, 1192), by DIDS and niflumic acid (594), and by glibenclamide (1133). No information is available on the structure.

D) EXTRACELLULAR ATP-ACTIVATED CL^- CURRENT. *I) Activation.* Purinergic activation of a Cl^- current has been demonstrated in atrial cells of the guinea pig heart (664) and in ventricular cells of the mouse (597) and the rat (500). Activation occurs with ATP, adenosine 5′-*O*-(3-thiotriphosphate), nonhydrolyzable ATP analogs, ADP, and AMP. Adenosine activates the channel in guinea pig but not in the mouse or rat. With respect to the receptors, no clear distinction is possible between P_1 and P_2, since ATP, adenosine, and AMP were equally active in the guinea pig (664). In rat ventricle, the receptor seems to be P_2 (500). The channel is insensitive to internal cAMP or $[Ca^{2+}]_i$. Activation is slow and takes seconds, suggesting the intervention of an intracellular messenger. Stimulation of phosphoinositol metabolism, with secondary activation of PKC by diacylglycerol (1125) is a possibility.

II) Permeation. The current shows outward rectification in normal solutions; in symmetrical conditions, the current-voltage relation is linear. Block occurs with DIDS (500); DIDS however also blocks P_2 receptors (597).

5. Nonselective cation channels and the I_f channel

In the heart, a number of nonselective cation channels (NSC) are activated by stimuli that also activate Cl^- currents, such as a rise in $[Ca^{2+}]_i$, an increase in $[ATP]_o$, and stretch. Others are activated by oxygen radicals and amphiphiles, by depolarization (sustained inward current or I_{st}), and by hyperpolarization (I_f current). Finally, a NSC current has been described with no known activation mechanism; it is spontaneously active and can thus be regarded as a background current. They all differ by permeability and block characteristics.

Both I_{st} (only described in the sinus) and I_f (present in nodal, Purkinje, and plain atrial and ventricular cells) play an important role in pacemaking under physiological and pathological conditions. The other NSC currents

seem only to be activated in pathological conditions (increase in $[Ca^{2+}]_i$, $[ATP]_o$, stretch, radicals, and amphiphiles). Because these currents carry inward current at negative E_m values, they cause the resting potential to be more positive than E_K and thus favor K^+ loss through K^+ channels, of which the conductance is increased in ischemic conditions.

A) INTRACELLULAR CA^{2+}-ACTIVATED CATION CHANNEL. Two types can be distinguished: one is activated by $[Ca^{2+}]_i$ alone, and the other, in addition, requires depolarization.

I) First type. The first type has been described in inside-out patches (172), cell-attached patches (251), and whole cell recordings (650).

A) Activation. Activation of the current occurs after exposure of the cell to Na^+-free medium (251), high $[Ca^{2+}]_o$, low $[K^+]_o$ solution (368), following intracellular injection of Ca^{2+} (650), application of caffeine, cell dialysis with solutions containing micromolar Ca^{2+} concentrations, and exposure to intracellular oxygen free radicals (472). The K_d for Ca^{2+} in inside-out patches is 1.2 μM with a Hill coefficient of 3. The channel activity is insensitive to large voltage changes (172, 251).

B) Permeation. The conductance of the single channel is 30–40 pS in cultured neonatal rat cells (172) and 15 pS in the guinea pig (251). The current-voltage relation is linear and shows a reversal at zero or slightly negative E_m values. The channel is equally permeable to Na^+, K^+, Li^+, and Cs^+ but excludes anions. Kinetics of the channel are voltage independent but largely determined by $[Ca^{2+}]_i$. At 10 μM, mean open times are 3.8 and 140 ms, and mean closed times are 1.8 and 15 ms (251). The long open time increases with $[Ca^{2+}]_i$ while closed times decrease; sometimes openings of many seconds are seen.

II) Second type. A second type of Ca^{2+}-activated cation channel, characterized by completely different activation and conductance parameters, has been found by incorporation of sarcolemmal vesicles isolated from adult canine ventricle in lipid bilayers (410). The channel has not been found in cell-attached or inside-out patches, a result that could be due to the channel being situated in the clefts of the sarcolemma.

The channel is activated by a combination of depolarization and rise in $[Ca^{2+}]_i$. In the presence of Ca^{2+} below 100 nM, the channel is activated for potentials at the *cis*-side (cytoplasmic side) positive to −60 mV. The K_d for Ca^{2+} is <1 μM, and the Hill coefficient is 2. Kinetics can be described by two open times (5.6 and 22 ms) and two closed times (9 and 29 ms). The current-voltage relation is linear, and the channel is equally permeable to Na^+ and K^+. The P_{Ca}/P_{Na} is 0.2.

B) EXTRACELLULAR ATP-ACTIVATED TRANSIENT CATION CHANNEL. *I) Activation.* A transient, rapidly desensitizing inward current is activated by $[ATP]_o$ in frog atrial myocytes (301), in rabbit SAN (899), in rabbit atrial cells and guinea pig atrial and ventricular cells (411, 752), and in rat ventricular myocytes (1180). The K_d for $[ATP]_o$ is 56 μM (301). Adenosine, AMP, and ADP are ineffective; channel activity is blocked by theophylline. On the basis of the action of agonists and antagonists, the receptor involved can be classified as P_{2x}. The channel is not sensitive to $[Ca^{2+}]_i$ (1180).

II) Permeation. The current-voltage relation is linear (49, 301) or slightly inwardly rectifying (411); the E_{rev} is close to 0 mV, suggesting a nonselective permeability. Activation of the channel leads to elevation of $[Ca^{2+}]_i$ (415). The current can be blocked by external Cd^{2+} (1180) but is insensitive to external Co^{2+} or Ni^{2+}. A P_{2x} receptor channel has been cloned from rat vas deferens (1008). Functionally, it resembles the cardiac channel activated by $[ATP]_o$. The structure has similarities with the inward rectifier channel, and its single-channel conductance is 15 pS at −100 mV. It is permeable to Na^+, K^+, and Ca^{2+} (P_{Ca}/P_{Na} is 4.8), Tris, and *N*-methyl-D-glucamine (NMDG) (1008). Inward rectification of the expressed channel is due to voltage-dependent gating and reduction in channel conductance (1183).

C) STRETCH-SENSITIVE CATION CHANNEL. *I) Activation.* Stretch- or swelling-activated NSC currents have been described in rat atrial cells (526), neonatal rat cells (188), cultured chick hearts (815), guinea pig ventricular cells (841), and dog ventricular myocytes (162).

II) Permeation. The channel in rat atrial cells is permeable to monovalent cations and Ca^{2+} (P_{Ca}/P_K estimated to be 0.9) (526) and potentially plays a role in generating Ca^{2+} overload and triggering atrial natriuretic factor secretion. The current-voltage relation is linear with a single-channel conductance of 21 pS (526).

Gadolinium ions block the cardiac channel and the accompanying volume changes in dog ventricular myocytes (162) and inhibit swelling induced by osmotic gradients in rabbit ventricular cells (946). Negative results were obtained in the rat atrial channel (526). Stretch-induced increase in $[Ca^{2+}]_i$, probably due to activation of the stretch-induced channel, is blocked by streptomycin (313).

A channel with a greater single-channel conductance of 120 pS, a preference of K^+ over Na^+ of 3.4, and impermeability to Ca^{2+} has been described in neonatal rats (188). The channel is insensitive to TEA (20 mM), 4-AP (1 mM), TTX (100 μM), or Cd^{2+} (1 mM).

D) NSC ACTIVATED BY AMPHIPHILES. In guinea pig ventricular cells (631) and in rabbit ventricular cells (114), a NSC channel is activated by amphiphiles. In the guinea pig, it shows a permeability sequence of $Cs^+ > K^+ > NMDG^+ > Na^+ > Ca^{2+}$. In the rabbit, the current shows some inward rectification and reverses at −18.5 mV in a solution containing a high Na^+ concentration. It is insensitive to TTX (10 μM) or Cd^{2+} (100 μM) but blocked by La^{3+}. Gadolinium blocks the channel in the rabbit but not in the guinea pig.

E) NSC ACTIVATED BY OXIDATIVE STRESS. A NSC is activated by exposure of guinea pig ventricular myocytes to extracellular radicals; an increase in $[Ca^{2+}]_i$ is not required (472). A similar current is activated by other oxidizing agents such as thimerosal and diamide (667, 881) and singlet oxygen (965). The channel activated by internal free radicals is blocked by Ni^{2+} and Gd^{3+} (472).

F) NSC BACKGROUND CHANNELS. An inward background current carried by cations has been described in SAN cells of the rabbit (356, 1038), in atrial and ventricular myocytes of the guinea pig heart (543), and in human atrial cells (191). In feline atrial myocytes, such a current is seen after addition of ACh (1062). In the SAN, the permeability sequence is $K^+ >> Rb^+ > Cs^+ > Na^+ > Li^+$. The NMG ion is not permeant, and 10 μM Gd^{3+} partially blocks the channel (356). In atrial and ventricular cells, the presence of a background current causes the resting potential to be less negative than E_K; in nodal cells it plays a role in pacemaker activity.

G) SUSTAINED INWARD CURRENT. *I) Activation, inactivation.* In SAN cells of the rabbit (346) and the guinea pig (345), an inward current is activated on depolarizations from -80 to -60 mV and more positive levels. In the potential range where spontaneous activity is seen, the current appears as a sustained inward current. Inactivation occurs slowly for relatively large depolarizations.

II) Permeation. The channel is permeable to monovalent cations; the current is nearly suppressed by depletion of extracellular Na^+. In contrast, it is not decreased by reducing $[Ca^{2+}]$ from 1.8 to 0.1 mM. Except for its permeation characteristics, the channel resembles the L-type Ca^{2+} channel; it is largely increased by isoproterenol and is blocked by DHP. The current is supposed to play an important role as inward current in pacemaking.

H) PACEMAKER CURRENT. The heartbeat in normal conditions finds its origin in the SAN. The electrical activity in the node is characterized by the existence of diastolic depolarization. Many currents participate in this process (730). One of the important currents, the pacemaker current I_f, is activated during the repolarization phase of the action potential and generates an inward current. The I_f current has been described primarily in SAN, AVN, and Purkinje cells (220) but is also present in atrial (124, 246, 779, 973) and ventricular cells (139, 1158). In the rabbit, the density decreases and the voltage dependence shifts in the negative direction from newborn to the adult stage (2, 803). The reverse shift occurs in primary cultures of rat ventricular cells and is accompanied by dedifferentiation (267). Recently, the channel protein has been cloned; the structure resembles that of other nucleotide-activated channels (318, 623).

I) Activation. Upon hyperpolarization of the SAN membrane from -40 mV to more negative levels, an inward current is activated that slowly increases to a steady level (see Ref. 730) (Fig. 11). On return to depolarized levels, the current is deactivated. Time constants are of the order of seconds at depolarized levels but become shorter with hyperpolarization. In SAN cells, the activation curve extends from -40 mV up to -100 mV, with a half-maximum value at -60 mV and a slope of 10 mV. It is not changed by $[K^+]_o$ (293). In Purkinje (115) and ventricular cells (1156), the activation curve lies more negative than in the SAN. The difference in voltage dependence is translated in the diastolic depolarization occurring at more hyperpolarized levels. The difference in activation voltages may be due to differences in sympathetic tone and adenylate cyclase activity. In ventricular cells, the current may become important in pathological conditions as a consequence of the shift in the activation curve, secondary to catecholamine secretion or to dedifferentiation (267). Important modulatory processes by neurotransmitters are described in section III, *A*, *D*, and *E*.

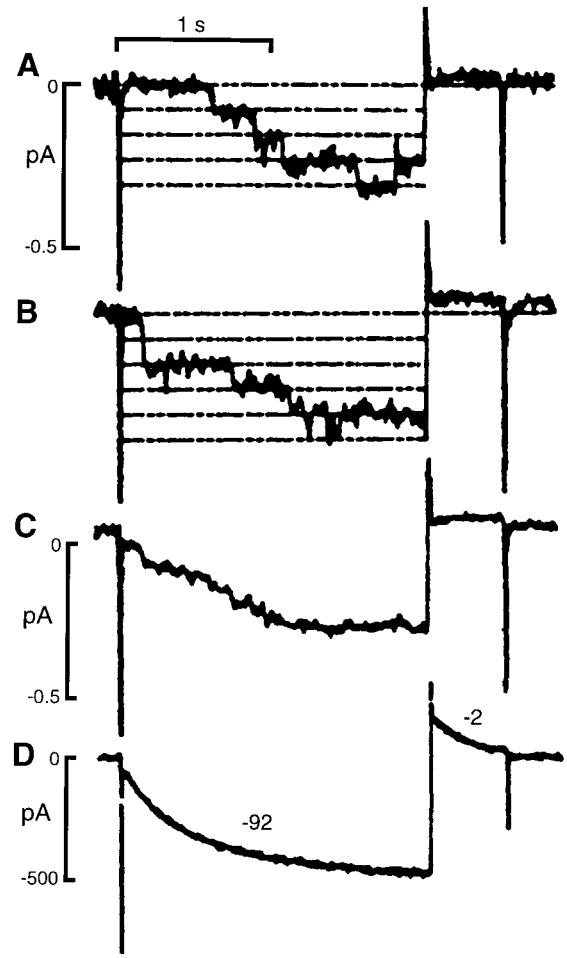


FIG. 11. Single-channel records (*A* and *B*) of the pacemaker current in cell-attached patch of a rabbit sinoatrial node cell during a hyperpolarizing pulse from -32 to -92 mV; *C* and *D* show average patch current and whole cell current in same cell. [From DiFrancesco (218). Copyright 1986 Macmillan Magazines Ltd.]

II) Permeation. The fully activated current-voltage relation is linear and reverses at -10 to -20 mV, suggest-

ing a nonselective channel, which passes K^+ and Na^+ ions (420). Permeability to other cations such as Li^+, Rb^+, and Cs^+ is small or nonexistent (217). In the range of diastolic potential, the current is inward and carried by Na^+. At the single-channel level, small currents have been recorded revealing a conductance of only 1 pS (218) (Fig. 11). Because the patches contained many small channels, information on the kinetics of single-channel activity could not be determined.

The amplitude of the fully activated current is sensitive to $[K^+]_o$ (217, 646); an increase of $[K^+]_o$ enhances the inward current through the channel (293). In K^+-free medium, the inward current is practically reduced to zero. The channel still undergoes activation, and upon depolarization a large outward current occurs. The voltage dependence of the activation and the kinetics of the macroscopic current, however, are not changed with $[K^+]_o$, suggesting no change in P_o but a modulation of the single-channel conductance. Selectivity for Na^+ is increased in low $[K^+]_o$ (reversal shifts in the depolarized direction) (420).

A rise of $[Ca^{2+}]_i$ from 10^{-10} to 10^{-7} M increases the amplitude of the I_f current and shifts the activation curve in the positive direction by more than 10 mV (354). From a simple screening effect, the opposite result is expected; the mechanism therefore is probably indirect (1172).

The permeation process is affected by the anion species in the extracellular medium. Substitution of Cl^- by organic anions results in a decrease of the macroscopic conductance without change in reversal and without shift of the activation curve (293). The channel is thus not permeable to anions, but its conductance is modulated by the anion species.

The current is inhibited by Cs^+ in a voltage-dependent way. Block by 2–5 mM is efficient and complete at hyperpolarized levels but incomplete close to the reversal (217). The effect of Cs^+ is asymmetric; inward current is specifically blocked by external Cs^+ but not by internal Cs^+ (when $[Na^+]_o$ is elevated) (293, 420).

6. *Electrogenic exchangers*

A) NA^+/CA^{2+} EXCHANGE. The Na^+/Ca^{2+} exchange (for review, see Ref. 765) plays an important role in the regulation of $[Ca^{2+}]_i$, in the excitation-contraction coupling process (increase of tension as well as relaxation), and in determining the time course of the action potential (56, 247) and of the electrical restitution following a stimulus (482).

The density of the exchanger in the heart differs with species (875). Its density is larger in the newborn (26), and with development, it shifts to the t tubules (294) or the plasma membrane overlying the junctional sarcoplasmic reticulum. This conclusion has been challenged (517). The expression is enhanced in failing hearts (387) and in conditions of increased $[Na^+]_i$ after ouabain or thyroid hormone exposure (426).

I) Activation and inactivation. Both Ca^{2+} and Na^+ are substrates of the exchange mechanism. In heart cells, the K_d for $[Na^+]_o$ in the forward mode is ~80 mM and for $[Ca^{2+}]_i$ is 0.6 μM; in the reverse mode, K_d for $[Na^+]_i$ is 21 mM and 1.4 mM for $[Ca^{2+}]_o$ (686). A higher value for $[Ca^{2+}]_i$ (20–30 μM) is obtained in macropatches (409). The K_d value for $[Na^+]_i$ is furthermore dependent on $[Ca^{2+}]_i$, suggesting the existence of competition (661). Affinity for Na^+ and even more for Ca^{2+} are thus highly asymmetric.

Intracellular Ca^{2+} not only serve as a substrate but exert a modulatory effect. A minimum of Ca^{2+} for instance is required for the function of the exchanger in the reverse mode. The apparent K_d for the modulatory site is 2 nM, a concentration much smaller than the K_d for Ca^{2+} as substrate (491, 534, 535).

The concentration of Ca^{2+} close to the transporter is modulated by the presence of negatively charged phospholipids, e.g., phosphatidylserine (408, 585, 1035) and phosphatidic acid, produced by phospholipase D action on plasmologens (360). The addition of negatively charged phospholipids or detergents stimulates the activity of the transporter, whereas positively charged detergents cause inhibition (766). The concentration of negatively charged phospholipids is regulated via a "flippase" reaction (408). Flippase or ATP-dependent aminophospholipid translocase transports phosphatidylserine or phosphatidylethanolamine from the outer to the inner side of the membrane, whereas floppase is responsible for the reverse transport.

Intracellular Na^+ stimulates the exchanger, but the effect shows inactivation (662) (Fig. 12). The process is comparable to the inactivation of a channel and depends on the binding of Na^+. Part of the exchanger is supposed to be sequestered and become inactive (389). In ATP-depleted, internal Na^+-loaded rat ventricular cells, maximum velocity (V_{max}) of the exchanger is drastically reduced, without change in the K_d for Na^+ or Ca^{2+} (389). The exchanger is not directly ATP driven, but ATP hydrolysis is required to keep $[Na^+]_i$ low via the Na^+-K^+ pump, to hold the negatively charged phospholipids in a critical concentration in the membrane, and/or to eliminate the inactivation process caused by Na^+ (67, 406, 662). In this respect, the role of PIP_2 has been emphasized. Inactivation by $[Na^+]_i$ can be removed by addition of PIP_2. The restituting effect of ATP is absent when synthesis of PIP_2 from phosphoinositol is inhibited (407).

II) Permeation: electrogenic character and reversal. In extruding Ca^{2+} from the cell against the electrochemical gradient, energy is spent. Energy is provided by the Na^+ distribution that allows a large passive Na^+ influx. To bring $[Ca^{2+}]_i$ down to submicromolar concentrations, it is necessary to transport about three Na^+ for one Ca^{2+}. This

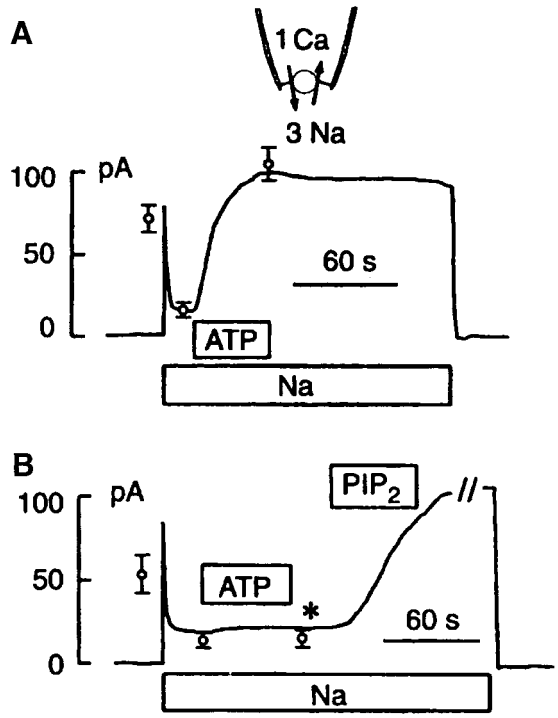


FIG. 12. Na^+/Ca^{2+} exchange current in a macropatch from a guinea pig ventricular myocyte. *A*: current activated by intracellular Na^+, inactivated rapidly to a steady level but increased again upon addition of ATP; effect persisted upon washout of ATP. *B*: ATP had no effect on a patch pretreated with a specific phosphoinositol-sensitive phospholipase C (PI-PLC), making phosphatidylinositol 4,5-bisphosphate (PIP_2) synthesis impossible. Addition of PIP_2 directly to inside of patch resulted in a marked recovery of current. [From Hilgemann and Ball (407). Copyright 1996 American Association for the Advancement of Science.]

stoichiometry has been verified experimentally, using efflux and voltage-clamp measurements (249, 309, 535, 661), and implies that the exchange mechanism is electrogenic and can be characterized by the E_{rev}, $E_{Na,Ca} = (nE_{Na} - 2E_{Ca})/n - 2$, or in the case of three Na^+ for one Ca^{2+} as $E_{Na,Ca} = 3E_{Na} - 2E_{Ca}$.

Under normal resting conditions, the E_{rev} can be calculated to be approximately −30 mV (249). Negative to this potential, Na^+ is moving in and Ca^{2+} out (forward mode) and inward current is generated; positive to this potential, Ca^{2+} is moving into the cell and Na^+ is extruded (reverse mode), and outward current is seen (409). The current-voltage relation shows outward rectification. During the initial part of the action potential, the carrier moves Ca^{2+} inward and favors repolarization. As Ca^{2+} is released from the SR, the E_{rev} of the carrier changes (see Ref. 75) with the result that the current reverses and slows repolarization. The exchanger is blocked by Ni^{2+} and Cd^{2+} (423, 535).

III) Molecular structure. The amino acid sequence of the transporter protein is known (see Ref. 765). The glycosylated NH_2 terminal is supposed to be in the extracellular compartment. The large hydrophilic linker between transmembrane segment 5 and 6 represents the regulatory part; it contains an EF Ca^{2+} binding site and the sites for Na^+ binding. A section of this regulatory link acts as an inhibitor when applied as a separate peptide (exchanger inhibitory peptide, XIP) (152, 663).

B) NA^+-K^+ PUMP. The function of the pump is to transport Na^+ to the outside and K^+ to the inside of the cell, which are directions opposite to the passive movements of these ions. The pump, therefore, has to consume energy to make this movement possible. Experiments have shown that for each ATP molecule consumed three Na^+ and two K^+ are transported. This means that the mechanism generates an electrical potential but also that it is sensitive to the E_m (for review, see Ref. 292).

Activation of the pump hyperpolarizes the resting E_m or exerts a repolarizing effect during the action potential. These aspects are especially pronounced during high-frequency stimulation, because of the increase in $[Na^+]_i$ that activates the pump. In the sinus node and Purkinje fibers, this hyperpolarizing effect counteracts the spontaneous diastolic depolarization and is known as overdrive suppression (see references in Ref. 1105).

The density of the pump sites per square micrometer varies from 1,200 in the guinea pig ventricle (714) to 2,500 in the rat ventricle (941). The density is larger in the ventricle than in the atria (1054), and in the ventricle it is greater in the subepicardial than in subendocardial cells. The turnover rate is ~75–100/s (714, 941).

I) Activation by extracellular K^+ and intracellular Na^+. The K_d value for $[K^+]_o$ is ~1 mM and for $[Na^+]_i$ is ~10 mM, with Hill coeficients of >1.0. In Na^+-free medium, the K_d for $[K^+]_o$ decreases to 0.2 mM, suggesting competition between $[Na^+]_o$ and $[K^+]_o$. The low K_d value for K^+ means that the pump rate is maximally stimulated at physiological $[K^+]_o$ values. The sensitivity to $[Na^+]_i$ decreases at higher $[K^+]_i$, although the maximum rate is not affected. At physiological concentrations, the current is directly proportional to $[Na^+]_i$, which explains the sensitivity of the pump rate to frequency of stimulation. In spontaneously active cells, an increase in frequency will result in an enhanced outward pump current acting as a negative feedback on the rate of firing, a phenomenon called overdrive suppression. The increased pump activity plays a role in the shortening of the action potential and suppression of spontaneous activity with frequency of stimulation (1025).

II) Permeation, voltage dependence, and E_{rev}. The transport through the pump is electrogenic and voltage dependent. The amount of energy consumed increases with hyperpolarization. When the energy required to move three Na^+ out and two K^+ in equals the energy obtained on hydrolysis of ATP, the pump will stop. At a given E_m called the E_{rev} of the pump, these two values are equal. The E_{rev} is thus determined by the free energy of ATP hydrolysis and the equilibrium potentials for Na^+

and K^+. Under normal conditions of $[Na^+]_i$, $[K^+]_o$, [ATP], [ADP], and $[P_i]$, E_{rev} is about −180 mV ($E_{rev} = \Delta G_{ATP}/F + 3E_{Na} - 2E_K$).

The E_{rev} shifts to less negative potentials when [ATP] decreases and [ADP] and $[P_i]$ increase, changes that occur during metabolic inhibition. When E_{rev} approaches the E_m, the pump rate will drastically reduce and eventually completely stop. These predictions have been verified experimentally (328). ΔG is normally 61.5 kJ/mol, but during ischemia it can fall to values of 49 or less (190). Under those conditions, the pump will stop at moderate E_m values (around −60 mV).

Pump rate as a function of E_m shows a broad maximum extending from −80 to ~0 mV; it declines for more negative potentials with an apparent reversal at very negative potentials around −180 mV. The pump rate also decreases at potentials positive to 0 mV (85). The voltage dependence with its positive and negative slope varies with $[Na^+]_o$ and $[K^+]_o$. When $[Na^+]_o$ is reduced, the positive slope of the current-voltage relation becomes less pronounced, i.e., pump rate increases at negative E_m values. Calculation of $[Na^+]_o$ dependence on E_m shows that the apparent affinity of the transport protein for $[Na^+]_o$ increases with hyperpolarization (K_d decreases). The negative slope at positive potentials can be changed in a similar way by increasing $[K^+]_o$; in other words, the apparent affinity for $[K^+]_o$ decreases with depolarization.

The voltage dependence of the pump has been explained by assuming that part of the molecule acts as a ion channel (310). In support of this hypothesis, gating currents have been measured (405). The voltage dependence is supposed to be due to cation binding sites of the pump buried in the membrane and only accessible from the extracellular side through a narrow channel. The actual concentration of Na^+ and K^+ at the binding sites will depend on the voltage gradient in this access channel. They are greater the more negative the E_m, and the reverse occurs at more positive potentials. The higher $[Na^+]$, the more difficult is the release of Na^+ to the extracellular medium. Also, $[K^+]$ will be greater at negative potentials, but this may not be effective if the concentration is already close to the saturating level in control conditions. At positive potentials, the actual $[K^+]$ will decrease and may fall below the saturating value and thus reduce the pump rate.

The pump is blocked by digitalis, but sensitivity is species dependent (84, 399); the rat is very insensitive with a K_d of 2.4×10^{-3} M for dihydroouabain (DHO) compared with 1.4×10^{-5} M in the guinea pig. Of theoretical but also practical importance is the observation that the sensitivity to digitalis decreases with increase of $[K^+]_o$, and the opposite effect is seen when $[Na^+]_i$ is elevated (942). DHO and K^+ however do not bind at the same site. An increase in $[K^+]_o$ increases the cycling of the enzyme between different states and decreases the time that the external site is available for DHO binding; an increase in $[Na^+]_i$, on the other hand, will force the enzyme to a state where Na^+ is delivered to the outside medium and thus a state where DHO can bind (84, 942). The antagonism between $[K^+]_o$ and ouabain is voltage dependent; the degree of inhibition by a given concentration decreases at more hyperpolarized potentials. This observation is consistent with the hypothesis of a binding site for ouabain within the membrane electrical field where the actual $[K^+]$ changes with E_m (84).

III) Molecular structure. The pump protein consists of one α- and one β-subunit and is probably present as a dimer. Both have been cloned. The α-subunit is a large protein of 110 kDa spanning the whole membrane; it carries the ATP binding and phosphorylation site at the intracellular portion and the digitalis binding site at the extracellular face. Three isoforms exist, of which the α_1 shows low affinity and α_2 and α_3 high affinity for ouabain (1054).

B. Ion Channels in Intracellular Organelles

1. SR channels

The SR membrane is a leaky membrane with a high conductance for K^+ and Cl^- (see review in Ref. 677). The only important gradient is that of Ca^{2+}, whereas K^+ and Cl^- seem to be equally distributed across the membrane. The concentration of free $[Ca^{2+}]$ has been estimated to be 700 μM (877). With a cytoplasmic concentration of ~0.1 μM, this means a gradient of 7.000. The Ca^{2+} E_{rev} is thus >200 mV, whereas the SR potential is estimated to be 0 mV. This means that the large chemical gradient is not antagonized by an electrical gradient. During Ca^{2+} release, the SR interior becomes negative but at most a few millivolts because of the counterion movement of K^+ and H^+ inward and Cl^- outward (315). Two release channels have been described: the ryanodine receptor channel and the inositol 1,4,5-trisphosphate (IP_3) receptor channel.

A) THE CA^{2+} RELEASE CHANNEL OR RYANODINE RECEPTOR (RYR). In mammalian cardiac cells, the SR represents the store from which Ca^{2+} is released during the action potential. In this process a Ca^{2+}-permeable channel in the SR plays an important role. The Ca^{2+} released also activates the Na^+/Ca^{2+} exchange current, a NSC current, and a Cl^- current, which may all participate in the genesis of early and delayed afterdepolarizations. In conditions of Ca^{2+} overload, the channel thus plays an indirect but important role in generating triggered activity. The channel is specifically blocked by ryanodine.

I) Activation. In cardiac cells, the trigger for activation of the channel seems to be the local rise in $[Ca^{2+}]_i$ consequent to Ca^{2+} influx during the action potential (261). A small proportion of the SR Ca^{2+} release may be activated directly by voltage in a way similar to that in

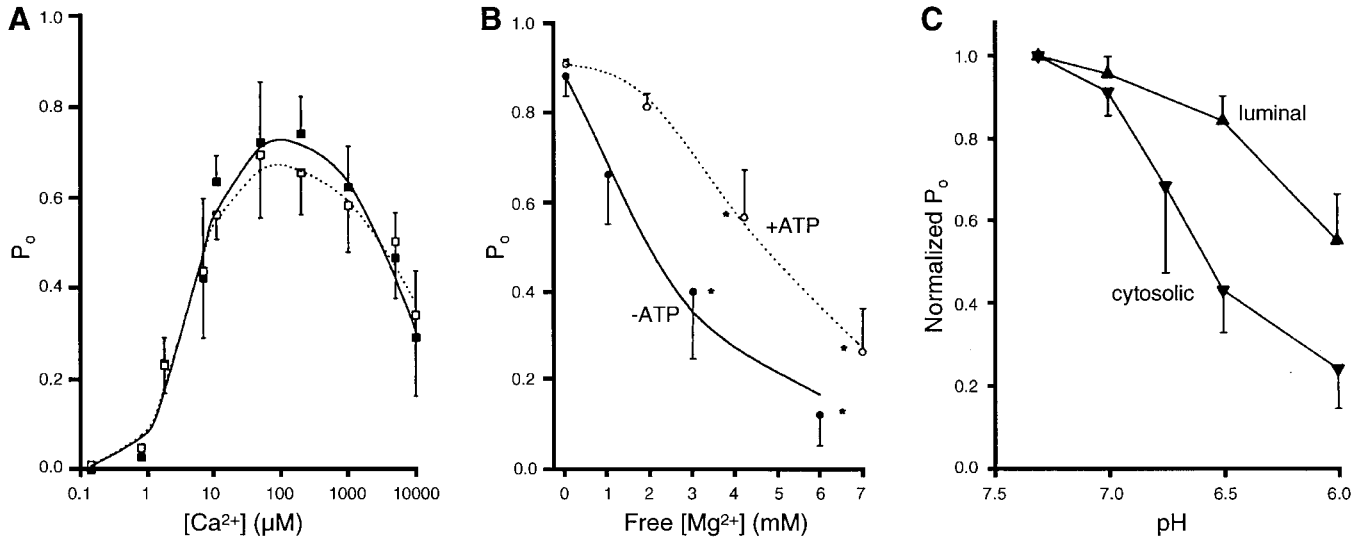


FIG. 13. Open probability of cardiac ryanodine receptor as a function of $[Ca^{2+}]$, $[Mg^{2+}]$, [ATP], and $[H^+]$. Single-channel activity was measured in planar lipid membranes, in which ryanodine receptors were incorporated from receptor-enriched vesicles. At high cytosolic $[Ca^{2+}]$ concentrations (*A*), channel inactivates. [ATP] increases activity while $[Mg^{2+}]$ (*B*) and $[H^+]$ (*C*) exert an inhibitory effect. Inhibitory effect of acidosis is greater at cytosolic compared with luminal side. [Adapted from Xu et al. (1120).]

skeletal muscle (424). The condition to observe this voltage-dependent mechanism seems to be the presence of cAMP (276, 424), and recent experiments have suggested that the Na^+ channel under those conditions may become permeable to Ca^{2+} (840).

The process of Ca^{2+}-induced Ca^{2+} release has been studied by measuring Ca^{2+} transients in vivo using fluorescence techniques, by flux studies of radioactive Ca^{2+} in isolated SR vesicles, and at the single-channel level after incorporation of the protein in artificial lipid bilayers. They have led to the following picture.

A) Ca^{2+}: the major activating mechanism. The primary and most important activator of the cardiac SR channel is the cytoplasmatic Ca^{2+} (120, 261). Information on the role of Ca^{2+} as trigger for Ca^{2+} release, derived from tension measurements in skinned preparations (261) and from $^{45}Ca^{2+}$ release in SR vesicles (678), is in favor of the existence of activation at low $[Ca^{2+}]$ and inactivation at elevated $[Ca^{2+}]$. The results show a biphasic activation curve as a function of cytosolic $[Ca^{2+}]$. The efficiency of Ca^{2+} as trigger also depends on the speed of Ca^{2+} application: the faster the rise in $[Ca^{2+}]$, the higher the tension developed (261). Experiments with flash photolysis of Ca^{2+} in native cells (709) and on pure proteins incorporated in lipid bilayers (154, 916) initially did not confirm the existence of inactivation at high $[Ca^{2+}]$. More recently, it has become clear (586) that the property of inactivation is lost during the isolation procedure of the protein or by the use of high concentrations of Cs^+ in the test medium. When the necessary precautions are taken, steady-state activation (K_d of 5 μM) and inactivation (K_d of 9 mM) have been demonstrated in lipid bialyers (1120) (Fig. 13). Similar experiments have further demonstrated the existence of adaptation: upon application of a constant step in $[Ca^{2+}]$, activation decreases with time, but release occurs again when $[Ca^{2+}]$ is increased by a second application, i.e., recovery is fast (349, 861, 1006). The rate of adaptation is markedly accelerated in the presence of $[Mg^{2+}]$ or after phosphorylation of the channel (1006). In accord with the existence of adaptation is the observation that the rate of decay of a Ca^{2+} spark is faster the greater the magnitude of the spark or the amount of Ca^{2+} released (629). Adaptation and/or inactivation are responsible for the nonregenerative behavior of the Ca^{2+} release channels under normal conditions. According to the original model of Fabiato (261, 262), Ca^{2+} bind to an activating site with high rate but low affinity and to a second inactivating site with slower association rate but high affinity. Modifications of this model have been proposed by others (120, 961).

Release is modulated by the presence of nucleotides: addition of [ATP] shifts the concentration effect curve to lower $[Ca^{2+}]$ and upward; the opposite effect occurs with [ADP] (1120) (Fig. 13). Phosphorylation (754, 1006) results in a shift of the P_o curve to lower $[Ca^{2+}]$ and [ATP] and in the upward direction; the gain is increased.

B) Influx of Ca^{2+} through L-type Ca^{2+} channels is the important source of activator Ca^{2+}. The stimulus for Ca^{2+} release during a contraction is provided by Ca^{2+} entering the cell through the plasma membrane via the L-type Ca^{2+} channel (120, 709). Release upon Ca^{2+} entry via T-type channels (913) and via reversed Na^+/Ca^{2+} exchange (589, 598, 914, 1073) occurs but is less important and much slower.

L-type Ca^{2+} channels are geometrically coupled to the release channels. In the heart, one L-type channel is coupled to more than one release channel, and the coupling is less tight than in skeletal muscle. The initial early Ca^{2+} influx is important and not the total Ca^{2+} influx. The first openings of the Ca^{2+} channel are determining, whereas reopenings are of no importance except as a loading function (120, 460, 1098). In cardiac hypertrophy and failure, the coupling may become deficient (333). The efficiency of Ca^{2+} influx in causing release or gain is variable. Gain, defined as the ratio between Ca^{2+} influx and Ca^{2+} release or as peak rate of Ca^{2+} release, increases with depolarization up to a maximum and decreases again at more positive potentials. This can be explained by the fact that the current through individual L-type Ca^{2+} channels is larger at more negative potentials because of the larger gradient; the effect on the release channels is great. At positive potentials close to the E_{rev}, the number of active channels is large, but the individual current through the channel is small; the final result is less or no activation of Ca^{2+} release. Although the total Ca^{2+} current through the plasma membrane may be the same in both cases, the result is quite different. The efficiency in releasing Ca^{2+} from the SR is greater when K^+ is used instead of Cs^+ as the major intracellular cation, suggesting that influx of K^+ into the SR through a K^+ channel probably accompanies Ca^{2+} release. The fact that the release induced via the exchanger also is improved can explain why the relationship between Ca^{2+} transient and voltage is less bell-shaped and stays constant up to very positive potentials (599).

C) Regenerative Ca^{2+} release: importance of luminal $[Ca^{2+}]$ or load. Under normal conditions, Ca^{2+} release is not propagated (987); the action potential is responsible for the propagation. The nonregenerative aspect of Ca^{2+} release has been shown by analyzing the behavior of sparks or microscopic Ca^{2+} release events (839). In the presence of a normal Ca^{2+} load, channels close rapidly, probably as a consequence of deactivation or fall in activator Ca^{2+} (460); the local $[Ca^{2+}]$ decreases quickly, due to diffusion from the small junctional space and by local pump reuptake. The time and space (width) constant of the spark are normally too short and too small to cause propagation of the release. The width of the spark can become larger by increasing the Ca^{2+} content of the SR and thus increasing the amplitude of the spark. At low Ca^{2+} load, release is linearly related to the Ca^{2+} present in the SR. Above given concentrations however, the P_o of the release channel is increased and the amount of Ca^{2+} released rises exponentially as a function of the preload (44). At the single-channel level, an increase in luminal $[Ca^{2+}]$ is accompanied upon release by longer and more frequent openings, and the conductance is greater (916). For a given Ca^{2+} load, the relative increase in P_o of the channel is also greater at low cytosolic $[Ca^{2+}]$ (628). Under conditions of high luminal and low cytosolic $[Ca^{2+}]$, Ca^{2+} may be released spontaneously from the SR, and this release may propagate along the cell; the gain is drastically increased (148, 628, 847, 987). Propagation from cell to cell, however, has a low probability (584). Increasing the Ca^{2+} load enhances the frequency of the spontaneous release events, such that the Ca^{2+} content of the SR and of the cell stays constant (986).

It is not clear how luminal $[Ca^{2+}]$ affects the threshold and the amount of Ca^{2+} released from the SR. Luminal $[Ca^{2+}]$ does not seem to activate the channel at the cytosolic side during its diffusion from the SR. This is concluded from the observation that Ca^{2+} release is not inhibited when Ba^{2+} or Sr^{2+} is added to the luminal side, although both ions permeate the channel from the luminal to the cytosolic side and block the channel when added to the cytosolic side (976, 999). In more recent experiments, however, a correlation has been found between activation-inactivation and Ca^{2+} flux through the channel (1121).

II) Permeation. The channel behaves as a high-conductance but poorly selective cation channel. It is permeable to bivalent (Ca^{2+}, Ba^{2+}, and even Mg^{2+}) and monovalent cations. The P_{Ca}/P_K is 6 in comparison with a ratio of >25 for the L-type Ca^{2+} channel. The permeability sequence for monovalent ions is as follows: $Cs^+ > Na^+ > K^+ > Li^+ > choline^+ > Tris^+$ (975). In theory, because of the high permeability, current carried by K^+ can be large. The absence of an important electrical or K^+ concentration gradient between lumen and cytoplasm, however, prevents this short-circuiting effect.

The conductance is large and differs with the type of permeating cation: ~100–150 pS for 50 mM $[Ca^{2+}]$ (luminal side), much smaller for Mg^{2+} (40 pS), and 750 pS for 250 mM $[K^+]$ (see Ref. 146). Saturation has been described for bivalent ions (975, 999). The presence of negative charges at the mouth of the channel regulates the local $[Ca^{2+}]$ and the conductance of the channel (999). Neutralization of carboxyl groups using carbodiimides reduces conduction. Addition of the negatively charged heparin on the other hand increases the local $[Ca^{2+}]$ and reduces the threshold for Ca^{2+} release (999).

Caffeine activates the channel in a Ca^{2+}-dependent way at low concentrations and in a Ca^{2+}-independent way at high concentrations (811). Ryanodine at low concentrations increases the P_o by forcing the channel in a substate in which it is more or less stabilized; at high concentrations it blocks the channel (813).

III) Molecular structure. From electron microscopic studies on skeletal muscle, the channel appears to form a square structure, with a fourfold symmetry, suggesting a tetrametric constitution (791). The structure displaces a central channel that branches into four radial channels. Cloning has revealed the existence of at least three different proteins: RyR1 (skeletal muscle), RyR2 (cardiac

muscle), and RyR3 (other tissues) (711). The NH_2 terminal and the shorter COOH terminal are supposed to protrude in the cytoplasm and form the foot structure seen in electron microscopic studies. The molecular site at which Ca^{2+} acts is not known (643) but is probably situated in the NH_2 terminal. Two smaller modulatory proteins have recently been described: triadin, which is supposed to mediate the interaction with calsequestrin, and FKBP12 (FK binding protein), which stabilizes the RyR and reduces the occurrence of substates in the skeletal muscle channel. When FKBP12 is inhibited by FK-506, the rate of Ca^{2+} release from the SR and the Ca^{2+} transient are increased (see Ref. 643).

B) IP_3 RECEPTOR. Although the role of IP_3 is well established in smooth muscle and nonexcitable cells (72), the evidence that IP_3 might play a role in the heart is circumstantial. Three aspects should be mentioned: *1*) IP_3 is released in cardiac cells, *2*) IP_3 receptors are present, and *3*) IP_3 causes release of Ca^{2+} (see review in Ref. 643).

1) Inositol 1,4,5-trisphosphate is released from the plasma membrane after activation of different receptors (M_2, M_1, P_2, endothelin) (394, 775, 936, 1041). Release of IP_3 is especially pronounced early during reperfusion, an effect mediated via activation of α-receptors (17, 1107).

2) Inositol 1,4,5-trisphosphate receptors can be localized by immunolocalization to the region of the intercalated disk (696). Expression is greater in Purkinje fibers than in other cells (335). Binding studies also found IP_3 receptors enriched in fractions containing the intercalated disks with little or no binding in fractions containing the longitudinal SR (521). Compared with RyR, the density is 50-fold less. Expression of IP_3 receptors is increased in failing hearts, whereas that of RyR is decreased (643).

From a structural point of view, the IP_3 receptor shares considerable homologies with the RyR, although the molecular weight is much less. The large NH_2 terminal lies in the cytoplasmic side with the IP_3 binding site at its end. The COOH terminal probably plays a role in activation because antibodies that bind to this region can induce or inhibit channel opening. Three isoforms have been isolated (252).

3) Inositol 1,4,5-trisphosphate causes Ca^{2+} release. Cardiac microsomes fused to planar bilayers exhibit channel activity modulated by IP_3. Four substates have been recognized. The conductance is smaller (85 pS) than that of the RyR, but the permeability series of bivalent ions is the same with a permeability ratio of bivalent over monovalent cations around 6 (78).

Tension measurements have shown that caffeine contracture is amplified, and Ca^{2+}-induced tension oscillations are enhanced in magnitude and frequency by IP_3 (321, 1043, 1185). The effects of IP_3 vary and depend on the state of Ca^{2+} loading of the SR and the cytoplasmic $[Ca^{2+}]$. The influence of cytoplasmic $[Ca^{2+}]$ is biphasic; the release for a given concentration of IP_3 increases with cytoplasmic $[Ca^{2+}]$, reaches a maximum at ~300 nM, after which it begins to be inhibitory (79). Like the RyR, the IP_3R may become regeneratively active in conditions of Ca^{2+} overload (374, 515). The IP_3 receptor is modulated by PKC, PKA, and Ca/calmodulin (CaM) kinase II, MgATP, and pH (see Ref. 451). The positive inotropic effect (775) as well as the proarrhythmic effect (887) of α-receptor stimulation has been related to IP_3 production. Aminoglycoside antibiotics block release (236) and suppress ventricular arrhythmias.

C) K^+, CL^-, AND H^+ CHANNELS IN THE SR. Outside the Ca^{2+}-release channel, K^+, Cl^-, and possibly H^+ channels constitute high-conductance pathways in the SR membrane. Because of their presence, the membrane of the SR is clamped at about zero potential. In this way, a large gradient forcing Ca^{2+} flowing from lumen to cytoplasm is guaranteed. The flux of K^+, H^+, or Cl^- as counterions facilitates the passive release as well as the active uptake of Ca^{2+} via the Ca^{2+}-ATPase.

I) H^+ channel. Whether H^+ movement occurs through channels is not known, but the importance of proton flux has been demonstrated by measurements of changes in E_m by pH in the presence of impermeant ions (679). Acidification occurs during the release, and alkalinization has been found during reuptake of Ca^{2+} (601).

II) K^+ channel. A K^+ channel with an elevated P_o at 0 mV exists in the SR membrane (810). The kinetics of the channel are very slow with open times of hundreds of milliseconds.

In isotonic $[K^+]$ solution, the single-channel conductance is 150 pS and the current-voltage relation is linear (810). The conductance is concentration dependent and saturates at 200 mM. The channel is blocked by Cs^+ (177) and TEA (1). The Cs^+ block is only slightly voltage dependent and can be relieved by addition of extra K^+. The block by Cs^+ explains the lower efficiency of Ca^{2+} as activator for Ca^{2+} release in Cs^+ solutions (599) and inhibition of spontaneous Ca^{2+} release in skinned preparations (510). Acidosis reduces P_o at 0 mV (576), but $[Ca^{2+}]$ or $[Mg^{2+}]$ does not seem to affect the channel (810).

III) Cl^- channel. In cardiac SR, a Cl^- channel stays open at 0 mV. Its voltage dependence is small (809) or nil (514). Gating of the single channel is characterized by two open times and two closed times (514).

The current-voltage relation is linear with a high conductance of 50–120 pS in cardiac cells (514, 809). The channel is permeable to Cl^-, less to SO_4^{2-}, and impermeable to gluconate (363). In skeletal muscle, the channel is also permeable to cations (Ca^{2+}), with a sequence of $P_{Cl}/P_{Tris}/P_{Ca} = 1.0/0.5/0.3$ (944). The existence of channels that are permeable to anions and cations is not exceptional (see mitochondrial voltage-dependent anion channel, Ref. 171).

Phosphorylation of the channel prevents the rundown of channels incorporated in lipid bilayers (514, 809). Phospholamban acts as a modulator (204), and its phosphorylation results in activation of the channnel.

2. Mitochondrial channels

In the mitochondria, energy is transferred from different substrates to ATP by way of oxidation. Because one of the key reactions exists in building up a proton gradient, the inner membrane of the mitochondrion is supposed to be relatively impermeable and to show a permeability that is highly regulated by electroneutral carriers and uniporters. The outer mitochondrion membrane, on the other hand, has long been considered to act as a sieve that allows fast exchange of ATP, ADP, acetyl CoA, and other metabolites. This classic view has changed dramatically during recent years.

A) THE OUTER MITOCHONDRIAL MEMBRANE. Permeability of the outer mitochondrial membrane has been studied after isolation and incorporation of protein fractions in artificial lipid bilayers. Two different types of channels have been found (637). One type is cation selective, of which the function is not clear. Because it is blocked by targeting peptides, it may play a role in protein transport (620). The second type is represented by a voltage-dependent channel permeable to anions and more specifically to ADP and ATP (Fig. 2). Together with the adenine nucleotide carrier in the inner membrane, it plays an important role in the traffic of ATP and ADP between the cytoplasm and the mitochondrial matrix.

I) Voltage-dependent anion channel. A) Activation. After incorporation in artificial lipid membranes the voltage-dependent anion channel (VDAC) shows a maximum P_o at zero E_m that falls off but not to zero on application of either a positive or negative voltage gradient. At the same time, the conductance shifts to a substate, and the channel changes selectivity, becoming more permeable to cations. The obvious question is whether this type of voltage sensitivity has functional implications. Although a direct measurement of a potential gradient over the outer membrane has not been made, it is generally assumed that a Donnan potential exists based on the presence of a difference in concentration of large, charged colloids between the cytoplasm and the intermembrane space. Phosphorylation and Ca^{2+} binding (during a contractile cycle) may modify this potential gradient (807). The intermembrane space contains metabolic enzymes such as adenylate cyclase, creatine kinase, nucleoside diphosphate kinase, and deoxyribonuclease.

Voltage gating is modulated by a modulatory protein and the NADH concentration. Sensitivity to voltage is increased in the presence of a modulatory protein (427) situated in the space between the inner and outer membrane. The increase in voltage dependence means that the channel closes for smaller changes in potential (0–5 mV) and becomes less permeable to anions (ATP, ADP). Voltage gating of the channel is further regulated by micromolar cytoplasmic concentrations of NADH that act as a ligand for the channel (1188). It is estimated that fluctuations of NADH could result in a sixfold change of the permeability to adenine nucleotides with consequent changes in the intensity of respiration (615). The VDAC should thus be considered a sensor of glycolysis, since cytoplasmic NADH is mainly determined by glycolysis. The closure of VDAC may be the mechanism by which glycolysis inhibits oxidative phosphorylation (Crabtree effect). In this connection it is of interest to note that the VDAC structure binds hexokinase and glycerolkinase.

B) Permeation of the VDAC. The VDAC is permeable for molecules up to 1 kDa. It stays open with a very large single-channel conductance of 650 pS at zero E_m and is slightly more permeable to anions than to cations (2/1). It allows passage of ATP and ADP. Permeability to anions ranges from 100/10/1 for Cl^-/succinate/ATP. The flux of ATP is high (10^6 molecules ATP/s, Ref. 807). The outer membrane behaves thus quite differently from the classic sieve but should be considered a highly regulated structure.

C) Structure of VDAC. The channel has been crystallized (972) and forms groups of six transmembrane pores, each pore consisting of a single 30- to 35-kDa VDAC polypeptide (87). The polypeptide contains numerous stretches of 10–14 amino acids that show an alternating polar and nonpolar character. Partial closure is supposed to be due to a positively charged stretch of the molecule moving out of the membrane (gating current, Ref. 925) and leaving the pore negatively charged and permeable to cations.

B) THE INNER MEMBRANE. The study of the permeability of the inner membrane (see Ref. 538) has been possible by application of the patch-clamp method to mitoplasts (928). Mitoplasts are mitochondria stripped of their outer membrane. Under physiological conditions, the permeability of the inner membrane is low. In ischemic conditions, however, this permeability barrier can be weakened or completely vanish by opening of large channels. Five different channels have been described (Fig. 2): *1*) a multiple conductance channel (MCC) or mega-channel permeable to large cations and anions, *2*) an ATP-dependent K^+ channel, *3*) a 107-pS slightly anion-selective channel, known as the mCS channel, acronym for mitochondrial centopicosiemens channel, and two channels activated when the matrix side is exposed to very alkaline conditions: *4*) a cation-selective 15-pS channel, and *5*) an anion-selective 45-pS channel.

I) The MCC or mitochondrial permeability transition pore. A) Activation. Under normal physiological conditions, the channel is closed mainly because of the high [ATP] and low [Ca^{2+}]. ATP is normally needed to main-

tain a low cellular [Ca^{2+}] and a negative mitochondrial E_m (908). The channel is activated by elevation of intramitochondrial [Ca^{2+}] in the micromolar range, in combination with low [ATP] (189, 244, 952). Activation is facilitated by long-chain acyl CoA (771), pro-oxidants, and dithiol oxidatives (reversed by disulfide reduction), high O_2 tension in combination with low [ATP], and high [Ca^{2+}]; it is inhibited by H^+, Mg^{2+}, and the immune suppressive drug cyclosporin (953). It is also sensitive to drugs that bind to the benzodiazepine receptor. Typical during activation is the increase of conductance in steps. A possible underlying mechanism is the assembly of subunits into a large channel by the action of Ca^{2+} (953).

The presence of a high conductance in the inner membrane poses a problem. Open pores spanning the inner membrane would dissipate the energy-transducing gradients and so uncouple oxidative phosphorylation (comparable to the effect of the uncoupling protein in brown fat cells). As intramitrochondrial [Ca^{2+}] is normally low and matrix [ATP] high, the MCC is not expected to be activated under normal conditions. In ischemia or Ca^{2+}-overload conditions, and especially upon reperfusion with high O_2 tension restored, the channel may be activated and is probably responsible for the permeability transition. Activation is accompanied by depolarization (see Fig. 19) and release of the mitochondrial content, especially Ca^{2+} itself. This release of Ca^{2+} may trigger a Ca^{2+} wave from one mitochondrion to the other. The mitochondrion may still be operating in a low-conductance mode, and the activation of the MCC remains reversible; the mitochondrion acts as an excitable organelle (449). If activation is steady, however, the cell will die (244, 953). Pore opening disrupts mitochondrial energy transduction by providing a short circuit for protons, bypassing the chemiosmotic proton circuit that normally links the oxidation of substrates with phosporylation of ADP. Once uncoupled, mitochondria act as a drain for glycolytic ATP. The molecular nature of the mega-channel as well as all other inner membrane channels is not known.

B) Permeation. The mega-channel has a variable conductance between 40 and 1,000 pS and is unselective to cations or anions. Large anions such as ADP and ATP can permeate.

II) The ATP-dependent K^+ channel. The ATP-dependent K^+ channel is similar to the channel in the plasma membranes of many cells (458). It has the same conductance and the same activation pattern. It opens by reduction of [ATP] below a critical concentration. It is also blocked by glibenclamide and sensitive to K^+ channel openers. The opening is facilitated by PKC-dependent phosphorylation (846). In contrast to the cardiac plasma membrane channel, it is sensitive to diazoxide and resembles more the pancreatic channel (316). Its role in mitochondria is not obvious; its activation may stabilize the mitochondrial E_m and avoid activation of the mega-channel. A role in preconditioning (316, 619, 846) and in volume regulation has been proposed; during ischemia, mitochondria swell, and opening of large-conductance channels may induce efflux of solutes and water and reduce volume.

III) The 107-pS anion channel or mCS channel. The 107-pS anion channel of mCS channel (928) is slightly anion selective. It is activated at positive matrix potentials, exponentially increasing with positivity. Its anion selectivity is poor (P_{Cl}/P_K of 4.5). It is not pH sensitive but inhibited by oxidative uncouplers. Under normal conditions, the channel is thus closed. For its function, different hypotheses have been offered: *1*) a function as uncoupling protein analogous to that in brown fat mitochondria, *2*) a role in volume homeostasis, and *3*) a function as import channel for proteins and mitobiogenesis. The majority of mitochondrial proteins are synthesized in the cytoplasm and hence have to be imported into the organelle; this requires a two channel assembly in tandem in the inner and outer membrane. *4*) Recently, it has been proposed (37) to function as a protective mechanism against a fall in mitochondrial potential. Permeability under physiological conditions is supposed to be small but large enough to allow passive equilibrium for Cl^-. In a situation of metabolic inhibition when the matrix negativity would normally drop, this process might be inhibited by opening of the mCS channel clamping the mitochondrial membrane at a very negative matrix potential.

IV) Alkaline-activated channels. When the matrix side of a mitoplast preparation is exposed to very alkaline solutions, two channels can be observed: one is cation selective and shows a conductance of 15 pS and a second is anion selective and shows a conductance of 45 pS. The role of these channels is unknown.

C. Gap Junction Channels

1. Introduction

Gap junctions are responsible for the syncitial nature of cardiac tissue. Their high conductance and permeability allow for fast conduction of the action potential (electrical coupling) and for an efficient flow from cell to cell of molecules or metabolites with a molecular mass up to 1.2 kDa (K^+, Na^+, Ca^{2+}, cAMP, cGMP, IP_3) (metabolic coupling). In the canine heart, each ventricular cell is connected to 11 other cells by way of gap junctions (824). Different values for the conductance between cells have been published from 250 to 2,500 nS/cell. For a normal conduction of the action potential between a pair of cells, ~35 gap channels seem to be sufficient (1078). Redundancy is thus great. This contrasts with the recent finding that mice heterozygous for connexin (Cx)43 null mutation

show a 45% reduction in conduction velocity of the action potential (344).

The distribution of gap junctions in the normal heart is nonuniform or anisotropic (761). Gap channels are found almost exclusively in the intercalated disks. Large intercalated disks exist at the end of the cells, smaller along the length. A small number is present at the SAN-atrium junction and the Purkinje-muscle junction. These observations explain why conduction in the transverse direction or between the SAN and the atrium or between the Purkinje system and the ventricular muscle can be critically reduced. In culture, the number of junctions is smaller, which translates in slower conduction of the action potential and absence of propagated Ca^{2+} waves.

2. *Formation of gap junctions and structural considerations*

Gap junctions can be induced by forcing two single cells into physical contact (1030). Precursors are present in the plasma membrane from which cell-to-cell channels can form rapidly upon pairing. Analysis with different biophysical techniques has shown that the channels responsible for the large conductance of the gap junction membrane consist of two hemichannels or connexons in two apposing cells (933). Each hemichannel is composed of six polypeptide subunits or connexins. Subunits derived from different genes differ in molecular mass varying between 26 and 50 kDa and in conductance between 40 and 160 pS. In the heart, three connexins are expressed: Cx40, Cx43, and Cx45 (197). They show a different distribution and differ in functional characteristics. Connexin43 is the most abundant. Connexin40 is preferentially present in atria, nodal tissue, and Purkinje system (501, 574). Connexin43 and Cx45 turnover quite rapidly (2–3 h) in cultured neonatal rat heart cells (197).

In the formation of gap junctions, hemichannels act as ligands for each other. Of importance for this activation is the presence of six cysteines in the extracellular loop of the connexin molecule. Docking and/or opening of channels involves disulfide exchange.

Each subunit or connexin is supposed to consist of four transmembrane segments, M1 to M4. M3 is amphiphatic and probably part of the pore structure. Amino acids at or near the NH_2 terminal and the M1/E1 border form part of a charged complex that may act as a voltage sensor. The COOH terminal together with the intracellular loop between M2 and M3 form the so-called proton gate (195).

The gap junction channel is gated via two important mechanisms called chemical and voltage gating.

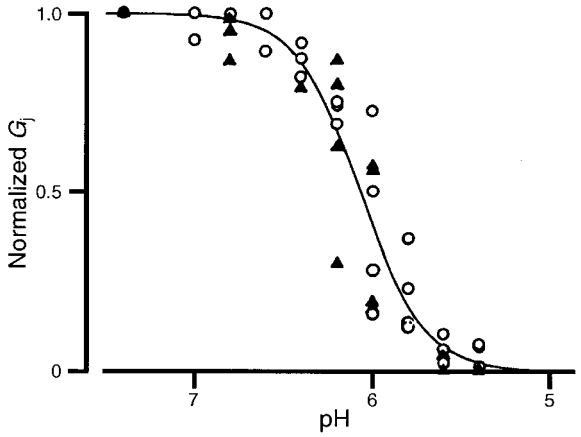


FIG. 14. Relationship between pH and junctional conductance (G_j) measured in cardiac paired cells of guinea pig. Most data were obtained in absence of free Ca^{2+} (○). In 4 experiments, results were obtained in presence of [Ca^{2+}] (pCa 6.8 and 7.0). At these concentrations, effect of pH was not different. [From Noma and Tsuboi (732).]

3. *Chemical and voltage gating*

A) CHEMICAL GATING BY PROTONS AND CALCIUM IONS: A SLOW PROCESS. Protons cause the gap channel to close (see Ref. 104) (Fig. 14). The mechanism is proposed to be a ball-receptor inactivation process; protons increase the positive charge of the receptor site and allow the negatively charged ball of the COOH terminal to bind and to occlude the channel pore. Deletion of part of the COOH terminal causes a significant fall in pH sensitivity (695). In the cytoplasmic loop between M2 and M3, which probably acts as the receptor site of the Cx43 gap junction, histidine residues are present that can be titrated by protons (933). The presence of positive charges at critical positions in this loop leads to channel closure.

An increase in [Ca^{2+}] also reduces the total junction conductance (109, 206, 285, 732, 984). In the experiments of Noma and Tsuboi (732), the sensitivity to block by Ca^{2+} was rather high under normal pH conditions (pK_{Ca} of 6.6 at pH 7.4), which means that concentrations of Ca^{2+} that close the channel may be reached during a normal contraction. Because uncoupling does not occur during a contraction, the authors suggested uncoupling to be rather slow or [Ca^{2+}] to be much smaller at the gap channels than at the myosin level. Larger increases in [Ca^{2+}] occur during ischemia and may induce uncoupling.

Possible underlying mechanisms are direct binding of Ca^{2+}/CaM to the channel or Ca^{2+}/CaM-activated phosphorylation (984). Phosphorylation of the COOH terminal increases the negativity and thus the ability to bind to the positively charged histidine of the loop between M2 and M3.

Information about interaction of Ca^{2+} and H^+ is controversial. The outcome is of importance, however, since simultaneous changes in the concentrations of these two ions occur frequently. A synergistic action of Ca^{2+} and H^+

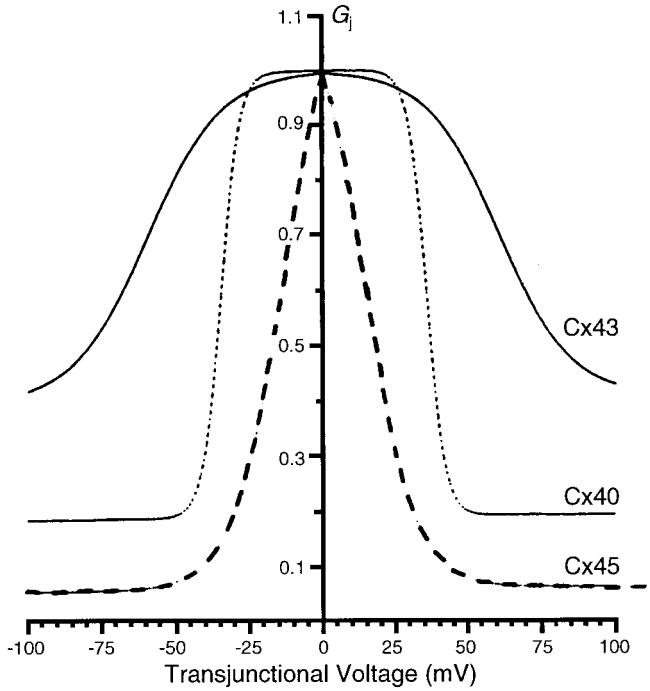


FIG. 15. Voltage sensitivity of cardiac connexins. Junctional conductance (G_j) is reduced, i.e., channel shows inactivation by application of a transjunctional voltage gradient of either sign. Sensitivity is different for different connexins (Cx). [From Spray and Fishman (933a). Copyright 1996 Kluwer Academic Publishers.]

has been demonstrated by studying dye movement between cells (109) or measuring electrical conductance (1091). A decrease of intracellular pH (pH_i) to 6.0 combined with an elevation of $[Ca^{2+}]_i$ to 425 nM fully blocked the transjunctional conductance. This conclusion is at variance to the findings of Noma and Tsuboi (732), who described a competition between Ca^{2+} and H^+.

B) VOLTAGE GATING: A FAST PROCESS. Gap junction channels in cardiac cells are sensitive to transjunctional voltage (691, 1053). Upon application of a voltage gradient, the instantaneous current-voltage relation is linear. With time, channels close however and the relation becomes S type, a process that can be compared with inactivation (1053) (Fig. 15). Such a process accelerates uncoupling during ischemia.

At the molecular level, amino acids at or near the NH_2 terminus and the M1/E1 border form part of a charged complex and thus may act as a voltage sensor (1040). The data are interpreted by supposing that each gate of the six connexins responds to the applied voltage in an independent way. By making chimeras, junctions with different time- and voltage-gating properties have been constructed; in this way, rectification may be generated, with a voltage independence on one side and closure on application of large polarities of a given sign on the other side. The transjunctional voltage dependence, together with the effects of Ca^{2+}, may explain the sealing phenomenon that occurs when part of the heart tissue is injured. During ischemia, this process is useful in isolating ischemic cells from viable functional cells. However, at the same time, it may be arrhythmogenic.

In native cardiac gap channels, a reduction of gap junction conductance following a decrease of transmembrane voltage has not been demonstrated yet, but if present, it might explain to a certain extent the uncoupling of cells when $[K^+]_o$ is increased in ischemia (see Ref. 581).

4. Permeation

The gap junction shows a high permeability and passes substances with a molecular mass up to 1 kDa, including IP_3 and cAMP (1030). The single-channel conductance decreases markedly for TEA and tetrabutylammonium. The ionic radius of these molecules is ~12 Å. This dimension may thus be close to the limiting pore diameter. Calcium ions easily permeate, but propagation of spontaneous Ca^{2+} waves, occurring under conditions of Ca^{2+} overload, is rather rare, with a probability of 0.15 (584).

Values for the single-channel conductance vary, and this variability may be related to the relative expression of the three connexins, the state of phosphorylation, and the voltage (111, 690, 955, 1031). Connexin43 has a conductance of 45–100 pS, depending on the phosphorylation state, is equally permeable to anions and cations, is highly permeable to dyes, but is insensitive to transjunctional voltage. Connexin40 shows a conductance of 120–160 pS, and its permeability to cations is about fivefold greater than to anions. Connexin45 has a smaller conductance of 30 pS, and its permeability to anions and dyes is limited; it is very sensitive to voltage (501). The current-voltage relation is linear, but rectification can be generated by expression of chimeras consisting of proteins with different voltage dependency (1040). If such asymmetry exists in native cells, it could play a role in unidirectional conductance.

For modulation by phosphorylation, see section III*D*.

III. ISCHEMIA SYNDROMES

Block of oxidative metabolism and fall in [ATP]/[ADP] causes important changes in ion concentrations ($[K^+]_o$, $[H^+]$, $[Na^+]_i$, $[Ca^{2+}]_i$, $[Mg^{2+}]_i$); disturbs lipid metabolism with accumulation of long-chain acylcarnitines, lysophosphoglycerides, fatty acids, and arachidonic acid; leads to the production of radicals, secretion of neurotransmitters, hormones, and metabolites, with concomitant stimulation of adrenergic, purinergic, and muscarinic receptors; and causes mechanical changes and stretch in the ischemic regions of the tissue. These changes have important modulatory effect on channels and carriers. In this section, this type of information is grouped in phys-

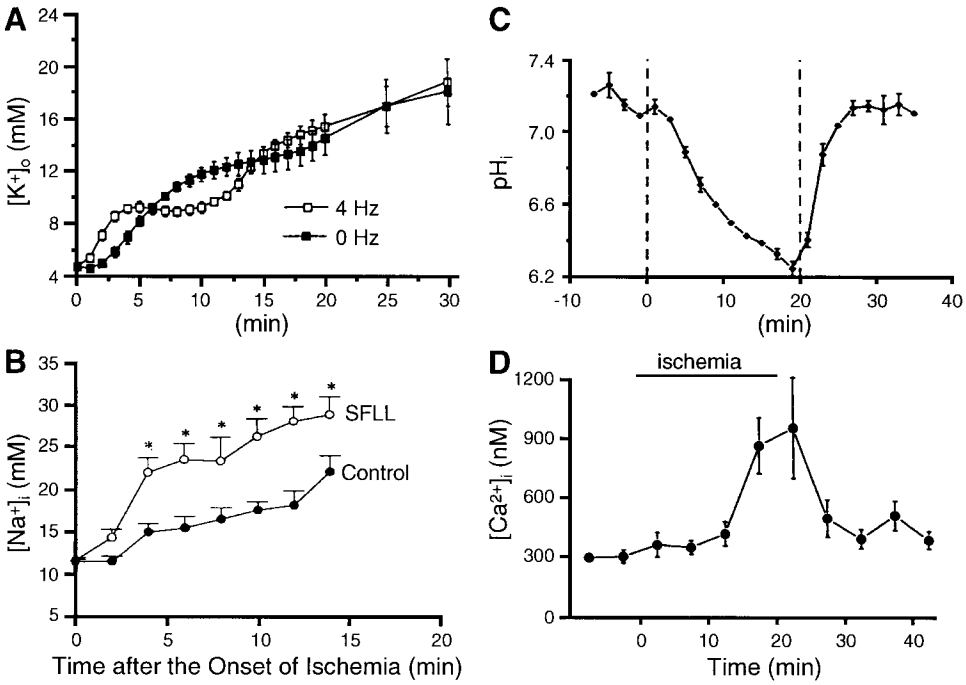


FIG. 16. *A*: increase of $[K^+]_o$ during ischemia in Langendorff-perfused quiescent and stimulated (4 Hz) rat hearts, measured with K^+-sensitive electrodes inserted in mid left myocardium. Note faster rise but lower plateau during stimulation; effect on plateau has been interpreted as due to activation of Na^+-K^+ pump. [Adapted from Wilde and Aksnes (1099).] *B*: increase of $[Na^+]_i$, measured by a floating Na^+-sensitive microelectrode, in a control group (●) and a group treated with a thrombin receptor activator (SFLL 100 μM; ○) after onset of ischemia in isolated, blood-perfused rabbit papillary muscles. Stimulation of thrombin receptors causes a larger and faster increase in $[Na^+]_i$. [Adapted from Yan et al. (1136).] *C*: fall in intracellular pH (^{31}P-NMR) during ischemia and fast recovery upon reperfusion in perfused ferret hearts. [Adapted from Marban et al. (640).] *D*: changes in free $[Ca^{2+}]_i$ (NMR with 5F-BAPTA) in perfused ferret hearts during 20 min of ischemia followed by reperfusion. Increase in $[Ca^{2+}]_i$ is delayed compared with changes in $[K^+]_o$, $[Na^+]_i$, or pH. [Adapted from Marban et al. (640).]

iopathological topics, characteristic for acute ischemia. The analysis of each syndrome includes *1*) a description of the changes, *2*) the mechanisms involved, *3*) the effect on channels and transporters, and *4*) the final outcome at the electrophysiological and arrhythmia level.

A. Changes in Ion Concentrations

In this section we distinguish successively accumulation of $[K^+]_o$, intra- and extracellular acidosis, accumulation of $[Na^+]_i$, accumulation of $[Ca^{2+}]_i$, and depletion of $[Mg^{2+}]_i$.

1. Increase of $[K^+]_o$

A) DESCRIPTION OF EXTRACELLULAR K^+ ACCUMULATION. Under aerobic conditions, $[K^+]_i$ is high and $[K^+]_o$ is low. Passive K^+ efflux is compensated by active K^+ influx via the Na^+-K^+ pump. During ischemia, this dynamic equilibrium is broken, and external K^+ accumulates. Potassium loss during ischemia typically occurs in three phases (Fig. 16*A*). Within 20 s after the occlusion of a coronary artery there is a fast accumulation of K^+ in the extracellular space that reaches a plateau after 3–10 min and is followed by a third slower increase starting between 15 and 30 min (see Ref. 1099).

The time to reach the plateau and the actual levels of $[K^+]_o$ differ widely among species, the models used (isolated perfused papillary or septum muscle, Langendorff-perfused heart, and in situ heart), the rate of beating, and the activation of the sympathetic nervous system. Plateau level of $[K^+]_o$ reached is highest (20 mM) in the isolated guinea pig heart (544), 10–11 mM in the in situ pig (288) and the rabbit perfused septum (1079), and 8–9 mM in the Langendorff-perfused rat (1100). The perfused rabbit papillary muscle shows a broad range of $[K^+]_o$ levels from 8 to 20 mM that depend on the diameter of the preparation. The difference has been correlated to a much more pronounced acidosis in the center of thick preparations (136).

Rate of stimulation is important between 0 and 60–90 min^{-1}, but not above this value (377, 418, 544, 1080). In the absence of stimulation, $[K^+]_o$ accumulation starts with a delay and its rate of accumulation is clearly less rapid; an outspoken plateau is not present.

A delay is also obtained in the presence of β-receptor blockade (418, 1097); the plateau level, however, was not different. Stimulation of the sympathetic nervous system on the other hand causes a faster rise in $[K^+]_o$ (1101).

During the plateau, $[K^+]_o$ is constant or slowly changing in the positive or negative direction (288, 418, 1101). The preparation remains excitable or regains its excitability. When stimulation is stopped, $[K^+]_o$ falls, suggesting an active Na^+-K^+ pump (1080). During the plateau, catecholamines are massively released from nerve endings and cells (865), glycolysis is stimulated, and lactate production is increased. The plateau level of $[K^+]_o$ is dependent on glycolytic activity (1101). When the heart is depleted of glycogen during successive ischemia periods, lactate production (348) and acidification (288) are less, and $[K^+]_o$ rises faster.

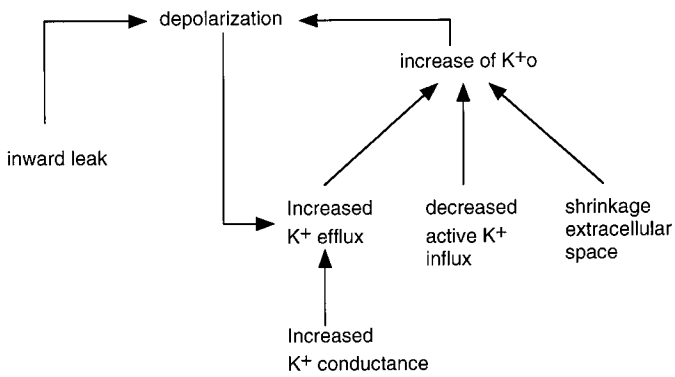
The changes in $[K^+]_o$ are far from homogeneous (857). They are most pronounced in the center of the

infarct. Higher levels are present in subepicardium compared with subendocardium.

When ischemia is continued over 20–30 min, $[K^+]_o$ starts to rise again. This phase is accompanied by inexcitability, an increase of longitudinal resistance, elevation of the resting tension, and development of rigor contracture (135, 1079). Lactate production stops (281), and extracellular pH (pH_o) remains at a low level. The metabolic changes suggest that anaerobic glycolysis is blocked.

Upon reperfusion, $[K^+]_o$ recovers completely and rapidly during the first rising phase or the plateau (8). The $[K^+]_o$ may even fall transiently below the control level. Recovery is incomplete, and $[K^+]_o$ remains elevated when reperfusion is started after 30 min of ischemia when the secondary rise of $[K^+]_o$ is already pronounced. Under those conditions, the resting tension of the muscle may increase and often shift into hypercontracture. At the same time, intracellular enzymes are released, evidencing an irreversible increase in membrane permeability.

B) MECHANISMS RESPONSIBLE FOR THE CHANGE IN $[K^+]_o$. Three main factors contribute to accumulation of $[K^+]$ in the extracellular space (scheme 1): shrinkage of the extracellular space, decrease of active K^+ influx, and increase of passive K^+ efflux.



SCHEME 1. Parameters determining depolarization and increase in extracellular $[K^+]$.

The multifactorial nature should be taken into account when discussing experiments in which only one factor is changed. In such situations, other factors may compensate.

1) Shift of water from the extracellular to the intracellular phase occurs as a consequence of increase in osmotically active particles in the cell, such as lactate and phosphate. Experimentally the amount has been evaluated and found to be ~20–35 mM, a value which is lower than expected on theoretical considerations (988). Much larger changes in osmotic pressure have been deduced from the changes in the concentration of the extracellular marker tetramethylammonium (552); however, a recent reevaluation, using higher concentrations of the marker, has yielded smaller values (1134). Taken together, it seems reasonable to assume that the increase in osmotically active particles can result in a restriction of the extracellular space by 15% after 10 min of ischemia. A similar value has been estimated in single rat ventricular myocytes exposed to silicon oil (282). Although small in itself, a decrease in the extracellular space acts as an amplification mechanism for any other mechanism involved in the increase of $[K^+]_o$.

2) There is a decrease of active K^+ influx by reduction of the Na^+-K^+ pump activity. Experimental evidence suggests partial inhibition of the pump. The block is incomplete; a transient arrest of electrical stimulation during ischemia results in a fall of $[K^+]_o$ (1080), whereas block of the pump by digitalis increases K^+ loss (544).

To justify the observed increase in $[K^+]_o$ however, the pump does not need to be blocked to a large extent. The capacity of the pump is remarkable, and a maximally active pump (150 pA or 3×10^{-15} mol/s) would totally deplete the extracellular space of K^+ (5 mM) in a very short time of 17 s (change in concentration is 18 $mmol \cdot l^{-1} \cdot min^{-1}$; extracellular space assumed to be 50% of the intracellular space or 10 pl/cell; $[K^+]_o$ is 5 mM; 1 Coul = 10^{-5} mol). Even if the pump is only working at half the maximal value and inhibition in early ischemia is only 50%, the change in $[K^+]_o$ will still be 4.5 $mmol \cdot l^{-1} \cdot min^{-1}$, which is more than needed.

Ischemic conditions make a moderate inhibition of the pump plausible: LCAC (886) and oxygen free radicals (880) have been found to reduce pump activity. Metabolic energy needed to fuel the pump becomes deficient. It is not so much the [ATP] alone, however, that should be considered (a fall to 150 μM which is the K_d value of the pump is probably an unrealistic low value during early ischemia) but much more the increase in [ADP] and $[P_i]$ which cause a substantial fall in the energy delivered from ATP hydrolysis or the phosphate potential. Although the normal value is ~61 kJ/mol and the E_{rev} of the pump is −180 mV, the phosphate potential may drop below 50 kJ/mol, and the E_{rev} of the pump to −60 mV, which is about the E_m of the cells during the plateau of $[K^+]_o$ accumulation. The pump, in other words, will stop functioning at the E_m expected under ischemic conditions (328). In accord with the assumption of a partial block of the pump activity, recent measurements reveal substantial increases in $[Na^+]_i$ (see sect. III*A3*) and internalization of pump molecules by endocytosis in [ATP] depletion (M. J. Shattock, personal communication).

3) There is an increase in K^+ outward movement. The first question is whether K^+ loss, in the form of potassium lactate or KCl cotransport, exists? On a molar basis, the amount of lactate production is four times larger than the net loss of K^+ (502). If only part of the lactate movement were linked to K^+ efflux, this process

could make an important contribution to $[K^+]_o$ accumulation. However, the following observations are against a tight coupling between K^+ and lactate: *1*) K^+ loss still occurs after blockade of anaerobic metabolism, when lactate production is strongly reduced; *2*) K^+ loss can be reduced by drugs, e.g., glibenclamide, whereas lactate production is not changed (502); and *3*) there is no evidence for an electrogenic transport of lactate (892). When cells are exposed to lactate, the intracellular medium becomes acidic, a process which is not accompanied by any current under voltage-clamp conditions or is not affected by a change in E_m. Acidification was saturable, partially stereospecific for L-lactate over D-lactate and inhibited by monocarboxylate carrier inhibitors, consistent with a carrier-mediated transport mechanism.

Part of K^+ moves out of the cell via a KCl cotransport, which is stimulated by an increase in cell volume as occurs in ischemia. Inhibition of the KCl cotransporter by bumetanide causes a decrease, and stimulation by ethylmaleimide an increase in $[K^+]_o$ accumulation (1136). The quantitative contribution of this mechanism is unknown.

The next question is whether evidence exists for electrogenic K^+ outward movement.

Quantitatively electrogenic K^+ movement can be expressed by the product of the conductance to K^+ and the electrochemical gradient, according to the equation: $I_K = g_K(E_m - E_K)$. It tells us that net outward movement will take place and increase when K^+ conductance (g_K) is elevated, but only when the E_m is positive to the equilibrium potential for K^+ (E_K). Evidence for the existence of increased g_K is given below. It should be stressed that an increase in g_K alone may be self-limiting because it causes hyperpolarization and moves the E_m closer to E_K. This is the reason why K^+ loss during ischemia is less when the preparation has been pretreated with a K^+ channel opener (499). As long as the preparation is excitable, K^+ loss occurs during the action potential, and loss will be greater when g_K is increased. Computations have shown that, although the action potential is shortened, net K^+ efflux is enhanced (134, and see Ref. 1099). This process is responsible for the initial phase of K^+ loss when the cells are still excitable. Later, when the cell becomes inexcitable, the rate of K^+ loss will be dependent on the existence of an inward leak current.

Evidence for an increased inward leak current as well as an increased K^+ conductance during ischemia has been obtained on models simulating the ischemic condition, because measurements of ionic currents using the voltage-clamp technique cannot be done in real ischemia. These models include multicellular and single cells exposed to hypoxia, metabolic inhibition, elevated $[K^+]_o$, and increased $[H^+]$. In preparations exposed to hypoxia or metabolic inhibitors (see Ref. 134), an increase in outward K^+ current has been measured. The current reverses at the expected E_K, and its current-voltage relation is linear or even outward rectifying, over a broad range of negative and positive potentials. The I_{K1} channel is an unlikely candidate, since the I_{K1} current is strongly inward rectifying, and inward rectification should even become more pronounced in the presence of an increased $[Mg^{2+}]_i$ (652), $[Na^+]_i$, and $[Ca^{2+}]_i$ (654, 671). The I_{K1} current furthermore was found to be decreased in the presence of lysophosphatidylcholine (LPC) (161, 542), free oxygen radicals (542), and intracellular acidosis (468).

A better candidate is the ATP-dependent K^+ channel, and a number of observations are consistent with this hypothesis. *1*) Single-channel activity typical for the ATP channel is seen in cell-attached patches during exposure of the cell to dinitrophenol (DNP) (990) or cyanide (CN) (729). A correlation in time is found between the shortening of the action potential and single-channel activity (694, 725); shortening of the action potential was reversed by intracellular injection of ATP (963). *2*) Glibenclamide, a blocker of the KATP, reverses the outward current induced by DNP or hypoxia (300) and the shortening of the action potential duration (317). Results on K^+ efflux and $[K^+]_o$ accumulation, however, are less convincing (1137).

A serious objection against the KATP channel being responsible for the increased efflux is its high sensitivity to ATP block (K_d 100 μM), whereas the [ATP] during the early minutes of ischemia remains at a sensibly higher level. The following remarks should be taken into consideration. *1*) During ischemia, the sensitivity of the channel to ATP block is decreased by the simultaneous rise in $[ADP]_i$ (970), P_i, and lactate (366, 516); acidosis (193, 266); the presence of free oxygen radicals (471) and extracellular adenosine (469, 540, 969); a decrease in taurine concentration (364); and the existence of stretch (1020). *2*) The [ATP] in the subsarcolemmal space or the concentration seen by the channel may be different from the bulk concentration. Block of anaerobic glycolysis, a pathway important for local subsarcolemmal [ATP] together with continued consumption of ATP by the Na^+-K^+-ATPase and adenylate cyclase may decrease the local [ATP] far below the bulk concentration (435, 781, 858, 998). *3*) Because of the large single-channel conductance and the high density of the channels, only a small proportion of the channels need to be activated to generate a relative large increase in conductance or current (725).

Two other candidate channels with outspoken outward rectifying properties are KNa and KAA. The KAA is a channel with a conductance slightly larger than the KATP; its P_o is enhanced in acidosis (527, 528) and by stretch (525), two conditions present during ischemia. With respect to the possible role of the KNa channel, the problem is similar to that of the ATP-dependent channel, the question being whether the $[Na^+]_i$ concentration can reach the high values needed to activate the channel (497, 625). Recent measurements usuing the NMR technique

show substantial increases in $[Na^+]_i$. It is furthermore important to realize that $[Na^+]_i$ close to the membrane can be much higher than in the bulk; gradients and local accumulation exist (642, 1085; and see Refs. 127, 591). For these reasons, activation of I_{KNa} during ischemia and especially during reperfusion (enhanced Na^+/H^+ exchange) remains a real possibility.

Three pathways for an increased inward leak probably participate in keeping E_m away from E_K: modified TTX-sensitive Na^+ channels, NSC channels, and Cl^- channels.

Sodium channels are modified by LPC in such a way that they open and show continuous activity at the resting potential level (107, 108, 1002). Inward current via NSC channels can be induced by stretch (188, 529, 815), $[ATP]_o$ (301, 411), free radicals (472), long-chain fatty acids (441), and a rise in $[Ca^{2+}]_i$ (172, 251, 410). Some of the NSC channels activated by stretch are equally permeable to Ca^{2+} (529). Calcium leak channels as such are induced by radicals (1056). An important Cl^- current is activated via β-receptor stimulation (443), a rise in $[Ca^{2+}]_i$ (910, 1192), hypotonic distension (357, 929, 992), and $[ATP]_o$ (500, 597, 664). Because E_{Cl} in cardiac cells is positive to the resting potential, an increase of Cl^- conductance generates an inward current at negative potentials. Outward movement of lactate through channels would generate an important inward current. At present, no evidence for such a mechanism exists, and the major efflux seems to occur through a proton-coupled carrier mechanism.

Finally, outward current is reduced through a partial block of the Na^+-K^+-ATPase by the fall in free enegy of hydrolysis, LCAC (959), and oxidative stress (880), which thus favors depolarization. In conclusion, it may be said that three K^+ channels with large conductance can be activated during metabolic inhibition, whereas a plethora of inward currents carried by Na^+, Ca^{2+}, and Cl^- are available to generate an electrical gradient for K^+ loss.

C) EFFECTS OF INCREASED $[K^+]_o$ ON CHANNELS AND CARRIERS. The effect of the increase in $[K^+]_o$ on channels, other than K^+ channels, is mainly indirect via the depolarization it causes (for references, see sect. II*A*). The extent of depolarization during acute ischemia is responsible for a partial or complete inactivation of the fast Na^+ channel, the T-type Ca^{2+} channel, the I_{to}, and the I_{Kur}. Recovery from inactivation of these currents is slowed and is accompanied by a decrease in excitability, prolongation of refractoriness, and conduction slowing of the action potential.

For a number of K^+ channels, but not for all, the conductance is increased when $[K^+]_o$ rises; the I_{K1} current (123) is the most sensitive, but voltage-activated currents such as I_{Kr} (851) and I_{to} (284) and ligand-gated K^+ channels such as KACh, KATP, KAA, and KNa all increase their conductance and carry more current at elevated $[K^+]_o$. In the case of I_{K1} and I_{Kr}, the mechanism is less pronounced inward rectification, consequent to a smaller block by intracellular cations (I_{K1}) or smaller inactivation (I_{Kr}). These changes again will stabilize the E_m and reduce excitability.

Worthwhile to mention is the enhanced conductance of the I_f current of which the Na^+-carrying capability is increased (293, 419). Because this current is only activated at hyperpolarized levels, the functional implications of this change remain limited, except transiently during reperfusion.

Extracellular K^+ stimulates the Na^+-K^+ pump, but because the normal K^+ concentration is already sensibly greater than the K_d of 1 mM (715), the increase in pump current via this mechanism is of no functional importance.

D) EFFECTS OF $[K^+]_o$ ON ELECTROPHYSIOLOGICAL CHARACTERISTICS. At the multicellular level, the changes by elevated $[K^+]_o$ will cause the cell to depolarize and the action potential to be reduced in amplitude, rate of rise, and duration. Inactivation of the Na^+ conductance, concomitant to the depolarization, is responsible for the fall in action potential amplitude and rate of rise and the decrease in excitability. Transiently, however, excitability may be increased, the reason being that the depolarization, although causing inactivation of the Na^+ current, at the same time moves the E_m closer to threshold with as result a reduction in the current required to reach threshold (231). At depolarized levels, the recovery from inactivation is slower; in the presence of increased $[K^+]_o$, the result is a prolongation of the postrepolarization refractoriness (see Ref. 480). Conduction velocity is depressed and slowed, especially during the relative refractory period (545). Shortening of the action potential is mainly due to an increase of the I_{K1} and I_{Kr} conductance and to a lesser extent to a decrease of Na^+ conductance. The changes in excitability, refractoriness, and conduction, together with the shortening of the action potential, favor the occurrence of reentry arrhythmias.

E) SYNOPSIS. Potassium loss during early ischemia occurs in three phases: a fast increase results in a plateau (10–20 mM) after 3–10 min and is followed (after 15–30 min) by a secondary and irreversible increase. Upon reperfusion before the irreversible phase, $[K^+]_o$ recovers rapidly. Three main factors contribute to the rise: shrinkage of the extracellular space, inhibition of active K^+ influx, and increase of passive K^+ efflux. The increase in K^+ efflux is due to the existence of an inward leak current (I_{Na}, I_{NSC} and I_{Cl}) which keeps the E_m positive to the E_K, concomitant with an increase in K^+ conductance (activation of I_{KATP}, I_{KAA} and I_{KNa}). The effect of elevated $[K^+]_o$, apart from causing an increase in conductance of K^+ channels, is indirect via the depolarization. The final result is inexcitability and block of conduction.

2. Intracellular and extracellular acidosis

A) PROTON DISTRIBUTION UNDER NORMAL AND ISCHEMIC CONDITIONS. Under normal perfusion and oxygenation, pH_i is slightly more acidic than pH_o. During ischemia, CO_2 retention and the net production of protons shift pH_i as well as pH_o in the acidic direction. External pH has been described to change rapidly and monotonically from 7.4 to values as low as 6.0 (159). Diverging results have been obtained on pH_i, depending mostly on the experimental model and the technique used [ion-sensitive electrodes (ISE), distribution of weak acids, ^{31}P-NMR spectroscopy, and fluorescence indicators]. With the use of NMR and fluorometric measurements, a pronounced fall in pH_i and pH_o has been found in preparations subjected to total ischemia. From a control value of 7.15–7.2, pH_i fell to 6.5 after ~4 min and to 6.2–6.0 after 10–20 min (rat, Refs. 116, 159, 962, 1047; ferret, Ref. 640; rabbit, Ref. 689) (Fig. 16*C*). A short delay of 1 min before the decline in pH_i has been observed by Vandenberg et al. (1011). Smaller changes in pH_i have been found using the ISE. This in part is due to a selection of superficially located cells for measurement that is an unavoidable bias with this technique. In a study on gas-superfused but blood-perfused papillary muscles of the rabbit heart subjected to ischemia (1135), internal pH was found to be rather resistant to any change when P_{CO_2} was held at the initial value; however, if the CO_2 tension in the experimental chamber surrounding the preparation was gradually increased to values that are probably present in vivo during ischemia, pH_i underwent a fall from 7.0 under control conditions to 6.55 after 18 min of ischemia. At the same time, changes in pH_o were more pronounced and decreased from 7.39 to 6.13. In other words, the pH ratio reversed, an effect that has been correlated to the higher buffering capacity of the intracellular medium. The finding that pH_i depends largely on P_{CO_2} predicts heterogeneity in pH_i changes, depending on the depth of the myocardial layer. The resistance of pH_i to undergo dramatic changes further demonstrates that protons continue to be transported against their electrochemical gradient even during ischemia. A more pronounced fall in pH_o than pH_i has been described in another study in which ischemia was simulated by bathing a papillary muscle in oil saturated with nitrogen (1018).

Changes in pH are less dramatic if the perfusion is not totally suppressed. In a comparative study where total ischemia and 10% perfusion were tested, pH_i was found to drop to 6.1 after 20 min of total ischemia, whereas this value was only 6.8 in 10% flow ischemia (769).

After an ischemic period short enough not to cause irreversible injury, pH on reperfusion recovers rapidly (398, 1011). Recovery is >50% in 1–1.5 min and may slow down a little during the following minutes.

B) REGULATION OF PROTON CONCENTRATION IN NORMAL, ISCHEMIC, AND REPERFUSION CONDITIONS. Intracellular proton concentration is kept at a much lower level than predicted for a passive distribution. For a −80 mV resting potential, the pH_i at equilibrium should approach 6.0. The fact that the pH_i is around 7.2 means that protons are actively transported outward. In aerobic conditions, the continuous production of protons is compensated by CO_2 elimination, Na^+/H^+ exchange, and Na^+-HCO_3^- cotransport, the latter two processes being ultimately coupled to the energy-consuming Na^+-K^+ pump (493, 595a, 616).

The Na^+/H^+ exchanger is activated by intracellular H^+, with a K_d of 7.4. Although this will make it an efficient mechanism (1114), the exchanger is far from equilibrium as can be learned from the $[Na^+]$ and $[H^+]$. The sensitivity of the exchanger to proton concentration is enhanced, and its activation curve shifted in the alkaline direction by α_1-receptor stimulation and activation of PKC (298). β-Receptor activation however inhibits (580). The exchanger is also inhibited by extracellular acidosis (1114).

The second important mechanism to protect the cell against acidosis is the Na^+-HCO_3^- cotransporter. According to a recent publication (7), it is electrogenic (2 HCO_3^- for 1 Na^+). In the guinea pig heart, the cotransporter is responsible under physiological conditions for about the same amount of acid extrusion as the Na^+/H^+ exchanger (198, 580). The cotransporter is also coupled to α- and β-receptors but in an opposite way to that of the Na^+/H^+ exchanger. α-Receptor stimulation in other words depresses whereas β-receptor stimulation enhances the activity of the cotransporter. The final effect will thus depend on the relative importance of the α versus β and cotransporter versus exchanger pathways. In the guinea pig, the α-effect is predominant over the β-effect, and the effect on the symporter is stronger than on the exchanger (580). Sympathetic stimulation therefore will decrease the rate of acid extrusion in this species. These effects are mimicked by application of extracellular ATP, whereas adenosine or ADP is without effect. In the rat, α-receptor stimulation has been reported to result in alkalinization, implying a larger effect on the exchanger (470). Lysophosphatidylcholine impairs the symporter and slows the recovery from an acid load (1129). The fact that two different mechanisms reduce the proton load explains why block of one does not necessarily result in acidosis.

In heart cells, two other processes, the Cl^-/HCO_3^- and Cl^-/OH^- exchange, act in the opposite way and may cause acid load (592, 948). They are stimulated by activation of β-receptors and $[ATP]_o$. Whether the stimulation involves a direct modification of the exchanger or is due to an indirect effect is not known. β-Receptor stimulation in addition stimulates glycolysis, which indirectly contributes to the acidification.

TABLE 1. *Effect of proton concentration on channels*

	Extracellular pH (luminal pH CaSR)			Cytoplasmic pH	
Channel	γ	P_o	Shift	P_o	Reference Nos.
Na	Decrease	Decrease	Positive	Decrease	1177
CaL	Decrease	Decrease		Decrease	784
CaT	Decrease	Decrease	Positive		1000
CaSR	Decrease			Decrease	1120
K1				Decrease	468
KNa				Decrease	1034
KATP				Increase	193, 266, 560
KAA				Increase	525
	Macroscopic conductance	Shift		Macroscopic conductance	
to		Positive		Decrease	938
Kr	Decrease	Positive			129
Gap				Decrease	109, 285, 732, 1091
MCC				Decrease	952

γ, Single-channel conductance; P_o, open probability; shift, voltage shift of activation-inactivation kinetics. External pH may affect γ, P_o, and cause shift; cytoplasmic pH only affects P_o at the single-channel level.

During ischemia, the fall in pH_i and pH_o is caused by an increased production of protons and insufficient removal (210).

1) Glycolytic ATP turnover in contrast to oxidative ATP turnover is accompanied by a net H^+ production. During ischemia, ATP production shifts from the mitochondrial system to glycolysis, and this shift is accompanied by an obligatory increase in protons. The finding that inhibition of glycolysis abolishes the acidosis during CN exposure is consistent with this explanation (10).

2) Net ATP hydrolysis is accompanied by net production of protons. One mole of ATP breakdown produces 0.8 mol H^+. This contrasts with the absorption of 0.35 mol H^+ upon breakdown of PCr which thus may shift the pH early in ischemia in the basic direction. The ratio of [PCr] to [ATP] is normally around two. Metabolic inhibition, at least in the beginning, can result in alcalosis or acidosis depending on the relative amounts of PCr or ATP that disappear (253). A delay in pH_i decline has been observed for global ischemia of the ferret heart (1011).

3) Removal of protons is insufficient. Under normal conditions, a large amount of the acidic load is removed by simple CO_2 diffusion. Although the production of CO_2 by aerobic metabolism ceases after coronary occlusion, it is still formed from HCO_3^- as a consequence of metabolic acidosis. The acidosis will thus be different across the ventricular wall with much more acidic conditions in the subendocardial regions (1077). Differences also exist between the central and border zones of the infarct. Acidosis will also greatly depend on the presence of buffer systems, e.g., blood or saline solutions (253, 390). These considerations should be taken into account when interpreting the data obtained in experimental models where the oxidative as well as the glycolytic pathways are inhibited by metabolic blockers but superfusion and elimination of CO_2 persist. The two other mechanisms for acid removal, i.e., the Na^+/H^+ exchanger and the Na^+-HCO_3^- cotransporter, are infavorably affected by the increase in $[Na^+]_i$. Upon reperfusion, pH in general rapidly recovers (398, 1011). The underlying mechanisms are washout of acid metabolites (H^+, $lactate^-$, and CO_2) and Na^+-coupled acid extrusion (Na^+-HCO_3^- cotransport and Na^+/H^+ exchange). The pH does not recover when hearts are perfused with HEPES buffer (no Na^+/HCO_3^- symport), and Na^+/H^+ exchanger and lactate transport are simultaneously inhibited. The most important of the three mechanisms is washout of CO_2 and lactate, whereas Na^+-dependent extrusion (Na^+/H^+ and Na^+/HCO_3^-) accounts for 20–35% of the recovery (1011). Among these two mechanisms, the relative contribution of Na^+-HCO_3^- cotransport is larger. The minor contribution of the Na^+/H^+ exchanger is evident from the small effect of blockers on the recovery of pH (398, 769, 864). Both extrusion mechanisms and the accompanying Na^+ influx, however, should be taken into account to understand the possible increase in Ca^{2+} load of the cell upon reperfusion. This explains why improved recovery of contractile behavior occurs (398), and arrhythmias are less frequent (29, 1123) when the exchanger is blocked.

C) EFFECTS OF THE PH CHANGES ON CHANNELS AND CARRIERS. Because channel proteins act like enzymes, large changes by protons may be expected. Most plasma membrane currents, with the exception of I_{KATP} and I_{KAA}, are inhibited, some by extracellular (I_{Na}, I_{Ca}, I_{to}, I_{Kr}, I_{Ks}), others by intracellular acidosis (I_{KNa}, I_{K1}) (for references, see Table 1) (Fig. 17). The mechanism is a decrease in single-channel conductance, P_o, or both and eventual voltage shifts in the activation-inactivation kinetics. The change in conductance in general is not voltage dependent; binding of protons outside the electrical field probably reduce the

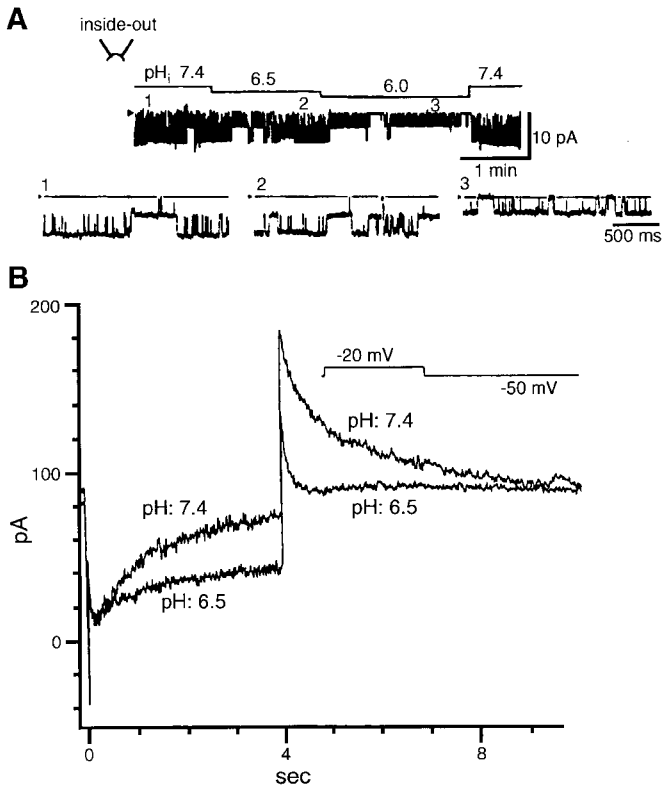


FIG. 17. Inhibition of I_{K1} by intracellular and of I_{Kr} by extracellular acidosis. *A*: inhibition by intracellular acidosis of single-channel I_{K1} current in an inside-out patch of a guinea pig ventricular myocyte. Open probability was reduced by pH changes from 7.4 to 6.5 and 6.0. Expanded records are given for times indicated by *1*, *2*, and *3* in top record. Similar changes of extracellular pH in outside-out patches had no effect (not shown). [Adapted from Ito et al. (468).] *B*: superimposed records of I_{Kr} obtained from rabbit ventricular myocytes in external pH of 7.4 and 6.5. Current during depolarization as well as tails are markedly diminished; rate of decay of tails is enhanced in acidosis. (From Carmeliet, unpublished data.)

local concentration of permeating cations or shift the conductance to a lower substate by an allosteric effect. The P_o is diminished by shortening of the open time (I_{Na}, I_{CaL}), prolongation of the closed time (I_{K1}), and increase in nulls (I_{CaL}). External acidosis normally causes also positive shifts in the activation-inactivation curves (I_{Na}, I_{CaT}, I_{to}, I_{Kr}). In case of shift, the effect on macroscopic current for a channel with activation-inactivation kinetics will thus depend on the resting (holding) E_m (938). In normal polarized cells, the current is not changed at positive test potentials but reduced for smaller depolarizations; the difference is due to the shift of the activation curve. When the resting potential is depolarized, however, the current may paradoxically be increased, an effect due the positive shift of the inactivation curve (442, 938). An exception to the rule of voltage independence can be found in the change of macroscopic conductance of the I_{Kr} current by extracellular protons (Fig. 17), which cause a larger reduction of the current at hyperpolarized potentials (129).

Among the plasma membrane channels, the KATP and KAA channels have to be mentioned separately, because they can show an increase in activity in the presence of elevated proton concentrations. Although in acidosis single-channel conductance is reduced and multiple substates become apparent (264), changes in P_o are more important and can be positive or negative depending on the presence or absence of ATP (193, 590). In the presence of ATP, the P_o (number of openings and open time) is increased for moderate decreases in pH (down to 6.0). The concentration response curve is shifted to higher [ATP] (560). The presence of Mg^{2+} seems required, which may suggest a change in phosphorylation of the channel, although this process has not been verified (193). For a fall of pH below 6.0, P_o is markedly reduced (560), an effect which may be caused by a faster rundown in inside-out patches. In the absence of [ATP], acidosis still increases mean open time but reduces overall P_o (193). In trypsin-treated patches, where rundown is absent, acidosis consistently increased activity in the presence of [ATP] (266, 304) but had no effect in ATP-free medium.

The gap junction channel is very sensitive to acidosis (109, 285, 732, 1091). Synergistic (109, 1091), additive (285), but also antagonistic interactions (732) have been described for simultaneous increases in $[H^+]$ and $[Ca^{2+}]$. The reduction in conductance by an increase of $[H^+]_i$ has been explained by a ball-receptor mechanism, comparable to the inactivation in voltage-operated channels, with the COOH terminal acting as the negatively charged ball and the M2-M3 link as the positively charged receptor (933).

Increase of the $[H^+]$ (pH change from 7.0 to 6.0) greatly reduced Ca^{2+} release from the SR; at the single-channel level, the conductance as well as P_o are diminished (812, 1120).

Among the transporters, the Na^+/Ca^{2+} exchanger is very sensitive to pH and strongly inhibited by acidosis (230, 764). Intracellular protons act as substrate on the Na^+/H^+ exchanger, but extracelluar acidosis inhibits (1029, 1114).

D) ELECTROPHYSIOLOGY AND PH CHANGES. The effects of acidosis at the channel level are translated at the multicellular level into a fall in resting potential, a reduction of upstroke velocity, a prolongation of the action potential duration, with eventual oscillations at the plateau level and occurrence of early afterdepolarizations (EAD) (175). Refractoriness is prolonged, due to a slowing of the Na^+ current recovery. Conduction of the action potential is retarded (319) as a consequence of the decrease in excitatory current and a fall in gap conductance. Of these changes, the prolongation of the action potential and the occurrence of EAD are not seen during ischemia but may be present upon reperfusion. During ischemia, the action potential is shortened as a consequence of extracellular K^+ accumulation and activation of outward currents,

mainly carried by KATP and KAA (see sects. III*A1* and IV*A*). All the acidosis-induced changes are proarrhythmic and may explain why the threshold for ventricular fibrillation is reduced in acidosis (950).

E) SYNOPSIS. Under aerobic conditions, the continuous production of protons is compensated by CO_2 elimination, Na^+/H^+ exchange, and Na^+-HCO_3^- cotransport. During ischemia, pH_i and pH_o fall by one pH unit or more. The fall is caused by an increased production and reduced removal of protons. Most plasma membrane channels with the exception of KATP and KAA, as well as gap junction channels, the SR Ca^{2+} release channel, and Na^+/Ca^{2+} exchange current are inhibited, some by extracellular and others by intracellular acidosis. The mechanism for the channels is a decrease in single-channel conductance and/or a reduction in P_o. In most instances, except for I_{Kr}, the effect is voltage independent. These changes result in depolarization, prolongation of the action potential, and occurrence of EAD.

3. Accumulation of $[Na^+]_i$

A) DESCRIPTION OF NA^+ DISTRIBUTION. Cytoplasmic free $[Na^+]$ concentration measured with ISE, NMR, and fluorescent dyes varies between 5 and 10 mM intracellular water for different animal species (see Refs. 30, 1037). Species differences exist, and $[Na^+]_i$ is higher in the rat and mouse ventricle cells (15–16 mM) (1143). In single cells, actual values depend on the cell isolation procedure; the presence of taurine, for instance, improves the quality of the cells and reduces $[Na^+]_i$ in rat cells from 20 to 10 mM (232, 945). The $[Na^+]_i$ is significantly higher in subepicardial than in subendocardial cells (173).

It is important to realize that $[Na^+]_i$ is not uniformly distributed in the cytoplasm. Recent estimations with electron-probe microanalysis (EPMA) (642, 1085), concomitant with evaluation of I_{KNa} activation (1085), show that the $[Na^+]$ in the subsarcolemmal space may differ substantially from the bulk cytoplasmic phase. Measurements of the time course of the Na^+ pump current (82) and the change in Na^+/Ca^{2+} exchange current (303) upon reactivation of the Na^+ pump are consistent with the presence of a fuzzy space in which diffusion of Na^+ is restricted (see Refs. 127, 591, 872). In mitochondria, the $[Na^+]$ is only half the cytoplasmic value (fluorometry, Ref. 232; EPMA, Ref. 1085).

A substantial monotonic increase in $[Na^+]_i$ occurs during ischemia or metabolic inhibition (NMR, Refs. 635, 769, 1015; fluorometry, Ref. 232). An increase of two- to fivefold with actual values of 20–25 mM have been recorded after 15–20 min of ischemia. These results stay in contrast to earlier results obtained using the ISE technique in the guinea pig (544, 1018) where no rise and even a small fall have been reported. A possible reason for the divergence in results is the preferential recording from superficial and thus from less ischemic, less acidotic cells with the ISE technique. A small fall in cytoplasmic $[Na^+]$ may be due to a shift from the cytoplasm to the mitochondria. Such a shift would be difficult to detect with other techniques. Recent experiments with ISE in the rabbit, however (1136), show a doubling of $[Na^+]_i$ after 15 min (Fig. 16*B*). Of importance was the observation that simultaneous stimulation of thrombin receptors caused a rise in $[Na^+]_i$ that was to 2.5 times greater, implying a role for thrombin receptors in the activation of a Na^+-permeant pathway (1136). Concomitantly, a greater accumulation of LPC in the membrane occurred, and the probability of arrhythmias increased (180, 751).

Information on recovery of $[Na^+]_i$ upon reperfusion is limited. If ischemia is restricted in duration to 20–30 min, $[Na^+]_i$ decreases monotonically and normalizes within 5 min (635). A transient slight increase at the very start of the reperfusion has been recorded in the rat (962). In the same species, recovery was fast initially but remained incomplete (769, 1015).

B) MECHANISMS INVOLVED IN THE CHANGES OF $[NA^+]_I$ DURING ISCHEMIA. During ischemia, an increase in $[Na^+]_i$ is caused by a reduced active outward movement and an increased passive inward leak.

A partial block of the pump during early ischemia is highly probable and may explain the observed changes in $[Na^+]_i$ (232). Inhibition of the pump is due to the fall in free energy of ATP hydrolysis as a result of changes in [ATP], [ADP], and $[P_i]$; a reduction in enzymatic activity under the influence of LCAC (959) and oxidative stress (880); and a reduction in the number of Na^+ pump molecules in the plasma membrane consequent to endocytosis (Shattock, personal communication). Theoretical calculations show that a reduction of the pump activity by one-fourth of its maximal activity will result in a change of $[Na^+]_i$ amounting to 3.4 $mmol \cdot l^{-1} \cdot min^{-1}$ (Na^+ influx is assumed to remain constant). Under normal conditions, the energy available from ATP hydrolysis amounts to 61.5 kJ/mol, but during ischemia, it can fall to 49 kJ/mol or less. Concomitant with this fall, the E_{rev} of the pump shifts in the depolarized direction and approaches the resting potential (190).

Inward leak of Na^+ occurs via carriers and channels. An important carrier is the Na^+/H^+ exchanger; extrusion of protons via the Na^+/H^+ exchanger causes Na^+ to move inward. Block of the Na^+/H^+ exchanger has been found to reduce $[Na^+]_i$ increase during ischemia (19, 769), although it had no effect on pH_i (398). In other experiments, the beneficial effect of Na^+/H^+ exchanger block was restricted to reperfusion (962).

Sodium ions move inward through TTX-sensitive Na^+ channels and NSC channels. The fast Na^+ channel is modulated by the rise in LPC to open repetitively and show continuous activity at the resting potential level

(107, 1002). Block of this pathway by lidocaine reduces this leak inward current (112, 1016).

Activity in NSC channels is induced by stretch (188, 529, 815), $[ATP]_o$ (301), free radicals (472), and a rise in $[Ca^{2+}]_i$ (172, 251, 410), all changes which occur very early during ischemia. Some of these NSC channels activated by stretch are equally permeable to Ca^{2+} (526). In pacemaker cells (Purkinje fibers), an important influx of Na^+ occurs via the I_f channel (327).

The fast recovery of $[Na^+]_i$ upon reperfusion is somewhat unexpected, since washout of protons from the extracellular space should stimulate Na^+/H^+ exchange and lead to a net Na^+ influx. Activation of the Na^+-K^+ pump however seems to prevail in most cases. Results may vary depending on the relative activity of the Na^+/H^+ exchanger and the pump. When glycogen is still available, glycolysis causes acidification and an increase in Na^+/H^+ exchange. In those instances, inhibition of the exchanger improves recovery of contractile activity and prevents arrhythmias (28, 29, 1123). When cells are depleted of glycogen, acidification is less, and activity of the exchanger remains limited. Under those conditions, $[Na^+]_i$ does not increase upon reperfusion, and only a rapid fall is observed.

C) EFFECT OF $[NA^+]_I$ INCREASE ON CHANNELS AND CARRIERS. Intracellular $[Na^+]$ if sufficiently elevated, activates specifically the I_{KNa} (497, 625), and enhances I_{Ks} (978) and I_{KACh} (943). In the case of I_{KNa}, an increase in $[Na^+]_i$ also reduces the single-channel conductance (1068), but this effect is largely compensated by the increase in P_o. Outward current through other K^+ channels, such as I_{K1} (654, 848) and I_{KATP} (494, 434), is blocked by intracellular Na^+; the block is voltage dependent, rapid, and seen as a decrease in overall conductance.

Intracellular Na^+ is the main substrate that activates the Na^+-K^+ pump current. The process becomes important during reperfusion when oxygen tension is restored. On the Na^+/Ca^{2+} exchanger, a rise in $[Na^+]_i$ favors reverse-mode activity, causing extra Ca^{2+} load. The relation between Na^+ and Ca^{2+} load explains the close relationship between $[Na^+]_i$ and the frequency of spontaneous Ca^{2+} release (216). Via interaction with the endogenous XIP region, a rise in $[Na^+]_i$ causes a state of inactivation of the exchanger (662, 663). The simultaneous rise in $[Ca^{2+}]_i$, however, will partly compensate for this inhibitory mechanism.

D) EFFECT OF $[NA^+]_I$ INCREASE ON ELECTROPHYSIOLOGY. All changes in channels and carriers, except for the reduction in I_{K1} and I_{KATP}, tend to hyperpolarize the membrane, but the effect will only be visible during reperfusion. The marked hyperpolarization of the diastolic E_m and the excessive shortening of the action potential, despite the low $[K^+]_o$, can be ascribed to stimulation of the pump and eventual activation of outward K^+ currents. Insufficient recovery of the Na^+ pump or excessive Na^+ influx via the Na^+/H^+ exchanger may be responsible for increased Ca^{2+} influx via the reversed Na^+/Ca^{2+} exchanger, causing Ca^{2+} overload, absence of recovery, and eventual arrhythmias. Prevention of Na^+ and Ca^{2+} overload by blocking the Na^+/H^+ exchanger (19, 548, 769, 863) explains the inhibitory effect of such intervention on reperfusion arrhythmias.

E) SYNOPSIS. During ischemia, $[Na^+]_i$ increases from 10 to 20 mM after 20–30 min. The mechanism is reduced active outward pumping (inhibited by the fall in free energy of ATP hydrolysis by LCAC and radicals) and an increased inward leak (through the Na^+/H^+ exchanger, I_{Na}, I_{NSC}, and I_{Cl}). The effect of increased $[Na^+]_i$ is activation of the pump and eventually of I_{KNa}. During reperfusion, these effects result in hyperpolarzation and short action potentials combined with low $[K^+]_o$. Dispersion is pronounced and is favorable to arrhythmias.

4. Changes in the distribution of $[Ca^{2+}]_i$

A) CA^{2+} DISTRIBUTION UNDER NORMAL CONDITIONS AND CHANGES DURING ISCHEMIA AND REPERFUSION. *I) Ca^{2+} distribution under normal conditions.* Under normal conditions, concentrations of Ca^{2+} differ in the cytosol, the SR, the mitochondria, and the nucleus (for review, see Ref. 773). In each compartment, it plays a different role. It modulates the activity of myofilaments in the cytosol and of ionic channels in the plasma membrane. The SR acts as a store for Ca^{2+} and modulates contraction and relaxation. In the mitochondrion, the free $[Ca^{2+}]$ regulates the activity of three dehydrogenases (pyruvate dehydrogenase, α-oxoglutarate dehydrogenase, and NAD-dependent isocitrate dehydrogenase) and adapts in that way energy production to energy utilization. In the nucleus, Ca^{2+} modulates gene expression.

Free $[Ca^{2+}]$ can be estimated in the cytoplasm (909) and mitochondria (341, 687) using phosphorescence, fluorometric methods, and NMR spectroscopy (640). For the measurement of $[Ca^{2+}]$ in mitochondria, the signal in the cytoplasm is Mn^{2+} quenched or the cytoplasm is unloaded from the indicator by washout.

Cytoplasmic $[Ca^{2+}]$ during diastole and at low stimulation frequencies is on the order of 100 nM (from 50 to 200 nM), with somewhat higher values in the subendocardial layers (116). During systole, this value may increase to between 500 and 1,000 nM or even higher values depending on the Ca^{2+} load in the SR; similar values are obtained on rapid application of caffeine, suggesting that most of the SR Ca^{2+} can be released during a contraction (see Ref. 73). From a comparison with values for total $[Ca^{2+}]$, it appears that only a very small fraction or $<0.03\%$ is present as the free ion at rest and most (order of 0.5 mM) is bound to cytoplasmic proteins; during activity, this fraction rises to 0.1%.

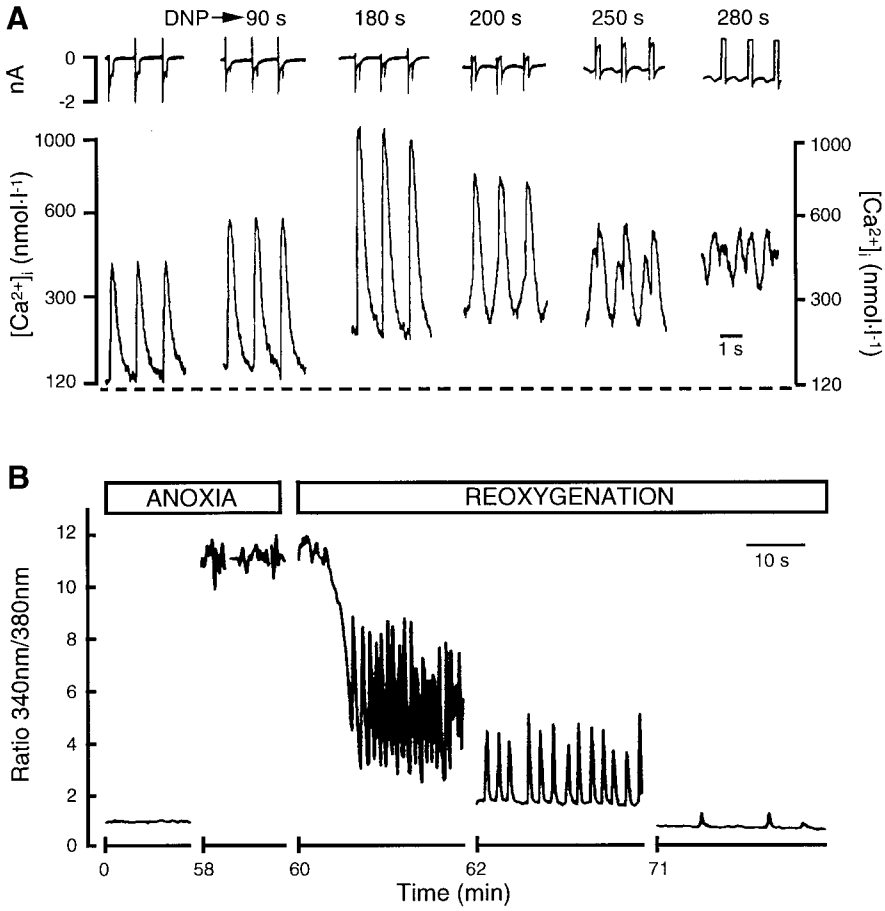


FIG. 18. *A*: changes in free $[Ca^{2+}]_i$ measured by indo 1 fluorescence in guinea pig myocytes upon metabolic inhibition by exposure to 0.1 mM dinitrophenol (DNP). Cells were voltage clamped from −80 to 0 mV for 160 ms, preceded by a prepulse to −45 mV to inactivate Na^+ current. Systolic $[Ca^{2+}]$ increased transiently, before declining and becoming oscillatory. Diastolic $[Ca^{2+}]$ gradually increased. Temporary increase in Ca^{2+} transient is not due to an increase in release from SR but has been explained by a block of Ca^{2+} absorption by mitochondria which normally takes place during systole. [From Isenberg et al. (461). Copyright 1993 Cardiovascular Research.] *B*: recovery of $[Ca^{2+}]$ in a rat single myocyte to normal values following an anoxia period. During early recovery, decelerating oscillations are seen, and spontaneous release continues during a certain time. [From Siegmund et al. (906).]

In the mitochondrion, the free $[Ca^{2+}]$ concentration at rest or during diastole is somewhat below the cytoplasmic $[Ca^{2+}]$ concentration (225, 312, 687, 870) or close to it (888). Because the mitochondrial matrix is negative with respect to the cytoplasm (estimations vary between −150 and −180 mV), this means that mitochondrial $[Ca^{2+}]$ is far from equilibrium.

The $[Ca^{2+}]$ in the mitochondrion is variable depending on the cytoplasmic load. When cytoplasmic $[Ca^{2+}]$ is <500 nM, the gradient of mitochondrial $[Ca^{2+}]$ to cytoplasmic $[Ca^{2+}]$ is less than unity but increases in an exponential way at higher values (687). Mitochondrial $[Ca^{2+}]$-to-cytoplasmic $[Ca^{2+}]$ ratios larger than unity have been measured during stimulation of isolated cells at 2 Hz (225). Total mitochondrial $[Ca^{2+}]$ measured by the electron probe transiently increases during a depolarization (461, 1084); the increase in free Ca^{2+} transient during metabolic inhibition has been interpreted as due to a fall in Ca^{2+} uptake by the mitochondria during the cardiac systole. The rise in free $[Ca^{2+}]$ has been confirmed using a combination of a fluorescence and confocal microscopy technique (140). In previous fluorescence measurements, such fast changes were not detected. The reason for this divergence is not clear but may be related to the buffer characteristics of the fluorescent probe (461). Mitochondria are thus no source of Ca^{2+} for the cytoplasm during stimulation but rather act as a buffer. Because mitochondria represent a large fraction of the cell volume, they can accumulate massive amounts of Ca^{2+} (43), a situation which occurs during pronounced and prolonged ischemia. The amount of Ca^{2+} stored in the mitochondrion is sufficient to lead to a contracture when the mitochondrial E_m breaks down after application of an uncoupler (1106).

In the cardiac SR, total $[Ca^{2+}]$ expressed per liter cytosolic volume has been measured to be between 100 and 200 μM (see Refs. 73, 323, 1024), with estimations of free $[Ca^{2+}]$ per liter SR volume at 700 μM (877). An enormous gradient of 7.000 thus exists between the SR and cytoplasm.

II) Changes of $[Ca^{2+}]_i$ during ischemia and reperfusion. During anoxia, metabolic inhibition or ischemia, diastolic free $[Ca^{2+}]_i$ rises with some delay. The delay is variable, and quite important differences in time course have been reported, depending on the model. In studies of ischemia (coronary ligation), the delay attained 10–20 min and the rise in $[Ca^{2+}]_i$ slightly preceded the development of contracture and uncoupling of the gap junctions (640, 1047) (Fig. 16*D*). A much shorter delay between 2 and 5 min has been described in other studies (519, 689). A large variation in time course also exists in experiments

on single cells subjected to hypoxia or metabolic inhibition (see references in Ref. 817). The reasons for these divergent results are not known but are probably related to the intensity of the metabolic blockade.

Results on systolic [Ca^{2+}] are variable. A transient rise may occur (Fig. 18*A*) (461) but is not always present [total heart (689, 1047), ventricular muscle (920), and singe cells (253, 461)] and is followed by a decrease when diastolic [$Ca^{2+}]_i$ rises (519, 640). The initial rise is not accompanied by an enhancement of the contraction and is probably due to a shift of Ca^{2+} from the mitochondrion to the SR (461) (Fig. 18*A*) and/or a release from binding sites by the increase in intracellular acidosis (312). The fall in systolic [$Ca^{2+}]_i$ may be accompanied by oscillations during diastole, caused by release from the SR (906).

In mitochondria, an increase is seen upon metabolic inhibition when the ratio of mitochontrial-to-cytoplasmic [Ca^{2+}] is low, a decrease when the starting level is greater than unity (225). In this last case, Ca^{2+} initially is taken by the SR and the Ca^{2+} transient is increased. Later, when the cytosolic [Ca^{2+}] increases, the mitochondrial [Ca^{2+}] simply follows and rises to the same extent. Release of Ca^{2+} from mitochondria and contracture development have been demonstrated when mitochondria are uncoupled (1106).

Changes in SR Ca^{2+} content during ischemia have not been measured directly. From the temporary increases in Ca^{2+} transient that have been recorded (461), a higher content early in ischemia can be deduced. Later on, less Ca^{2+} will be stored in the SR as a consequence of inhibition of the SR Ca^{2+}-ATPase activity (503).

Upon reperfusion, the change in diastolic [Ca^{2+}] can follow three different time courses: *1*) a rapid decline to the normal value (ferret, Ref. 920; rat, Refs. 641, 1047); *2*) an initial fall to an intermediate value followed by oscillations that can end with a return to normal or be followed by a secondary increase (773, 817, 906) (Fig. 18*B*); at the multicellular level oscillations may then trigger arrhythmias (703); *3*) no recovery but an immediate rise to elevated values in the micromolar range accompanied by contracture and irreversible injury (11, 397, 906, 962). Irreversibility is often the outcome when the cytoplasmic [Ca^{2+}] has risen above 1 μM, and this occurs when the ischemic period is extended over 20 min.

Data on changes in mitochondrial [Ca^{2+}] upon reoxygenation or reperfusion are scarce. On theoretical grounds an increase in mitochondrial [Ca^{2+}] may be expected when oxidative metabolism restarts the mitochondrial battery and increases the matrix negativity. No change in mitochondrial [Ca^{2+}] was seen on reoxygenation after 50 min of hypoxia, but a dramatic 10-fold increase was seen after 80 min of hypoxia. Addition of ruthenium red that blocks Ca^{2+} uptake in the mitochondria exerted a protective effect (11). Large increases in mitochondrial [Ca^{2+}] are accompanied by irreversible changes and hypercontracture (687). The mitochondrial damage is not due to Ca^{2+} alone but to the concomitant increase in phosphate, LCAC, proton concentration, oxidative stress, and low [ATP], changes that result in the opening of the mitochondrial mega-channel. This contention is based on the observation that large Ca^{2+} overload caused by digitalis is easily reversible when it is not accompanied by energy shortage (595).

Recordings of Ca^{2+} transients suggest that reuptake of Ca^{2+} in the SR is quickly restored upon reperfusion. In some cases, however, overload is the consequence with spontaneous oscillatory release and generation of EAD or DAD.

B) MECHANISMS FOR [$CA^{2+}]_I$ CHANGES. The increase in cytoplasmic [Ca^{2+}] in the cells submitted to ischemia is due to a less efficient removal from the cell via the Na^+/Ca^{2+} exchanger and a reduced Ca^{2+} uptake in the SR, an increased inward leak through the plasma membrane, and displacement of Ca^{2+} from binding sites in the cytoplasm and in mitochondria by protons. Mitochondria are probably not responsible for an increase in cytoplasmic [Ca^{2+}] except in excessive overload; in most instances, the mitochondria absorb Ca^{2+} during ischemia.

In the early stage of ischemia, free [Ca^{2+}] in the cytoplasm increases due to a displacement of Ca^{2+} by H^+ from binding sites (312). The experimental basis for this mechanism was provided by the observation that an increase of CO_2 tension from 5 to 20% caused [Ca^{2+}] in the cytoplasm to rise from 130 to 221 nM. The effect was not changed by application of ryanodine (block of the SR Ca^{2+} channel) or ruthenium red (block of mitochondrion uniporter).

Calcium influx and efflux through the plasma membrane that are normally in equilibrium (986, 1057) go out of balance during ischemia with Ca^{2+} influx exceeding Ca^{2+} efflux. The most important mechanism for removal of Ca^{2+} from the cell is the Na^+/Ca^{2+} exchanger, and it is responsible for 77% of the Ca^{2+} extrusion (583, 986). During ischemia, the Na^+/Ca^{2+} exchanger is less efficient because of an increase in $[Na^+]_i$ and $[H^+]_i$. An increase in $[Na^+]_i$ promotes the reversed mode of the Na^+/Ca^{2+} exchanger whereby Ca^{2+} is entering the cell and Na^+ is removed (358, 920, 962). All mechanisms responsible for an increase in $[Na^+]_i$ thus also contribute to the increase in $[Ca^{2+}]_i$ via the exchanger. The efficiency of the exchanger is furthermore reduced by the acidosis (230, 764) and the influence of radicals (163).

Whereas Ca^{2+} efflux is reduced, Ca^{2+} influx is increased during metabolic ihibition. The role of T- and L-type Ca^{2+} channels in this respect is negligible. Background channels carrying Ca^{2+} inward become more important during ischemia as they are activated by radicals (472, 1056). Inward leak of Ca^{2+} also occurs via NSC channels (see sect. III*A5*) that are activated by $[ATP]_o$

(301, 411), mechanical stretch (188, 526, 841), and a rise in $[Ca^{2+}]_i$ itself (172, 251, 650).

During ischemia, less Ca^{2+} is stored in the SR, an effect mainly due to block of Ca^{2+}-ATPase activity, whereas changes in the release mechanism are less important. The decrease in Ca^{2+} reuptake is due to a reduction of V_{max}, not to a change in K_d (0.5–0.7 μM) or the Hill coefficient (503). The effect is related to the reduction in free energy change of ATP hydrolysis (339, 644) and modification of the carrier by oxygen radicals (disulfide bridge formation) (565). Although the SR Ca^{2+} channel can become activated by exposure to oxygen radicals (429) or to arachidonic acid and its metabolites (196), the increased levels of $[H^+]$, $[Mg^{2+}]$, and $[PO_4^{3-}]$ exert a blocking effect. The overall result is probably of less importance.

Influx as well as efflux of Ca^{2+} in the mitochondria depend on the electrical and proton gradient (Fig. 2). Influx through the uniporter is driven by the electrical potential difference that is estimated to be −150 to −180 mV. Efflux from the mitochondria depends on the proton gradient and is continuously guaranteed by the Na^+/Ca^{2+} exchanger and the Na^+/H^+ exchanger which act in concert to keep intramitochondrial $[Na^+]$ and $[Ca^{2+}]$ at low levels. The immediate source of energy lies in the Na^+ gradient, but the final energy source is respiration and the proton gradient. Block of the oxidative metabolism, resulting in the disappearance of the electrical and proton gradients, is thus expected to result in equilibration of the mitochondrial $[Ca^{2+}]$ and the cytoplasmic $[Ca^{2+}]$ with a ratio of mitochondrial-to-cytoplasmic $[Ca^{2+}]$ approaching unity. Because the cell volume fraction of mitochondria is 30–36%, they may absorb a large amount of Ca^{2+} and act as a buffer.

There are mechanisms responsible for $[Ca^{2+}]_i$ changes during reperfusion. Depending on the severity and duration of the ischemic period, the cytosolic $[Ca^{2+}]$ concentration may normalize or on the contrary increase to dramatic levels leading to cell death.

The rapid initial recovery phase seems to depend on the activity of the SR Ca^{2+}-ATPase (see Refs. 503, 697). Although the Ca^{2+}-ATPase activity and the number of ryanodine receptors (1005) are reduced, uptake in the SR and increase of the luminal $[Ca^{2+}]$ may be so large that threshold for release is attained resulting in oscillations (987). If, in the mean time, removal of Ca^{2+} from the cell through the Na^+/Ca^{2+} exchanger is restored, the Ca^{2+} level may return to normal values (773, 906). In some cases, however, the oscillations are followed by a secondary increase. Recovery depends much on the restoration of the Na^+ gradient and is facilitated by blockade of the Na^+/H^+ exchanger (578, 864) or acidosis (see Ref. 91). Protection against Ca^{2+} overload contracture is also provided by the natriuretic peptide that stimulates guanylate cyclase (397). On the other hand, production of IP_3 secondary to α_1-receptor stimulation may inhibit recovery (17, 236).

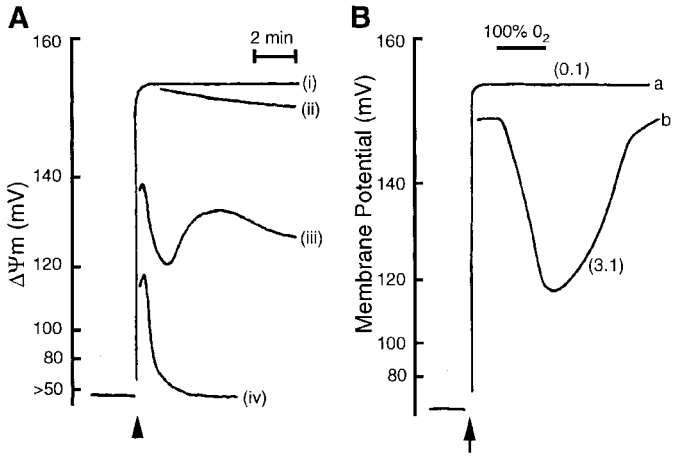


FIG. 19. *A*: effect of free $[Ca^{2+}]$ on membrane potential ($\Delta\psi_m$) of inner mitochondrial membrane measured by accumulation of tetraphenyl phosphonium in membrane. Arrow indicates start of respiration by addition of succinate to isolated mitochondria (rat heart). $[Ca^{2+}]$ was increased from 0.1 μM (*i*) to 1.0 μM (*ii*), 3 μM (*iii*), and 5 μM (*iv*); high $[Ca^{2+}]$ resulted in breakdown of electrical gradient. This effect could be counteracted by adding ATP (data not shown). [From Duchen et al. (244). Copyright 1993 Cardiovascular Research.] *B*: effect of high oxygen tension on membrane potential of isolated mitochondria is modulated by free $[Ca^{2+}]$. Arrow indicates start of respiration after addition of succinate; [ATP] was 0.2 μM. Oxygen tension is increased to 100% (horizontal line). In presence of 0.1 μM $[Ca^{2+}]$ (*a*), rise in oxygen tension has no effect, but in presence of 3.1 μM (*b*), a breakdown of electrical gradient occurs. [From Crompton and Andreeva (189). Copyright 1993 Steinkopff Verlag.]

A critical situation may occur when the ischemic period has been too long and restoration of the Na^+-pump activity has been retarded. In this case, the $[Ca^{2+}]$ remains elevated. On restoration of the oxygen supply, the electron flow through the oxidative chain generates an electrical gradient in the mitochondrion causing a massive absorption of Ca^{2+} from the cytoplasm. The presence of such an abnormally high $[Ca^{2+}]$ together with low [ATP] and oxidative stress may cause the opening of the mitochondrial mega-channel (Figs. 2 and 19). The ATP, instead of being exported to the cytoplasm, may even be hydrolyzed to generate a proton gradient and limit the absorption of Ca^{2+} in the mitochondrion. The final result is disappearance of all mitochondrial gradients, hypercontracture, and cell death (244, 340). It is important to note that high $[Ca^{2+}]$ alone does not cause the permeability transition as shown by experiments with toxic concentrations of digitalis in which $[Ca^{2+}]$ increases to much higher levels than in ischemia and cells remain viable (595).

C) EFFECTS OF $[CA^{2+}]_I$ ON CURRENTS AND CARRIERS. An increase in $[Ca^{2+}]_i$ modulates multiple currents in cardiac cells. The L-type Ca^{2+} current itself is very sensitive to increases in $[Ca^{2+}]_i$ and shows an increased rate of inactivation, limiting in this way the Ca^{2+} load of the cell (see Ref. 673). In contrast, the current may also undergo facil-

itation for moderate increases of the Ca^{2+} level (505) (see sect. II*A2*).

Some currents are dependent on $[Ca^{2+}]_i$ for their activation: $I_{Cl(Ca)}$ (910, 1192), I_{NSC} (172, 251), the Ca^{2+} release channel (120, 261), and the Na^+/Ca^{2+} exchanger (686). An increase in $[Ca^{2+}]_i$ stimulates the Na^+/Ca^{2+} exchanger and counteracts in this way the Na^+-induced inactivation (663). Other currents are modulated; an increase is seen for the T-type Ca^{2+} current (994), I_{Ks} (728, 978), I_{Kr} (851), I_f (354), the Na^+-K^+ pump (314), and the mitochondrial MCC (189, 952). Two currents are inhibited: the I_{K1} (654, 670, 671), a phenomenon comparable to the block by $[Mg^{2+}]_i$, and the gap junction channel (206, 732).

The $[Ca^{2+}]_i$ influences furthermore a number of enzymes. Adenylyl cyclase is inhibited (1154), and others are stimulated, e.g., mitochondrial dehydrogenases (692), the ATP synthase (199), PKC, phosphatases, and proteases (causing rundown of channels) (see Ref. 496).

D) ELECTROPHYSIOLOGICAL EFFECTS OF $[CA^{2+}]_i$. Enhancement of Ca^{2+}-induced inactivation of the Ca^{2+} current, increase of the transient Ca^{2+}-activated Cl^- current, and of the two delayed K^+ currents will lead to shortening of the action potential.

When the rise in $[Ca^{2+}]$ is more pronounced (Ca^{2+} overload), EAD as well as DAD are generated, and arrhythmias may result (519, 971). One of the mechanisms underlying EAD is reactivation of L-type Ca^{2+} current (684), but also release of Ca^{2+} from the SR (782), followed by activation of the "transient inward current" can cause EAD. The transient inward current (not described as a separate current in this review) is a composite current and is carried through the Cl^- channel (747, 912), the NSC channels (121, 368), and the Na^+/Ca^{2+} exchanger (369, 579). Although the primary activator for Ca^{2+} release is the cytoplasmic Ca^{2+}, other factors such as ATP (1120), the phosphorylation state of the channel (754, 1006), and luminal $[Ca^{2+}]$ (917) determine the efficiency of the release mechanism or, what has been called, the gain of the system (120). Under conditions of moderate Ca^{2+} load, the release is linearly related to the luminal $[Ca^{2+}]$ concentration and not propagated; the gain is constant. With increase of Ca^{2+} load however, gain increases nonproportionally, and release becomes regenerative and propagated (44, 367, 476, 847, 940). Under these conditions, release may be spontaneous and occur in the absence of trigger Ca^{2+}. The result is EAD during the action potential or DAD, following repolarization. Important to note is that Ca^{2+} overload also increases the tendency in Purkinje fibers to become spontaneously active; the activation curve of the I_f current is shifted in the positive direction (354, 1172), which favors activation of the current and increases the rate of diastolic depolarization. The fall in gap junction conductance by $[Ca^{2+}]_i$ further enhances the probability for arrhythmias by reducing conduction (206, 732). In a final stage, activation of the mitochondrial mega-channel uncouples the ATP synthase, causes hydrolysis of ATP, and leads to rigor and contracture (189, 244). High $[Ca^{2+}]$ alone, however, is not sufficient (595); a simultaneous fall in [ATP] and the presence of oxidative stress that may occur during reperfusion creates a favorable condition for activation of the channel (189, 244) (Fig. 19). Once activated, the opening of the MCC channel leads to irreversible processes and cell death.

E) SYNOPSIS. The $[Ca^{2+}]$ differs in the cytoplasm, the SR, and the mitochondria. Whereas systolic $[Ca^{2+}]$ may transiently increase during the first minutes of ischemia, diastolic $[Ca^{2+}]$ only increases after a delay of 10–20 min. In the mitochondria, $[Ca^{2+}]$ follows the changes in cytosolic level, although there may be an initial release if the $[Ca^{2+}]$ in the mitochondria is higher than in the cytoplasm at the start of ischemia. The increase in cytosolic $[Ca^{2+}]$ is due to a less efficient removal to the extracellular space via the Na^+/Ca^{2+} exchanger, a reduced uptake in the SR, an increased inward leak, and a displacement by protons from binding sites. An increase of cytosolic $[Ca^{2+}]$ activates a number of channels, carriers, and enzymes (I_{NSC}, I_{Cl}, and $I_{Na,Ca}$) and modulates others (I_{CaL}, I_{CaT}, I_{Ks},I_{Kr}, I_f, $I_{Na,K}$, and gap junction channels). The result is the occurrence of EAD, DAD, and arrhythmias. If on reperfusion, removal of Ca^{2+} to the extracellular space and into the SR is insufficient, while O_2 tension is restored and the mitochondrion E_m energized, a massive absorption of Ca^{2+} may occur in the mitochondrion and result in activation of the mitochondrial mega-channel, which signals irreversibility.

5. *Changes in $[Mg^{2+}]_i$*

A) DISTRIBUTION AND REGULATION OF $[MG^{2+}]$ UNDER AEROBIC CONDITIONS AND ISCHEMIC CONDITIONS. Estimations of total Mg^{2+} in heart cells using EPMA (see Ref. 701) vary between 50 and 80 mmol/kg dry wt for the cytoplasm (A band), 20–80 mmol/kg dry wt for mitochondria, and 20–75 mmol/kg dry wt for junctional SR. From the estimated dry weight-to-wet weight ratio of 10% for the cytoplasm and 25% for mitochondria, these values are translated in 5–8 mM for the cytoplasm and 6–20 mM for mitochondria.

Measurements of free $[Mg^{2+}]$ in the cytoplasm, using ISE, NMR, and fluorescence techniques, have yielded data between 0.5 and 0.8 mM intracellular water (299, 701). In contrast to Ca^{2+}, which is largely concentrated in the SR, Mg^{2+} does not seem to be concentrated in a particular intracellular compartment. The free $[Mg^{2+}]$ in mitochondria, estimated to be 0.8 mM (818), is similar to the cytoplasmic $[Mg^{2+}]$. Isolated mitochondria can absorb and extrude Mg^{2+} in a respiration-dependent way, but in vivo this does not seem to result in any large gradient.

In the cytoplasm, only about one-tenth of the total $[Mg^{2+}]$ is free; the rest is bound to nucleotides (ATP) and

proteins. A competition between Mg^{2+} and protons has been demonstrated by the fall in free $[Mg^{2+}]$ when the cell is subjected to a pulse of alkalosis (299).

After 10–15 min of ischemia, free $[Mg^{2+}]$ has been found to increase from 0.5–0.8 mM to 2–6 mM (539, 702, 869). Upon reperfusion, the level of free $[Mg^{2+}]_i$ decreases to the original level (869) but may remain transiently elevated at 1.5 mM (539, 702).

B) MECHANISMS OF $[MG^{2+}]$ REGULATION. Free $[Mg^{2+}]$ in the cytoplasm is of the same order of magnitude as the extracellular concentration. With the fact that the intracellular medium is negative taken into account, the distribution of free $[Mg^{2+}]$ is thus far from equilibrium. Thermodynamically active transport is required. Two mechanisms are involved: a Mg^{2+}-ATPase and a Na^+/Mg^{2+} exchange process (361, 907). In favor of a Na^+/Mg^{2+} exchange mechanism are the observations that $[Mg^{2+}]_i$ increases upon application of ouabain. Reduction of $[Na^+]_o$ also results in an increase of $[Mg^{2+}]_i$ (214). The $[Mg^{2+}]$ content can be modulated by β-receptor stimulation, causing a release of intracellular Mg^{2+}, and by M_2 receptor stimulation, leading to the opposite effect. These changes may be secondary to mobilization of the mitochondrial $[Mg^{2+}]$ pool by an effect on the adenine nucleotide metabolism.

The most important mechanism for the increase in $[Mg^{2+}]_i$ during ischemia is the net hydrolysis of ATP to which Mg^{2+} was bound. Block of the Mg^{2+}-ATPase and reduction of the activity of Na^+/Mg^{2+} exchange mechanism, however, should not be neglected.

C) ELECTROPHYSIOLOGICAL EFFECTS OF INTRACELLULAR MG^{2+}. Intracellular $[Mg^{2+}]$ is needed to activate enzymes that phosphorylate or dephosphorylate channels (Na^+, Ca^{2+}, K^+, Cl^-, and f channels). On the other hand, as a cation interacting with the pore structure, it blocks a number of channels, and in the case of K^+ channels generates inward rectification.

An increase in $[Mg^{2+}]_i$ blocks outward current through the fast Na^+ channel. The block is slightly voltage dependent with an electrical distance of 0.18 from inside. The phenomenon has only a biophysical importance (66).

On the Ca^{2+} channel, $[Mg^{2+}]_i$ exerts two effects: it is necessary for the phosphorylation of the channel, but an increase to concentration levels reached during ischemia reduces the current. The effect is direct through block and indirect via a change in phosphorylation state (1090, 1132) or modulation by Mg-nucleotide complexes (743). Sensitivity for Mg^{2+} block is dependent on the state of phosphorylation and increases in the dephosphorylated state (1131). Conductance is not changed, but the number of functional channels and the P_o are reduced (1130). At very high concentrations of 9 mM, the current is completely blocked (see Ref. 5).

Outward currents through I_{K1} (652), I_{KACh}, and I_{KATP} (1127), and the delayed rectifier I_{Ks} (243) are reduced by $[Mg^{2+}]_i$. In the case of I_{K1}, I_{KACh}, and I_{KATP}, the block is an open channel block and voltage dependent (see sect. II*A3*). The I_{KNa} is inhibited by a shift to a lower substate conductance (1068). Inhibition of I_{Ks} is not due to a voltage-dependent block but to a fall in fully activated current that may be secondary to activation of phosphatase (243).

The Ca^{2+} SR release channel is blocked, and single-channel conductance is reduced (Fig. 13). The K^+ channel in the SR is also inhibited (810). In mitochondria, high $[Mg^{2+}]$ protects against Ca^{2+} overload, probably by blocking the transition pore (1189).

At the multicellular level, the effect of an increase in $[Mg^{2+}]$ is difficult to predict. Depending on the relative contribution of Ca^{2+} or K^+ currents, the action potential may be shortened or prolonged. The effect on the SR and mitochondria can be regarded as stabilizing and protective against irreversible changes.

D) SYNOPSIS. Free $[Mg^{2+}]$ in the cytoplasm and the mitochondrion has been estimated to be 0.5–0.8 mM or 10% of the total amount present. During ischemia, the free concentration may increase to 2–6 mM. This change is due to hydrolysis of ATP to which Mg^{2+} are bound, and partly to a deficient removal via a Mg^{2+}-ATPase and the Na^+/Mg^{2+} exchanger. The behavior of channels and carriers is changed by Mg^{2+} via effects on phosphorylation, by blocking the pore (Na^+ and Ca^{2+} channels), or by causing inward rectification in the case of K^+ channels. At the multicellular level, Mg^{2+} may be considered to exert a stabilizing effect.

B. Amphiphiles and Fatty Acids

1. *Accumulation of LCAC, lysophosphoglycerides, fatty acids, and arachidonic acid*

Under aerobic conditions, fatty acids (FA) are taken up from the extracellular medium or derived from hydrolysis of triacylglycerols and phospholipids and transformed to FA-CoA (acyl CoA). At the outer leaflet of the inner mitochondrion membrane, FA-CoA is transformed to FA-carnitine under the influence of the enzyme carnitine acyltranferase I (CAT-I) and then transferred to the matrix via the translocase. At the inner site of the membrane, the reverse reaction (CAT-II) results again in the formation of FA-CoA. Degradation occurs by sequential removal of two carbon units (β-oxidation) (1014). Under anaerobic conditions, the arrest of β-oxidation results in accumulation of FA-carnitine or LCAC and with some delay in the increase of fatty acids.

The concentration of FA rises because of enhanced breakdown of membrane phospholipids, while at the same time import in the cell continues. The result is a 4–7 times increase in FA during global ischemia in rats and even more up to 20 times during reperfusion (1014).

Metabolism of phospholipids is drastically disturbed by activation of a number of lipases. Arachidonic acid is released from phosphatidylcholine (PC) and PIP_2 secondarily to activation of a number of phospholipases (PL), such as PLA_2, PLC, PLD, and diacylglycerollipase. At what time this activation occurs during ischemia is still a matter of discussion (1013). Under the influence of lipoxygenase, AA is metabolized in stimulatory metabolites, such as leukotrienes (LT) while the cyclooxygenase pathway results in inhibitory metabolites, such as prostaglandins and thromboxanes (see Ref. 1014).

Concomitantly with AA, formed by PLA_2 activation, lysophosphoglyceride (LPG) and more specifically lysophosphatidylcholine (LPC) concentrations increase (see Refs. 674, 675). Under aerobic conditions, catabolism of LPC is so efficient that the concentration never rises substantially. In ischemia, catabolism is inhibited and production is stimulated. Enzymes responsible for the formation of LPC are activated through the rise in $[Ca^{2+}]_i$, acidosis, α_1-receptor stimulation, LT, radicals, and thrombin, whereas acidosis and LCAC inhibit the enzymes active in the catabolic pathway.

In the further analysis and description, distinction is made between amphiphiles (LCAC and LPG) and fatty acids (including AA). They exert quite different effects on channels and carriers, effects which may be related to their different electrical charge: amphiphiles act as zwitterions or cations at physiological pH, whereas fatty acids are negatively charged.

2. *Mechanisms of action and specific effects on channels and carriers*

A) AMPHIPHILES (LCAC AND LPG). *I) Mechanisms of action.* Amphiphiles are molecules with an hydrophilic, electrically charged part and an aliphatic, hydrophobic part. The most important amphiphiles in heart are LCAC and LPG, of which LPC is an important representative. At normal pH, LCAC act as cations, and LPG act as zwitterions.

Different mechanisms have been proposed to explain the effect of amphiphiles on channels and transporters.

1) Relatively low concentrations of LCAC or lysophospholipids may interact directly with the channel protein (582). Because of their charge, they can affect the permeation pathway or change the local concentrations of the substrate ion or other important ions. Such a mechanism is, for example, consistent with the observed reduction of K^+ channel conductance by positively charged LCAC.

2) By interfering with the charge of the phospholipids surrounding the channels, the gating characteristics, i.e., activation and inactivation of channels are shifted on the voltage axis (457). A reduction of negative charges by LCAC has been demonstrated in myocytes and red blood cells by measuring a decrease in electrophoretic mobility (343, 682). The effect has been compared with the action of an increase in $[Ca^{2+}]_o$ (886).

3) High concentrations affect membrane fluidity and/or disrupt the cytoskeleton. Mechanical destabilization is related to the conical form of the lyso-compounds compared with the cylindrical form of the diacylphospholipids (630). Incorporation of lysocompounds in the membrane has been measured experimentally (343) and results in a significant perturbation of the bilayer structure. An increase in membrane fluidity has been correlated with the apparent high density of α-receptors. The increased number of receptors is caused by an uncovering of receptors already present in the membrane (185) (see Ref. 268).

Mechanical destabilization and dysfunction of membrane proteins can also be the result of disruption of the cytoskeleton. Such destabilization has been shown to occur with lyso-compounds (1003).

II) Specific effects on channel and transporter function. The current through the fast Na^+ channels is sensibly affected in such a way that peak current is inhibited (845), but inactivation slowed (1111) and repetitive channel activity generated at rather negative E_m values, causing an important inward leak. This persistent Na^+ current does not deactivate on hyperpolarization and is characterized at the single-channel level by a bursting mode of openings (107, 108, 1002, 1003). It plays an important role as leak current. In this context, it is important to note that a nonselective cation current (I_{NSC}) is activated by lysocompounds (113, 631).

The L-type Ca^{2+} current has been reported to be reduced (161, 1110), or not changed (614).

Potassium currents are inhibited. Lysophophatidylcholine reduces the single-channel conductance of I_{K1} with no change in P_o, whereas L-palmitoylcarnitine has no effect on the single-channel conductance but reduces the P_o (161, 541, 542, 844). In both cases, current is markedly reduced. Also, I_{KATP} is inhibited by LCAC (528).

Lysophosphatidylcholine inhibits the Na^+/Ca^{2+} exchanger (74) and the Na^+-HCO_3^- symporter (1129), and LCAC block outward current through the Na^+ pump (886, 959).

Long-chain acylcarnitines specifically are concentrated in gap junctions during ischemia even during short intervals and cause a marked decrease in the single-channel P_o (1112, 1124). Long-chain acylcarnitines may thus be responsible for the change in conduction that occurs after 10 min of ischemia, concomitantly with the secondary rise in $[K^+]_o$. The rise in LCAC and the increase in longitudinal resistance are delayed by blockers of the CAT enzyme. Simultaneous increases in $[Ca^{2+}]_i$, which is also favored by LCAC, may exert an additive effect.

In the mitochondria, LCAC, in conjunction with Ca^{2+}, phosphate and oxidative stress, facilitate the activation of the mega-channel (771). Activation of the channel causes

complete uncoupling of oxidation from ATP synthesis and signals irreversible changes in ischemia.

B) FA AND AA: MECHANISM OF ACTION AND SPECIFIC EFFECTS. *I) Mechanism of action*. To a certain extent FA affect channel behavior via the same mechanisms as amphiphiles; more specific effects are related to AA metabolites. Fatty acids interact with channels in different ways. *1*) Fatty acids affect the concentration of permeating ions by adding negative charge to the channel protein, and more specifically cause an increase of cations (stimulation of cation channels) but a decrease in anions (inhibition of Cl^- channels). This hypothesis has received support by experiments on smooth muscle K^+ channel activation in which incorporation of lipophilic substances with negative or positive charge (763), respectively, caused activation or inhibition of K^+ channels. *2*) At high concentrations, FA exert a detergent action and increase membrane fluidity with consequent destabilization (1112). *3*) A third type of interaction is due to the metabolites of AA, such as LT, which interact with and activate G proteins and PKC (859, 1128).

II) Effects of FA and AA on channels and carriers. In contradistinction to amphiphiles, FA and AA activate outward currents and stimulate the Na^+/Ca^{2+} exchanger. Gap junction is, however, inhibited.

Polyunsaturated FA inhibit the Na^+ current, shift the inactivation curve to the left in neonatal rat cells (1117) and in the expressed α-subunit of the human channel (1118), and reduce the gating current (57).

L-type Ca^{2+} current is variably affected: an increase by FA and AA has been observed in guinea pig ventricular cells (441); on the contrary, inhibition occurs in the frog (762) by AA and in rat ventricular myocytes by polyunsaturated FA (1116).

Among the K^+ currents, I_{KACh} and I_{Kur} are stimulated, whereas I_{to} and I_{KATP} are inhibited. Activation of I_{KACh} in the absence of ACh or other agonists occurs upon application of AA. The effect is indirect after metabolism to leukotrienes. The LT_4 activate the G protein by stimulating GDP/GTP exchange (570, 859, 1128). Also, the stimulatory effect of AA on I_{Kur} is due to its metabolites (89).

An outwardly rectifying K^+ current (I_{KAA}) with large single-channel conductance (60–90 pS) is activated in rat ventricular myocytes by AA and other unsaturated FA (527, 528). The same channel is stimulated by stretch and acidosis and its P_o is slightly increased with depolarization (525, 1050) (Fig. 9). The channel is different from the KATP, which is inhibited by AA (527, 1050). The stimulation by stretch does not occur through activation of phospholipases and generation of FA (742), because addition of albumin which binds to FA does not eliminate the effect (525). Polyunsaturated FA and AA cause inhibition of I_{to} while increasing I_{Kur} (89).

The Na^+/Ca^{2+} exchanger is stimulated by negatively charged FA and especially unsaturated FA (766). This effect is secondary to an increase of the local $[Ca^{2+}]$ concentration at the intracellular side secondary to a translocation of phosphatidylserine (408, 585).

Gap junction conductance is inhibited by concentrations of AA and FA in the micromolar concentration (110, 289, 647). Fatty acids are highly concentrated in gap junctions during ischemia and probably destabilize the channels (1112). Among the FA the long-chain unsaturated and short-chain saturated FA are the most effective (110).

3. Electrophysiological effects of amphiphiles and fatty acids

On the basis of the effects on individual channels and carriers, predicted electrophysiological effects are different for amphiphiles and FA. Long-chain acylcarnitines and LPC generate preferentially depolarizing currents, whereas FA and AA and metabolites increase outward currents. The simultaneous occurrence of these apparently opposing effects is important, however, in the genesis of K^+ loss: opening of K^+ channels will facilitate K^+ loss but only when the E_m is kept away from the E_{rev} of K^+ by an inward leak.

Both LCAC and LPC, by favoring inward over outward current and inhibiting transporters, cause a fall in resting potential, reduction of upstroke velocity, initial prolongation of the action potential, appearance of DAD, and rhythmic activity (see Ref. 675). On the contrary, FA and AA do not cause prolongation but rather shortening of the action potential mainly by activation of K^+ outward current (528, 1050).

Amphiphiles as well as FA (1112) destabilize the gap junction, reduce its conductance, uncouple cells, and decrease conduction of the action potential. The combination of all these effects provides the conditions for the initiation and the maintenance of arrhythmias. Because of the role of LCAC and AA in the generation of arrhythmias, possible therapies are based on inhibition of their production by acting on the CAT-I and PLA_2 enzymes (183, 675).

4. Synopsis

During ischemia, amphiphiles (LCAC, LPG) and FA including AA and its metabolites accumulate in the plasma membrane, the gap junction, and intracellular membranes of the SR and the mitochondrion. Amphiphiles and FA may interact directly with channel proteins, with the phospholipids surrounding the channel proteins, or change the membrane fluidity. Amphiphiles increase inward current at the resting potential (fast Na^+ channel and I_{NSC}) with simultaneous reduction of outward current through K^+ channels (I_{K1} and I_{KATP}). Carriers such as the Na^+/Ca^{2+} exchanger and Na^+-K^+ pump are inhibited. On the contrary, FA activate outward currents, such as I_{KACh}, I_{Kur}, and I_{KAA} and stimulate the Na^+/Ca^{2+} exchanger. The simultaneous activation of inward and outward currents

favors K^+ loss and Ca^{2+} overload. Together with the induced cell uncoupling, conditions are created to generate arrhythmias.

C. Radicals

1. Generation of oxidative stress and basic mechanism of interaction

Oxidative stress results from the excessive generation of radicals, peroxides, and singlet oxygen on one hand, and from the deficiency of protective mechanisms (enzymes and scavengers) that normally eliminate these radicals on the other hand. Free radicals are molecules with one or more unpaired electrons; singlet oxygen is an oxygen molecule with an unpaired electron moved to a higher orbital. The unpaired electron alters the chemical reactivity of an atom or molecule, making it usually more reactive. The lifetime of radicals is in the order of 10^{-6} to 10^{-9} s.

Free radicals are a normal product of the metabolic chain and, in some instances, functionally very useful (e.g., NO). During aerobic metabolism, between 1 and 5% of total oxygen is reduced to superoxide (52). During aberrant metabolism (ischemia or reperfusion) however, when high-energy electrons leak out of the metabolic chain, radicals are formed to a higher degree (564, 736).

Superoxide radical is normally generated from O_2 during the oxidation of NADPH to $NADP^+$. In the presence of sufficient superoxide dismutase (SOD), the superoxide anion is directly transformed with H^+ to H_2O_2. Superoxide anion and H_2O_2 are also formed during the oxidation of xanthine by xanthine oxidase, auto-oxidation of catecholamines, and during arachidonic acid metabolism and other reactions such as those catalyzed by monoaminooxidase, tyrosine hydrolase, and L-aminooxidase. The danger arises when superoxide radical, NO radical, and H_2O_2 lead to the formation of the very reactive hydroxyl radical.

During ischemia, the formation of radicals is amplified. The excessive amount of radicals leads to changes in proteins (oxidation of sulhydryl groups), DNA and RNA molecules, but especially in membrane lipids. Lipids undergo peroxidation; polyunsaturated fatty acids more specifically are an easy target because of the easier removal of protons from the double bounds. The result is a lipid peroxyradical. These peroxyradicals oxidize proteins, cholesterol, and other FA especially polyunsaturated FA, thus propagating the reaction in a chain-type way (561). Two peroxyl radicals can collide and result in the generation of singlet oxygen, which can then directly oxidize polyunsaturated FA to hydroxyperoxides. Decomposition of lipid peroxides generates new radicals and noxious products, such as malondialdehyde; these latter reactions occur only late during ischemia (90 min of anoxia).

In many cases, evidence for the generation of radicals during ischemia or during reperfusion is indirect and based on findings in which agents that inhibit the production of radicals or speed up their elimination also reduce the expected electrophysiological changes. More recent experiments using electron-spin resonance, chemiluminescence, or HPLC with salicylate as the trapping agent have added direct evidence for the production of radicals at least during reperfusion. The intensity of radical production during the first minutes (2–5 min) of reperfusion depends on the duration of the preceding ischemia and is already evident after 15 min of ischemia (see Refs. 561, 564, 736).

A second important factor in the genesis of oxidative stress is deficiency in the protection. The cell is protected against abnormal generation or too long persistence of radicals by a number of enzymatic reactions and the presence of antioxidants or scavengers, but in ischemia and reperfusion, these substances are less available.

1) Among the enzymatic reactions that eliminate free radicals, SOD, catalase, glutathione oxidase, and peroxidase play an important role. Superoxide dismutase transforms the superoxide anion with protons to H_2O_2 and O_2, and catalase or glutathione peroxidase finishes the reaction with the formation of water from H_2O_2. Glutathione oxidase also eliminates lipid peroxides under simultaneous oxidation of two molecules of reduced glutathione. Mice lacking glutathione peroxidase are susceptible to reperfusion injury (1153), whereas mice with overexpression of catalase are more resistant to ischemia-reperfusion injury (603).

2) A number of scavengers or substances normally present in the cell neutralize radicals. Examples are vitamin E or α-tocopherol, vitamin C, glutathione, histidine and other amino acids, carotenoids, and flavonoids. These substances react with radicals and in the process become themselves radicals; the reactivity of these latter however is much less pronounced.

2. Effects of oxygen radicals on ion channels and transporters

Radicals attack proteins and lipids (80). Sulfhydryl groups of proteins are oxidized and disulfide bridges are formed, resulting in disturbances of the ion permeation or gating of ionic channels, decrease of transport capacity of carrier molecules, and activation of enzymes (957, 1070). Membrane lipids undergo peroxidation and change indirectly the behavior of channels.

Although Na^+ and Ca^{2+} currents are inhibited, the cells are sensitive to Ca^{2+} overload; this is the consequence of induction of leak channels, inhibition of the Na^+-K^+ pump and of the Na^+/Ca^{2+} exchanger, and block of K^+ current causing depolarization. In a later stage,

I_{KATP} is activated either by depletion of [ATP] or a direct effect of free radicals on the channel.

Information on the effect of free radicals on the Na^+ channel is scarce. In the frog ventricle, application of *tert*-butylhydroxyperoxide causes a gradual reduction in fast Na^+ current with a shift of the E_{rev} in the negative direction. Transiently, window current shows a dramatic increase (12-fold) due to an increased overlap of activation and inactivation curves (81). Such an increase in window current was not seen in feline ventricular myocytes (40). In rat ventricular cells, the most prominent effect of H_2O_2 is a slowing of inactivation of the TTX-sensitive current, an effect dependent on activation of PKC (1070). Oxidation of sulfhydryl groups in the pore-forming structures by agents like NO radicals inhibit the Na^+ current (151).

Inhibition of the I_{CaL} occurs in the guinea pig (138) and is accompanied by a fall in Ca^{2+} transient (330). In the ferret, the effect depends on the type of radicals; exposure to oxygen free radicals and *S*-nitrosothiols increases the current, whereas the opposite effect is obtained with the NO radical (119). The latter effect is probably due to activation of guanylate cyclase.

Under the influence of H_2O_2, a Ca^{2+}-permeable leak channel is induced in cultured rat ventricular myocytes (1056). The channel resembles the Ca^{2+}-permeable channel described by Coulombe et al. (187); it has a larger single-channel conductance than the channel described by Rosenberg et al. (806) but is also insensitive to DHP.

A nonselective cation channel is activated in guinea pig ventricular myocytes after extracellular or intracellular exposure of radicals (472). It has the characteristics of the $[Ca^{2+}]_i$-induced I_{NSC} with a reversal at -20 mV. Activation by oxygen radicals, however, is independent of changes in $[Ca^{2+}]_i$, since it persists in the presence of EGTA or ryanodine. Activation can be prevented by dithiothreitol and stimulated by oxidizing agents such as diamide and thimerosal. The data suggest activation by extracellular attack of the channel protein and oxidation of sulfhydryl groups (472). A similar conclusion was reached for the effect of singlet oxygen on the frog ventricle (965). Because radicals cause Ca^{2+} overload, activation of a I_{NSC} via $[Ca^{2+}]_i$ is also possible; in this case, the induction of a I_{NSC} by extracellular oxygen radicals is blocked by buffering intracellular Ca^{2+} (881). Because in ischemic conditions a rise in $[Ca^{2+}]_i$ occurs, the two inducing processes will cooperate.

Most of K^+ currents with the exception of I_{KATP} are inhibited by exposure to oxygen radicals.

The I_{to} current in rat atrial myocytes is decreased secondary to a shift in the inactivation curve to the left by exposure to 1 mM *tert*-butylhydroperoxide or after photoactivation of 100 nM Rose Bengal (768). A decrease also occurs by exposure to oxygen radicals for the delayed K^+ current (138, 966, 1007) and the I_{K1} current (471, 881). In the frog, the interesting observation was made that block of the delayed K^+ current is dependent on the state of the channel (966). The largest block was obtained when singlet oxygen was exposed to the channel in the rested state (activation gate closed); on the other hand, if the channel was activated before application of singlet oxygen, the blocking effect was less rapid and less pronounced (1007). In contrast to the well-documented decrease in delayed K^+ current by exposure to oxygen radicals, exposure to thimerosal, known to oxidize sulfhydryl groups into disulfide bridges (1144), increases I_{Ks} in canine ventricular myocytes. Bisulfide group formation between the NH_2-terminal ball and part of the channel protein, thus immobilizing the inactivation gate (816), is the mechanism proposed for the marked slowing of inactivation in expressed Kv.1.4 channels. The effect was reversible upon addition of reducing agents such as glutathione or dithiothreitol.

An outward K^+ current with the characterisitcs of the I_{KATP} appears after some delay upon exposure to oxidative stress (471). The delay may correspond to the time required to deplete the cell of [ATP] (980), but a direct effect on the channel with changes in the affinity for ATP is another possible explanation (450). Oxidation of sulfhydryl groups by thimerosal treatment indeed changes the ATP sensitivity of the channel and causes faster activation during ischemia (164). A decrease in sensitivity to ATP has been described after a period of ischemia, but the type of covalent chemical change is not known (213). Similar effects can be obtained by partial proteolysis in the presence of trypsin (266).

Direct measurement of the of the Na^+/Ca^{2+} exchange current in patch-clamp experiments with controlled Ca^{2+} and Na^+ concentrations showed a decrease under oxidative stress (163). In contrast, stimulatory effects have been described in flux studies in cardiac vesicles (799), in hearts subjected to hypoxia-reoxygenation (228), and in voltage-clamped guinea pig ventricular myocytes treated with H_2O_2 (329). In those cases, Ca^{2+} and Na^+ concentrations were not "clamped," and stimulation may be due to indirect concentration changes.

The Na^+-K^+ pump current is reduced (880). The inhibition correlates with a decrease in specific ouabain binding and enzyme activity (495, 533).

Oxidative stress activates the SR Ca^{2+} channel probably by disulfide bound formation (511, 879). Binding of ryanodine is decreased (428). The Ca^{2+}-ATPase of the SR is blocked in a sensitive way by oxidative stress, and the inhibition can be neutralized by dithiothreitol (254, 565).

The mitochondrial mega-channel is activated in severe metabolic challenge by oxygen radicals in combination with Ca^{2+}, phosphate overload, and ATP shortage (189, 244). As a consequence, the mitochondrial E_m breaks down, which is the sign of irreversible damage (Fig. 19).

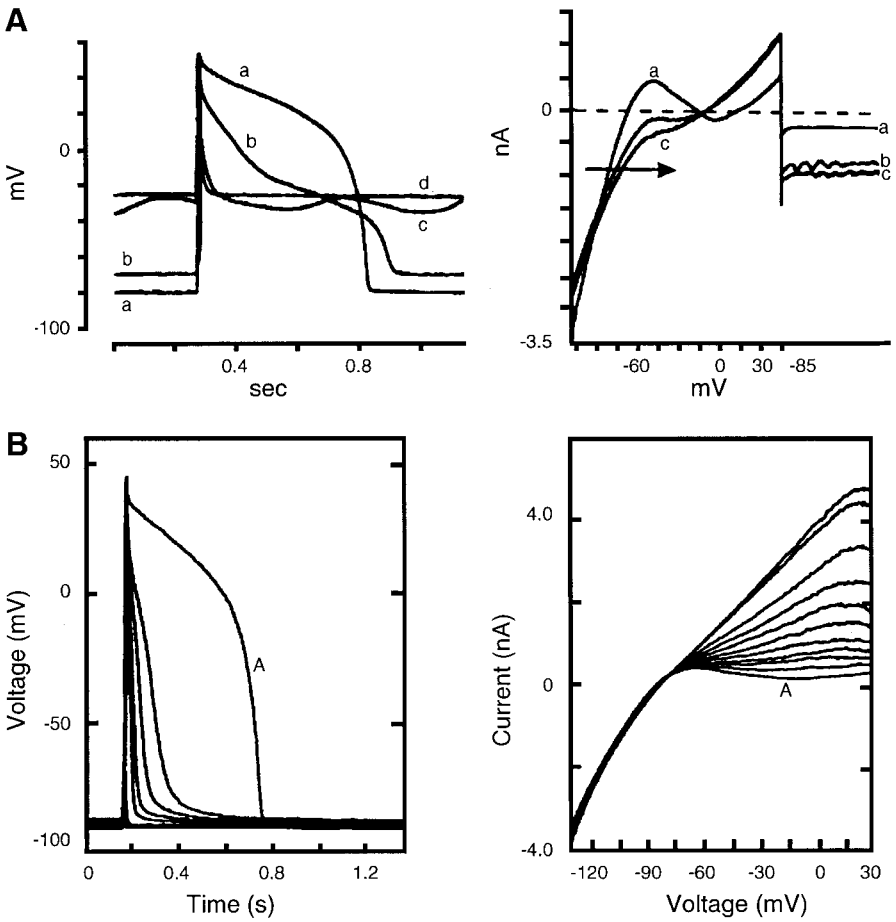


FIG. 20. *A*: early changes in electrical activity and ionic currents during exposure to free radical stress in a guinea pig ventricular myocyte. *Trace a* was obtained upon gaining cell access, and *traces b—d* were obtained after 9, 11, and 13 min of free radical exposure, respectively. Depolarization of diastolic membrane potential was accompanied by a loss of plateau, and membrane potential finally stabilized at −30 mV (*left*). Current tracings (*right*) were obtained from ramps that followed voltage recordings. Note positive shift in reversal potential, increase in outward current positive to −20 mV, negative shift in holding current, and transient oscillations after stepping back to holding potential. Changes can be explained by genesis of a nonselective cation current and a decrease in I_{K1}. [From Jabr and Cole (472). Copyright 1995 American Heart Association.] *B*: late changes in action potential duration (*left*) and current-voltage relation (*right*) during oxygen free radicals exposure in a guinea pig ventricular myocyte. Action potential shortened while an important outward current with outward rectification (I_{KATP}) developed. [From Jabr and Cole (471). Copyright 1993 American Heart Association.]

3. Electrophysiological changes caused by radicals

Two stages can be distinguished in the development of symptoms in cells exposed to oxygen radical-generating systems (Fig. 20). In a first stage, upstroke velocity and conduction of the action potential are reduced; the plateau is prolonged, and EAD may appear on the slow phase of repolarization (283, 391, 716, 746, 1070). Repolarization is followed by DAD (283, 391, 565). Eventually, the cell may depolarize and show continuous oscillations at the plateau level. In a second stage, extra systoles or spontaneous activity is still present, but the action potential gradually decreases in duration (68). When the action potential becomes very short (471), the diastolic potential shifts in the hyperpolarized direction (41), and the cell becomes inexcitable and goes into irreversible contracture.

An explanation for the electrophysiological changes can be given in terms of the changes in ionic currents described above. In the early stage, outward currents (I_{to}, I_{Ks}, I_{K1}, and the Na^+-K^+ pump current) are reduced, whereas inward currents (NSC, Ca^{2+}leak, and Na^+ current) are increased. These changes explain the depolarization at rest and the prolongation of the action potential with eventual EAD and oscillatory behavior during the plateau. The reduction of action potential amplitude and upstroke velocity are due to inhibition of the fast Na^+ current, partly as a direct effect, partly secundary to the depolarization. These changes result in a marked depression of the conduction of the action potential, an effect potentiated by a fall in gap junction conductance when the cell becomes Ca^{2+} overloaded. Calcium overload is due to facilitated Ca^{2+} influx, in addition to a reduced removal from the cell via the Na^+/Ca^{2+} exchanger and the Ca^{2+}-ATPase and reduced active uptake in the SR. Calcium overload also provides an explanation for the occurrence of DAD.

The shortening of the action potential in the second stage accompanied by hyperpolarization is probably due to activation of I_{KATP}, by a direct effect of oxygen radicals on the channel, and a decrease in ATP sensitivity or secondary to ATP depletion. Such depletion is accentuated when the mitochondrial mega-channel is activated.

Although proarrhythmic effects of oxygen radicals can be expected (881) from the occurrence of EAD and DAD and from the fall in upstroke velocity and gap junction conductance, their role in (reperfusion) arrhythmias has been answered with pros and cons. Arguments have been based on the use of antioxidants, but their effect seems to be species dependent. In the rat, an antioxygen

radical treatment mostly provides protection against arrhythmias (71, 1108; but see Ref. 353), whereas such protection is absent in the dog (260). Antioxygen radical treatment may provide a better protection against delayed rather than early ventricular arrhythmias (166). Effects furthermore may be due to changes at the level of the coronary vasculature, radicals being responsible for the no-reflow phenomenon upon reperfusion (6).

4. Synopsis

Oxidative stress during ischemia and especially upon reperfusion results from the excessive generation of radicals and the deficiency of protection by enzymes (SOD, catalase, and glutathione oxidase) and scavengers. Radicals attack proteins (sulfhydryl oxidation) and cause lipid peroxidation, resulting in increased leak current (I_{NSC}), block of most K^+ currents, activation of the SR Ca^{2+} release channel, and eventually the mitochondrial megachannel. Electrophysiologically, upstroke and conduction velocity of the action potential are reduced, and the plateau is prolonged with the appearance of EAD and eventual depolarization to the plateau level. In a second stage, the cell may repolarize, again showing very short action potentials (activation of I_{KATP}). In a final stage, the cell becomes inexcitable and goes into contracture.

D. Catecholamines

1. Release of catecholamines during ischemia

During ischemia, two distinct periods of catecholamine release occur. An immediate release in the systemic circulation occurs after stimulation of pain receptors and afferent nerve fibers in the ischemic zone (depolarization due to increased $[K^+]_o$ and shortage of O_2). At that time, the local release is negligible; during a 5-min ischemia (angioplasty) in humans, no local norepinephrine spillover occurs, despite evidence for a generalized sympathicoadrenal activation (721). This early stimulation is of short duration, and the local release is rapidly inhibited by α_2-receptor activation at the presynaptic level, inhibition of the exocytotic process by acidosis, and inexcitability of the nerve fibers due to the rise in $[K^+]_o$ (866). At ~10–15 min, a second "metabolic" release phase starts that is quantitatively much more important (100–1,000 times larger). The underlying mechanism of this phase is different from the first "exocytotic" phase and is accompanied by a reversal of the Na^+-dependent carrier that normally is responsible for the transmitter reuptake. Because of shortage of metabolic energy, the Na^+-K^+-ATPase is blocked, and $[Na^+]_i$ rises and causes the Na^+-dependent carrier to reverse. The increase in Na^+ is amplified by an acidosis-enhanced Na^+/H^+ exchange. Storage of the neurotransmitter in the vesicles furthermore falls because of inhibition of the H^+-ATPase. Evidence for reversal of the transporter has been provided by the use of desipramine, which blocks the carrier and markedly reduces the second release (865).

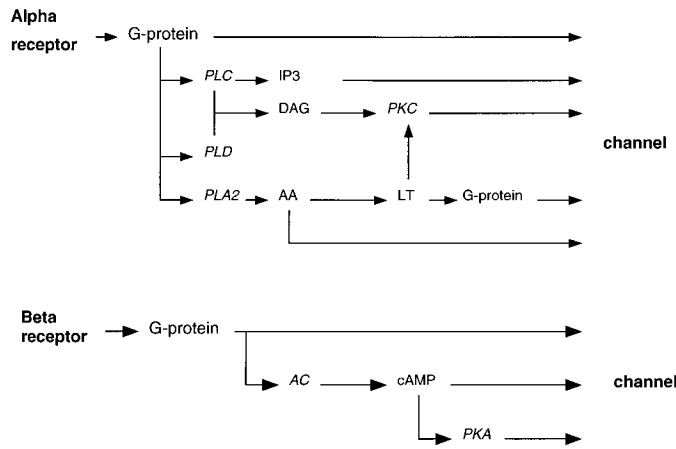
The effect of this excessive release of catecholamines is amplified by increases in α- and β-receptor densities during ischemia. The underlying mechanism for β-receptor upregulation is a translocation from an intracellular pool to the plasma membrane (632). For α-receptors (185), it is not a translocation but an uncovering process by the increased level of LCAC and consequent increase of membrane fluidity.

Although β-receptor density is upregulated during ischemia (632), β-receptor kinase, which inactivates the receptor, is stimulated at the same time (1004) with less cAMP as a result (1028). Initially during ischemia, the response may be increased, but later desensitization takes over.

2. Receptors and coupling to effectors

Catecholamines can bind to different types of receptors. In the heart, one distinguishes α- and β-receptors, which are further subdivided on a pharmacological basis in α_1- [α_{1A} (or $\alpha_{A/C}$), α_{1B} and others], α_2-, β_1-, β_2-, and β_3-receptors. About 80% of the α-receptors are of the α_{1B}-type (268). The density of α-receptors is variable with species. In the rat, the receptors are numerous, and the ratio of β- to α-receptors is 1.0. In primates and humans (937), α_1-receptors are less expressed, and the ratio of β- to α-receptors is 2.5. Similarly to α-receptors, the ratio of β_1- to β_2-receptors is species dependent. In the adult mammalian heart, this ratio is 0.8, whereas the reverse situation occurs in the frog (919).

The biochemical pathways involved in the modulation of ionic channels and transporters by α-receptor stimulation in heart tissue are multiple (268) (scheme 2).



SCHEME 2. Coupling of α- and β-receptors to channels.

This complexity may explain the various effects described in the literature.

α-Receptors are connected to effectors via G proteins. In most cases, the nature of the G protein is of the pertussis toxin-insensitive type, belonging to G_q and G_h type (336). In canine Purkinje fibers (23) and in hamster cardiac myocytes (873), pertussis toxin-sensitive pathways have also been described. Different phospholipases (PLC, PLD, and PLA_2) are activated via α-receptors (336). Phospholipase C activation, which is the most important mechanism, increases turnover of inositol phospholipids and leads to the formation of IP_3 (775) (or inositol 1,4-bisphosphate, Ref. 16) and DAG. Diacylglycerol in turn activates PKC, and more specifically the classical or Ca^{2+}-activated PKC and the novel or Ca^{2+}-independent PKC. Phospholipase A_2 stimulation causes an increase in lysophospholipids and in AA with subsequent formation of leukotrienes; leukotrienes in turn may again activate PKC. α_2-Receptors are negatively coupled to adenylate cyclase, and stimulation leads to a decrease of cAMP. In other instances, they are positively coupled to a Ca^{2+}/CaM-activated phosphodiesterase (PDE) with the same result, i.e., a decrease in cAMP (833).

For β_1-receptors, three types of coupling with their effectors have been described (scheme 2): a direct coupling via a G protein, a second type of coupling via cAMP without phosphorylation, and a third way via phosphorylation by PKA, and probably other kinases. For β_2-receptors, a coupling to adenylate cyclase has been demonstrated (919).

Coupling between β_1-receptor and effector has been best studied in the case of the I_{Ca}, the I_f, and the I_{Cl}.

Direct coupling of the β-receptor to the L-type Ca^{2+} channel via a stimulatory G protein has been demonstrated in excised patches and in channels incorporated in lipid bilayers (453) and is further suggested by the development of the effect in two phases with time constants of 150 ms and 35 s (see Ref. 1147). A direct coupling may not be universal, however, since a fast phase is not present in all species. In preparations of the frog, rat, and guinea pig, with only a slow phase, the whole effect can be blocked by PKA inhibitors (380). A direct coupling has also been proposed for stimulation of the I_f current in the SAN (1148), but its functional importance is not clear.

A second type of coupling between β-receptor and effector occurs via a direct effect of cAMP on the effector and has been demonstrated for the I_f current in the SAN (223).

The third type of coupling consists of PKA-dependent phosphorylation of the channel and has been clearly demonstrated for the I_{Ca}, I_f, and I_{Cl}. Other currents also are probably stimulated via this pathway: I_{st}, I_{Ks}, I_{Kur}, I_{KATP}, I_{gap}, and I_{CaSR}. The pathway involved in the inhibition of I_{K1}, I_{Kss}, and I_{to} is not known.

In the Ca^{2+} channel, two sites probably undergo phosphorylation: phosphorylation of *site 1* makes the Ca^{2+} channel available and phosphorylation of *site 2* causes the shift in the modal gating (720, 737). The existence of two phosphorylation sites is consistent with the observation that stimulatory but differential effects are obtained using specific phosphatase inhibitors (720, 1096): okadaic acid (acting on phosphatase 2A) promotes *mode 2* behavior, while calyculin A (acting on phosphatase 1) increases availability. The α-subunit may undergo phosphorylation (1152), but expression of the β-subunit seems required to observe the increase in current (549).

Phosphorylation of the I_f channel is of prime importance for modulation of the pacemaker current in ventricular and Purkinje cells. Experimental manipulations, using forskolin, PDE inhibitors, and permeable cAMP analogs (143), shift the activation curve in the positive direction while the opposite effect is seen when phosphorylation is inhibited using different PKA inhibitors. Inhibition of phosphatase by calyculin A shifts the activation curve in the positive direction with no change in the maximal conductance (1157, 1158). Whether PKA-dependent phosphorylation of the I_f is also occurring in the SAN remains controversial.

Activation of a Cl^- current by β-receptor stimulation occurs via a two-step phoshorylation of the regulatory domain of the CFTR protein. Phosphorylation allows binding and hydrolysis of ATP. The two steps correspond to two levels of activity, one characterized by short openings, the second by long-duration openings (34, 446, 448).

3. Effect of α-receptor stimulation

A) EFFECT OF α-RECEPTOR STIMULATION ON CHANNELS AND CARRIERS. Major effects of α-receptor stimulation occur on the K^+ currents (decrease and increase), gap junction (decrease), and exchangers (stimulation).

Stimulation of α_{1B}-receptors coexpressed with the human cardiac Na^+ channel in oocytes (704) causes a 40% reduction in current without changes in kinetics. The same effect is obtained with phorbol esters, suggesting the involvement of PKC stimulation.

For Ca^{2+} channels the results are variable: an increase (103, 402, 1179), no change (24, 402), and a decrease in L-type Ca^{2+} current (92, 379) have been described. The variable effects could be due to differences in washout via the pipette of intracellular components required for modulation of the channels (1179). Antagonism of the β-receptor stimulation was characterized at the single-channel level by a shortening of the open time and an increase in blank sweeps but no change in the single-channel conductance (147). The T-type channel activity is mostly reported to be increased (14, 993); such an effect may be important for Ca^{2+} loading of the cell, since the T-type channel is activated at rather negative E_m values. Stimulation of PKC leads to results similar to

α-receptor stimulation, i.e., variable effects on the L-type (233, 577) and stimulation of the T-type current (993). It is not known whether the T-type channel protein is phosphorylated. An indirect effect via alkalinization secondary to activation of the Na^+/H^+ exchanger by PKC can be excluded, since pH_i changes between 6.5 and 8.0 do not induce changes in the T-type current (1000). Facilitation by increased $[Ca^{2+}]_i$, secondary to Ca^{2+} release by IP_3, is a possibility that remains to be investigated. Arachidonic acid and leukotrienes that are produced during α-receptor stimulation enhance the Ca^{2+} current (441).

Among the K^+ currents, some (I_{to}, I_{Kur}, and I_{K1}) are decreased, and others (I_{Ks}, I_{KACh}, I_{KATP}, and I_{KAA}) are enhanced. α-Receptor stimulation substantially decreases I_{to} in rat (24, 256, 796, 977) and rabbit ventricular myocytes (270). A pertussis toxin-insensitive G protein plays the role of transduction factor, but the subsequent biochemical pathway is not known. Phorbol ester application and PKC activation yielded divergent results, and an increase as well as decrease have been described. In some cases, α-receptor stimulation decreased the current, whereas PKC activation had the opposite effect (270). In Kv.4.2 and Kv.4.3 expressed channels, PKC stimulation inhibited the current (713).

α-Receptor activation reduces I_{Kur} (human atrial cells, Ref. 605; rat ventricular myocytes, Ref. 796) and the inward rectifier I_{K1} (24, 256, 796, 843, 874). The addition of purified PKC to inside-out patches, containing I_{K1} channels, has the same effect (843).

The effect on I_{Ks} is species dependent. In the guinea pig, α-receptor stimulation activates PKC and causes enhancement of the current (979, 1051); the effect is additive to PKA activation (978). In the mouse (431) and rat (24), activation of PKC reduces I_{Ks}. It is interesting to note that the cloned minK protein, which can be regarded as a modulator or an essential part of the KvLQT1 channel, differs by only one amino acid in these species (404).

In oocytes, α_{1C} ($\alpha_{1A/C}$)-expression causes a rapid upregulation of the K_{min} current by elevating $[Ca^{2+}]_i$ (996). The I_{KACh} current is enhanced by α-receptor stimulation via activation of PLA_2, generation of AA and subsequently of LT. Leukotrienes activate directly the pertussis toxin-sensitive G protein connected to the KACh channel (570).

The I_{KATP} in human and rabbit ventricular myocytes is activated by norepinephrine via stimulation of PKC (439); the simultaneous increase of $[Ca^{2+}]_i$ facilitates the activation of PKC (1063). Excessive turnover of cAMP, a result of massive β-receptor stimulation, may furthermore deplete subsarcolemmal [ATP], which again enhances I_{KATP} (435, 858). Currents with characteristics similar to, but not identical to, I_{KATP} are activated by AA (527) or by LPC (1050).

Information on Cl^- channels is scanty. Although a Cl^- channel is activated by PKC (168, 1052, 1178), the role of α-receptors has not been verified. The swelling-induced Cl^- current is reduced by α-receptor stimulation (238, 241).

Phosphorylation by PKC of the gap junction causes a fall in single-channel conductance but an increase in P_o (690, 699); the overall effect of phenylephrine is a reduction of the gap junction conductance by 45% (209). Such a reduction may play a role in the genesis of reentry arrhythmias.

α-Receptor stimulation activates the Na^+-K^+ pump current (874, 1103). The α-receptor involved is α_{1B}, which is connected via a pertussis toxin-sensitive G protein (874) to PKC; sensitivity of the coupling is increased at elevated $[Ca^{2+}]_i$ (1059). In part, the stimulation of the pump may be secondary to an increase in $[Na^+]_i$ as a consequence of an enhanced Na^+/H^+ exchange.

Activity of the Na^+/Ca^{2+} exchanger is enhanced (939) probably after PKC stimulation. The increase in activity can occur in the absence of pH_i change (939), but the simultaneous activation of Na^+/H^+ exchange (470) will add to the final effect, since alkalinization as such is a strong stimulator of Na^+/Ca^{2+} exchange (230, 245).

B) ELECTROPHYSIOLOGICAL ASPECTS OF α-RECEPTOR STIMULATION. Because pump current is enhanced but I_{K1} is inhibited, the outcome of α-receptor stimulation on the resting potential is difficult to predict and will depend on the relative contribution of the two currents to the resting potential. A depolarization of the resting potential is seen in the rat atrium, whereas hyperpolarization occurs in the rat ventricle (256, 977). Inhibition of the I_{K1} has been invoked to explain the positive chronotropy in canine Purkinje fibers (874) and Langendorff-perfused guinea pig hearts (150).

α_1-Receptor stimulation prolongs the action potential in most preparations (see Ref. 268), the underlying mechanisms being block of I_{to}, I_{Kur}, and I_{K1}. Exception to this rule is the guinea pig, where no effect or even shortening of the action potential is observed (227). This effect is related to the fact that in this species I_{to} is not pronounced; in contrast, I_{Ks} current is well represented, and this current is increased after PKC activation.

Delayed afterdepolarizations can be enhanced or inhibited by α-receptor stimulation, depending on the mechanism by which they are generated. When elicited after exposure to high $[Ca^{2+}]_o$, α-receptor stimulation potentiates DAD (274). The potentiating effect could be due to reduction of the resting K^+ conductance (I_{K1}), to enhanced IP_3 production with release of intracellular Ca^{2+}, and to increase of I_{Cl} and $I_{Na,Ca}$. In this context, it can be mentioned that α-receptor stimulation favors reperfusion arrhythmias (757), an effect that has been related to Ca^{2+} overload. Once started by abnormal automaticity, the arrhythmia may be stabilized as a reentry arrhythmia by cell uncoupling as a consequence of a fall in gap junction conductance (209). When DAD are caused by strophanthidin-induced block of the Na^+-K^+ pump (274), α-recep-

tor stimulation inhibits DAD, an effect which can be explained by the stimulatory effect on the pump, reducing $[Ca^{2+}]_i$ overload. An inhibitory effect was also seen when DAD were elicited by β-receptor activation (833); in this case, a decrease in cAMP by activation of α_2-receptors is a possible explanation.

4. Effects of β-receptor stimulation

A) EFFECTS OF β-RECEPTOR STIMULATION ON CHANNELS AND CARRIERS. β-Receptor activation results in stimulation of a number of currents [I_{Ca}, I_f, I_{st}, I_{Ks}, I_{Kur}, I_{KATP}, I_{Cl}, I_{gap}, $I_{Ca(SR)}$], whereas only a few are reduced (I_{to}, I_{Kss}, and I_{K1}).

The effect of β-receptor stimulation on the Na^+ channel has been a controversial issue in cardiac electrophysiology. All published effects can be explained, however, when it is assumed that the basic mechanism consists of a shift of both activation and inactivation curves to the left, as shown experimentally (738). The shift explains why an increase in current is seen when both the holding potential and the test potential are rather negative, why no change is seen when the test potential is positive and holding potential negative, and why a decrease is obtained when the holding potential is less negative (see references in Ref. 738). In depolarized, but still excitable cells (early ischemia, border zone), the negative shift of the inactivation curve will cause further inhibition of the Na^+ current, with reduction in upstroke velocity and in conduction of the action potential. This inhibitory effect, however, may be counteracted by some hyperpolarization of the resting potential.

β-Receptor stimulation by epinephrine and norepinephrine increases peak I_{Ca} and slows inactivation (see Ref. 673); the slowing is clearly seen when Ba^{2+} is the charge carrier but is very variable with Ca^{2+} because of Ca^{2+}-induced inactivation. Activation and inactivation curves are shifted to the left. At the single-channel level, the conductance is not changed, but the P_o during a pulse as well as the number of functional channels are increased (decrease of blank sweeps). A shift from short to long openings is manifest and explains the slowing of inactivation (1160). Gating current is not changed, suggesting that coupling between gating and channel opening is improved.

β-Receptor stimulation has been reported to be ineffective on the T-type Ca^{2+} current (48, 355, 1001), but recently a facilitatory effect has been described in bullfrog atria (13). β-Receptor stimulation furthermore may indirectly increase the currrent via an increase in $[Ca^{2+}]_i$, which is known to facilitate the current (994). The increase in current by ouabain can be explained in a similar way (13).

In canine Purkinje fibers, the first component (time constant of 50 ms) of the transient outward current I_{to} is decreased by β-receptor stimulation, whereas the slowly inactivating component (partly I_{Kur}?) is substantially increased, such that the ratio of fast to slow component changes from 0.65 to 0.15 (717). The effect is duplicated by forskolin and cAMP. In contrast, in feline ventricular cells, forskolin or isoproterenol has no effect on I_{to} (268).

Analysis of the effect of β-receptor stimulation on I_{Kur} and I_{Kss} currents is incomplete. In canine Purkinje fibers, the steady-state current that follows the two phases of the I_{to} is increased (717); it is possible, however, that the records included I_{Cl}. An increase in I_{Kur} was observed in the human atrium (605) and in cultured neonatal rat ventricular myocytes (345). The I_{Kss} in rat ventricular myocytes on the other hand was very sensitively reduced (nanomolar concentrations) (849).

β-Receptor stimulation does not change I_{Kr} but upgrades the I_{Ks} current after phosphorylation of the channel by PKA (65, 384, 1051, 1149). Enhancement of the current may also occur in a phosphorylation-independent way (296). In the frog, the total conductance is dramatically increased, and the activation curve is shifted in the negative direction (243). The single-channel conductance is not changed, but P_o is increased (242).

The I_{K1} current is reduced, an effect opposite to M_2 stimulation (559).

Activation of I_{KATP} on the other hand is facilitated by β-receptor stimulation (435, 858). Subsarcolemmal depletion of [ATP] due to ATP consumption by a stimulated Na^+-K^+ pump and by enhanced cAMP synthesis (435, 858) has been proposed to be the underlying mechanism. The same mechanism may be responsible for activation of I_{KATP} upon simultaneous application of isoproterenol and a subthreshold concentration of a potassium channel opener; the effect does not require phosphorylation (995).

β-Receptor stimulation activates a Cl^- current in a number of ventricular preparations (see Ref. 308). The current is activated by a two-step phoshorylation of the regulatory domain of the CFTR protein (34, 446, 448). The results on the swelling-induced Cl^- current are divergent; forskolin increases (745), whereas cAMP inhibits the current (362).

β-Adrenergic stimulation increases the pacemaker current I_f by inducing a shift in the positive direction of the activation curve without changing the fully activated current (779) (Fig. 21) or the single-channel conductance (220, 222). Such an effect implies that the current activates at an earlier stage during the final repolarization of the action potential. Diastolic depolarization is faster, and frequency of spontaneous activity is increased. The effect on I_f is obtained at concentrations that also enhance I_{Ca} [K_d = 7 nM (1173) for I_{Ca} and 14 nM for I_f].

β-Receptor stimulation strongly enhances a sustained inward current in the SAN of the rabbit (346). The current carried by Na^+ is activated by depolarizations to −70 mV and more positive and shows minimal inactivation in the range of potentials corresponding to the dia-

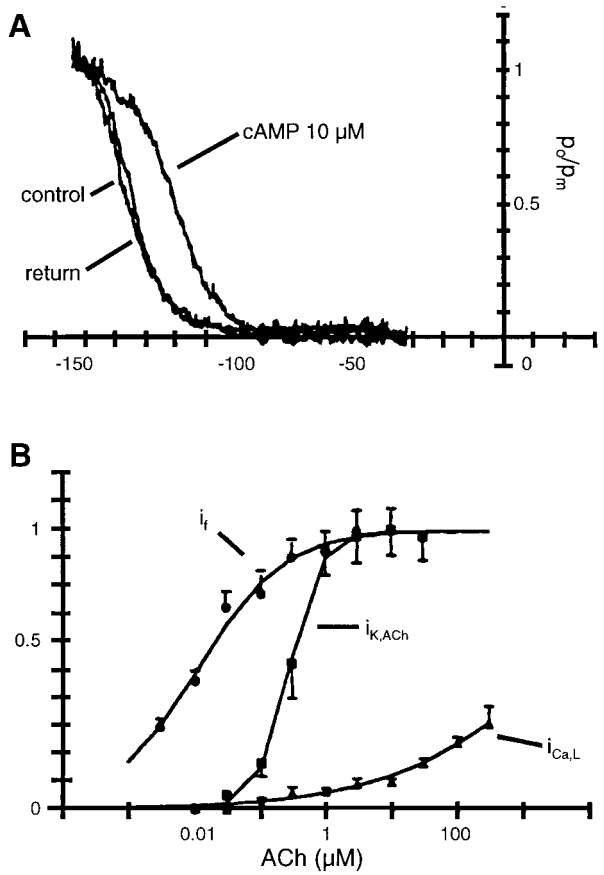


FIG. 21. *A*: action of cAMP on probability of channel opening in a macropatch of a rabbit sinoatrial node cell as measured by ramp method. cAMP at 10 μM shifts activation curve of I_f to the right by 14 mV. [From DiFrancesco and Mangoni (222).] *B*: dose-response curve for I_f, I_{KACh}, and I_{CaL}. I_f is most sensitive current to ACh. [From DiFrancesco et al. (221). Copyright 1989 American Association for the Advancement of Science.]

stolic depolarization. Its enhancement by β-receptor stimulation favors spontaneous activity in the sinus node.

Earlier experiments on multicellular preparations using radioactive K^+ uptake (706) are consistent with stimulation of the Na^+-K^+ pump after β-receptor activation. Direct measurements of the pump current have revealed variable effects. A stimulatory effect is present in sheep multicellular Purkinje preparations but not in the single cell, suggesting that the increase might have been indirect and due to extracellular $[K^+]_o$ accumulation (326). However, in single cells from the rabbit, $[Na^+]_i$ is reduced (211). Little or no response was seen in rat myocytes (464). In guinea pig ventricular myocytes, the effect requires PKA-dependent phosphorylation, does not depend on changes in $[Na^+]_i$ or $[K^+]_o$, but is highly dependent on $[Ca^{2+}]_i$ (314). Inhibition of the pump by isoproterenol occurs at low $[Ca^{2+}]_i$ and stimulation at high $[Ca^{2+}]_i$. Gao et al. (314) conclude that the direct effect of β-receptor stimulation by isoproterenol is inhibition (similarly to results of biochemical studies), but stimulation of the pump may occur as a result of the simultaneous increase in $[Ca^{2+}]_i$ to the micromolar range and the concomitant shift of the current-voltage relation of the pump in the negative direction. The stimulatory effect is especially pronounced at hyperpolarized potentials.

Phosphorylation of the SR Ca^{2+} channel by PKA shifts the P_o curve as a function of pCa or of [ATP] upward and to the left (see Ref. 359). Open times are increased and sensitivity to Mg^{2+} block is decreased (1006).

Coupling between cells is improved by cAMP (207, 823). Phosphorylation of the gap channel protein by PKA causes a fall in single-channel conductance (690), but gap junction conductance increases (208, 823), suggesting a rise in P_o at the channel level.

B) ELECTROPHYSIOLOGICAL EFFECTS OF β-RECEPTOR STIMULATION. β-Receptor stimulation increases spontaneous pacemaker frequency in the SAN secondary to an enhanced rate of diastolic depolarization and a shortening of the systolic period (101), improves conduction in the AVN, elevates the plateau level, and shortens the action potential duration in atrial and ventricular cells (506). Excessive stimulation may result in Ca^{2+} overload and genesis of triggered spontaneous activity.

Rate of diastolic depolarization in SAN cells is enhanced due to increase of L-type Ca^{2+} current, an increase of the sustained Na^+ inward current, and a positive shift of the activation curve of I_f. Elevation of the plateau level is explained by the increase in I_{CaL}. At the same time, the larger depolarization allows for greater activation of the I_{Ks} current, which is itself also increased by β-receptor stimulation. Rate of repolarization may further be enhanced by stimulation of the Na^+ pump and by activation of Cl^- channels, but the presence of the latter current in pacemaker cells has not been documented.

Similar changes in action potential occur in atrial and ventricular cells: the plateau is shifted in the positive direction and its duration is shortened (506). Shortening of the action potential is amplified when the cell is partially depleted of [ATP] during ischemia; β-receptor stimulation enhances local [ATP] depletion, via consumption by the Na^+-K^+ pump, the contractile system, and cAMP synthesis, resulting in activation of KATP.

β-Receptor stimulation enhances the tendency to spontaneous activity in atrial and ventricular cells. The mechanism is twofold: possible activation of I_f current and facilitation of triggered activity. The I_f current is present in Purkinje cells but also in plain atrial and ventricular cells. Because β-receptor stimulation shifts the activation curve in the positive direction, threshold may be reached at the resting potential. Triggered activity is favored by the Ca^{2+} overload that accompanies excessive β-receptor stimulation (782). Calcium influx is increased via the L-type Ca^{2+} channel and the amount of Ca^{2+}

stored in the SR is enhanced, by stimulation of the Ca^{2+}-ATPase, secondary to phospholamban phosphorylation (see Ref. 503). The high level of luminal Ca^{2+} in the SR may result in spontaneous release and genesis of DAD or EAD. The occurrence of EAD is especially favored by the increased I_{Ca}. In depolarized cells, e.g., in the presence of increased $[K^+]_o$ concentration, enhancement of the Ca^{2+} current may generate Ca^{2+}-dependent action potentials (132). These action potentials are slowly conducted and may play a role in reentry arrhythmias.

5. Synopsis

Obstruction of the coronary circulation is immediately accompanied by a release of catecholamines in the systemic circulation, followed later after 10 min by a local release caused by block of the reuptake mechanism in nerve endings. At the same time, the number of α- and β-receptors in the plasma membrane are increased. Both α- and β-receptors are coupled to their effector channels via complex pathways: the coupling may be direct via a G protein or indirect via a number of phospholipases (α-receptors) or adenylate cyclase (β-receptors). α-Receptor stimulation causes increase of some but decrease of other K^+ curents, decrease in gap junction conductance, and stimulation of the Na^+/Ca^{2+}, Na^+/K^+, and Na^+/H^+ exchangers. At the cellular level, the resting potential may depolarize or hyperpolarize; the action potential in most preparations is prolonged. Delayed afterdepolarizations are enhanced when they have been caused by increased inward Ca^{2+} leak; they may be inhibited when they have been induced by block of Na^+-K^+ pump inhibition. β-Receptor activation results in stimulation of most plasma membrane currents (inward and outward currents), gap junction channel, and SR Ca^{2+} release channel. Electrophysiologically, β-receptor activation increases pacemaker activity, improves nodal conduction, and elevates and shortens the plateau of the action potential. Excessive stimulation may result in Ca^{2+} overload and triggered activity.

E. Extracellular ATP, Adenosine, and ACh

1. Extracellular ATP

A) RELEASE OF ATP. Extracellular ATP is generated from two sources: *1*) sympathetic nerve endings secrete ATP upon electrical stimulation together with other neurotransmitters; and *2*) as a metabolite, ATP is released from cardiac cells exposed to hypoxia (203). A possible pathway for the release in the extracellular medium is the PKA-activated anion channel (871). During a limited time, [ATP] may reach the micromolar concentration range and induce multiple functional changes in cardiac cells (1027). In the extracellular space, it is rapidly degraded to adenosine by ectonucleotidases.

B) RECEPTORS AND COUPLING. The effects of ATP occur via stimulation of specific P_2 receptors. At least five subtypes of P_2 receptors can be distinguished, among which the P_{2x} and P_{2y} are the most important for cardiac cells. The P_{2x} receptor molecule should be mentioned separately because the molecule expressed in vas deferens is itself a NSC channel, permeable to Na^+, Ca^{2+}, and K^+. The structure resembles the inward-rectifying K^+ channel (1008). Whether the P_{2x} receptor forms a NSC channel in heart is not known. Other P_2 receptors (P_{2y}) are linked directly to ion channels via a G_s protein (I_{CaL}, Refs. 853, 1180; I_{Ks}, Ref. 668) or act indirectly via stimulation of adenylate cyclase (I_{KATP}) (31), activation of PLC and secondarily of PKC (I_{CaL}) (852), or possibly activation of tyrosine kinase (649).

C) EFFECTS OF $[ATP]_o$ ON CHANNELS. The effect of $[ATP]_o$ is usually of short duration because ATP is rapidly broken down in the extracellular medium.

Activation and inactivation of the Na^+ current are shifted in the negative direction, causing inhibition of the Na^+ current when the depolarization starts from a negative resting potential ($K_{0.5}$ is 0.7 μM for [ATP]) (855).

The effects on the L-type Ca^{2+} current vary with species and the experimental condition: stimulation as well as inhibition have been described. In the frog (12) and the rat (854, 1180), the L-type Ca^{2+} current is enhanced, activation and inactivation curves are shifted in the negative direction, while single-channel conductance is not affected. In the ferret (789), the guinea pig SAN (787), and the hamster (1045), only inhibition has been observed.

The $[ATP]_o$ sensibly increases T-type Ca^{2+} current in bullfrog atrial cells (12, 1026). The effect probably does not occur via PKC, since activation of this enzyme inhibits the T-type current (994), at least in the mammalian heart.

A K^+ current with strong resemblance to the KACh channel is activated upon exposure to $[ATP]_o$ in atria of the bullfrog (301), guinea pig (665), and bovine (302). In guinea pig atrial (668) and ventricular cells (649), a delayed K^+ current is also activated. In rat ventricular myocytes, activation of I_{KATP} is facilitated by application of $[ATP]_o$; the effect occurs via enhanced adenylate cyclase activity and subsarcolemmal [ATP] depletion (31).

An I_{Cl} with outward going rectification is activated in atrial cells of the guinea pig (664) and in ventricular cells of the mouse (597) and rat (500). The presence of P_{2y} receptors has been demonstrated in the rat, but the coupling to the Cl^- channels has not been elucidated.

A NSC channel is transiently activated by $[ATP]_o$ in atrial cells of the frog (301), atrial cells of the rabbit, atrial and ventricular cells from the guinea pig (411, 752), and ventricular cells of the rat (1180). The current carries bivalent as well as monovalent ions. Its E_{rev} is close to zero, and its current-voltage relation is linear or slightly inward rectifying (899). At the present time, it is not clear

whether activation occurs directly by binding to the P_{2x} channel (1008) or indirectly by activation of a Cl^-/HCO_3^- exchanger, intracellular acidification, and release of Ca^{2+} or after phosphorylation of extracellular proteins (see Ref. 1027).

D) ELECTROPHYSIOLOGICAL EFFECTS OF $[ATP]_o$. Changes in electrophysiological properties may be different in ischemic versus nonischemic conditions. In normal conditions, the overall initial effect of $[ATP]_o$ in most preparations is depolarization, due to activation of the I_{NSC} and the I_{Cl} with concomitant activation of I_{Na} and I_{Ca} (especially T-type I_{Ca}). These effects seem to overrule the smaller stimulation of K^+ currents. The depolarization may be conducive to the generation of spontaneous activity (1027). In ischemic conditions, with cells partly depleted of [ATP], stimulation of adenylate cyclase by $[ATP]_o$ may further deplete local subsarcolemmal [ATP] and activate I_{KATP} (31). This will lead to hyperpolarization and shortening of the action potential. The $[ATP]_o$ may thus cause opposite changes in healthy tissue and in ischemic cells. In vivo, the effect is of short duration, because ATP is rapidly broken down to adenosine.

2. Adenosine and ACh

A) RELEASE OF ADENOSINE AND ACETYLCHOLINE. Adenosine is generated either intracellularly or extracellularly. Extracellularly it is formed from ATP under the influence of ectonucleotidases. In the cell it is normally formed from AMP under the action of 5′-nucleotidase but again rapidly synthesized to AMP under the influence of adenosine kinase (203). Because ATP is consumed in this high turnover process, the cycle may be regarded as futile; there are, however, some advantages to it. When oxygen tension is reduced below a critical threshold, adenosine kinase becomes inhibited, and adenosine genesis is amplified and released from the cell, possibly via the PKA-activated anion channel (871). The high concentration reached permits rapid signaling, causing for instance vasodilation that counteracts the hypoxic stimulus.

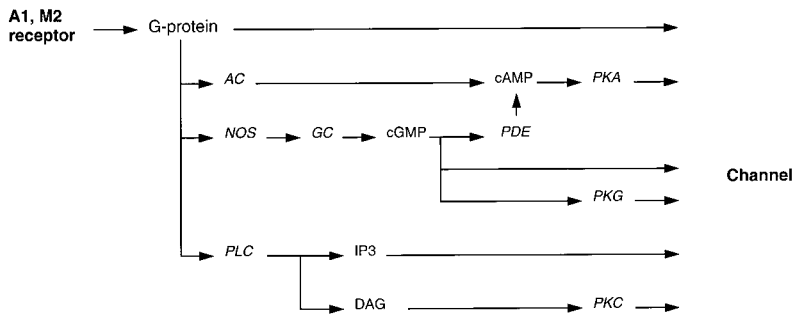
Acetylcholine is secreted from parasympathetic nerve endings. Its importance during ischemia is not well defined. Vagal afferent and subsequent efferent activity is increased upon coronary obstruction (797). In this way, SAN and AVN activity are changed.

B) RECEPTORS AND COUPLING. The effect of adenosine occurs via stimulation of P_1 purinergic receptors. In the group of P_1 receptors, four subtypes have been described, of which A_1 is the cardiac receptor. The effect of ACh occurs via stimulation of M_2 receptors (M_1 receptors are also present in the rat and may be responsible for the stimulatory effect at high concentrations (878; but see Ref. 658).

A_1 and M_2 receptors are linked to the effector in more than one way (scheme 3).

A first possibility is a direct connection between a G protein and the channel protein. Indirect pathways have been described for activation of phospholipases and subsequently of PKC, activation of NO synthase and phosphatase, or inhibition of adenylate cyclase.

A direct stimulatory pathway exists for the KACh (467) and KATP channels (469, 540, 969). In the case of the KATP channel, activation also occurs secondary to stimulation of PKC (608, 619, 1062). The pathway involved is probably activation of PLC with subsequent formation of IP_3 and DAG. A_1 receptors (790) and M_2 receptors (286, 401, 960) are negatively coupled to adenylate cyclase, resulting in a fall of cAMP concentration and reduction in PKA-mediated phosphorylation of the I_{Ca} and I_f channel. Indirect inhibitory effects via reduction in adenylate cyclase activity are of functional importance in the presence of β-receptor stimulation (accentuated antagonism) (286, 401). Withdrawal of ACh under those conditions causes transient increases in I_{Ca}. Inhibitory effects also occur via activation of NO synthase (NOS3) (645, 792, 895) and secondary increase in cGMP. Guanosine 3′,5′-cyclic monophosphate has been proposed to activate PDE II and reduce the cAMP concentration (681, 792) or activate PKG with phosphorylation and reduced activity of the effector (600, 680, 947, but see Ref. 365 for a facilitatory effect via PKG). In accord with the PKG hypothesis, NO donors have been shown to reduce I_{Ca} in the presence of



SCHEME 3. Coupling of A_1 and M_2 receptors to channels.

nonhydrolyzable cAMP, whereas the effect disappears with block of guanylate cyclase or of PKG (1048). Consistent with the proposal of PDE II activation, a fall in cAMP concentration has been demonstrated in the SAN, secondary to NO production upon cholinergic receptor activation; the effect was absent when NO synthase was inhibited (371). It should be added, however, that low concentrations of cGMP have been found to inhibit PDE III in mammalian cells and thus enhance the concentration of cAMP (740). Inhibition of PDE III has also been implied in the rebound stimulation of I_{Ca} upon withdrawal of ACh (1064). Guanosine 3′,5′-cyclic monophosphate may not only affect PDE or PKG but exert a direct activating effect, similar to cAMP, e.g., on the I_f current (223). In this context, it should be mentioned that exogenous NO can enhance the I_f current and increase the rate of beating (705); this effect probably occurs via a direct stimulatory effect of cGMP on the channel protein (219).

The G protein involved in the activation of NOS3 is not known. Stimulation of the synthase, however, may be secondary to IP_3 formation, which may cause an increase of free $[Ca^{2+}]$ and activation of the enzyme. Formation of IP_3 probably results from PLC stimulation (see Ref. 643).

C) EFFECTS OF ADENOSINE AND ACETYLCHOLINE ON CHANNELS. In general, the effects of adenosine and ACh are opposite to β-receptor stimulation, but exceptions to this rule exist.

A decrease in cAMP and reduction in PKA-mediated phosphorylation causes reduction in I_{Ca}, I_{Cl}, I_f, and gap conductance. The concentration of cAMP is reduced via inhibition of adenylate cyclase activity or by increased breakdown (NO pathway). Stimulation of the NO pathway can further result in PKG-induced phosphorylation of the channel (see above).

The I_{Ca} is reduced by a decrease in the number of functional channels and a fall in P_o (286, 401, 712, 790). Information of eventual changes in I_{Cl} is scarce, but a decrease of the PKA-activated current occurs in the presence of Na^+ substitutes (1072) and of tetramethylammonium compounds (1169) which stimulate M_2 receptors. Adenosine reduces Cl^- background current induced by isoproterenol (793).

Acetylcholine or muscarinic stimulation causes the activation curve of I_f to shift in the negative direction (891). This effect occurs at concentrations much smaller (K_d 20 nM) than necessary to open the KACh channel (0.5 μM, Ref. 221) or to inhibit I_{Ca} (1173) (Fig. 21). In the SAN, ACh has an effect on its own; in secondary pacemakers like the Purkinje fibers, it only reverses the effect of β-receptor stimulation (144). Adenosine exerts an effect similar to ACh via an analogous pathway (1174).

Gap junction conductance is decreased by carbachol or cGMP, an effect opposite to that of catecholamines or cAMP (572, 955).

Among the K^+ currents, I_{KACh} and I_{KATP} are activated, whereas I_{K1} and I_{Ks} are inhibited. Adenosine and ACh efficiently activate the I_{KATP} in ventricles (469, 531, 540, 969) and the I_{KACh} in the SAN, AVN, atria, and ventricles (55). For both types of currents, the link is direct via a G protein. The direct coupling is not universally present in mammalian ventricles but exists in the human (95, 558), rat (676), ferret (95), and dog (1142). In addition to a direct coupling, the increase in I_{KATP} also occurs indirectly via activation of PKC and is supposed to play a role in preconditioning (439, 608, 619, 1062). At first sight, stimulation of PKC however has two opposing effects. It inhibits maximum activity of KATP but at the same time reduces the slope or Hill coefficient from 2.2 to 1.0 (609). From the fall in slope, activation of the channel at millimolar [ATP] was predicted and later confirmed (610). The effect is antagonized by protein kinase inhibitors or phosphatases.

Inhibition of I_{K1} (559) and I_{Ks} (748) occurs when the currents have been upgraded by β-receptor stimulation and increase in cAMP (accentuated antagonism).

D) ELECTROPHYSIOLOGICAL EFFECTS OF ADENOSINE AND ACETYLCHOLINE. In contrast to ATP, the effect of adenosine and ACh results in hyperpolarization and shortening of the action potential, slowing of the rate of diastolic depolarization in pacemaker cells, and inhibition of EAD and DAD (55, 927). In general, adenosine and ACh are considered to act as cardioprotective and stabilizing agents. Mice overexpressing A_1 receptors show an increased resistance against ischemia evidenced by a longer time to development of contracture and improved functional recovery upon reperfusion (648). The electrophysiological changes are the result of K^+ current activation (KACh and or KATP) and inhibition of I_{Ca}, I_f, and I_{Cl}. In the SAN, the rate is slowed and pacemaker shifts may occur (53, 1086). In AVN cells, the action potential is inhibited resulting in conduction slowing or block. In the clinical context, this blocking effect is used to suppress paroxysmal supraventricular tachycardia (226).

As antiadrenergic agents adenosine and ACh suppress catecholamine-induced early and late afterdepolarizations (927), and reduce inward background current (792, 793). This effect may play an anti-arrhythmic role during ischemia and reperfusion. A protective effect of vagal stimulation on reperfusion arrhythmias has been demonstrated in cats (1190). Stimulation of M_2 receptors with oxotremorine markedly reduced the occurrence of ventricular fibrillation in a feline model subjected to acute ischemia and stimulation of the left ganglion stellate (1190). Animals selected for their elevated vagal tone were less susceptible to ventricular fibrillation upon acute ischemia (170).

Indirect inhibitory effects via adenylate cyclase are of functional importance in the presence of β-receptor stimulation (accentuated antagonism) (286, 401). Withdrawal of ACh under those conditions causes transient increases in I_{Ca}, lengthening of the action potential duration, and induction of DAD and spontaneous activity (438, 698, 1060).

In contrast to the stabilizing effects described above, high concentrations of ACh induce a TTX-insensitive Na^+ current in guinea pig ventricular myocytes (658, 896). The current reverses at −25 mV in normal Tyrode solution, suggesting selectivity for Na^+. M_2 receptor activation with consequent stimulation of PLC, IP_3 formation, and activation of PKC is a possible scheme (658). The result is an increase of $[Na^+]_i$ (822) and $[Ca^{2+}]_i$ (557) and an enhancement of Ca^{2+} transients and contraction (785).

3. Synopsis

Release of ATP during metabolic inhibition is increased. Because of its rapid metabolization in the extracellular space, its functional importance is limited. Information on the coupling between receptor and effector is incomplete. Adenosine 5′-triphosphate stimulates I_{NSC} and I_{Cl}, facilitates I_{CaT}, and activates some K^+ currents (I_{KACh}, I_{Ks}, and I_{KATP}). Prolongation as well as shortening of the action potential have been described. Shortening may become important during ischemia.

Adenosine release as well as vagal reflexes are enhanced during ischemia. A_1 receptors (adenosine) and M_2 receptors (ACh) are linked to their effectors directly via a G protein or indirectly via changes in the activity of PLC, NO synthase, and adenylate cyclase. In general, the effects are opposite to β-receptor stimulation. More specifically, I_{Ca}, I_{Cl}, I_f, and gap junction conductance are negatively affected, whereas activation occurs for I_{KACh} and I_{KATP}. Electrophysiologically adenosine and ACh cause hyperpolarization, shortening of the action potential, slowing of diastolic depolarization, and inhibition of EAD and DAD.

F. Stretch and Volume Changes

1. Existence of stretch

Stretch can take two different forms, depending on whether it is caused by tension applied to the cell or after an increase in cell volume due to intracellular hyperosmosis. Stretching of cells occurs in a passive way during the filling of the ventricles. Stretch will be pronounced in Purkinje or endocardial cells surviving an infarct zone. Cells of the ischemic zone will elongate during the active contraction of viable cells surrounding the infarcted zone. This type of stretch is characterized by an increase in the longitudinal dimension but a decrease of the transversal direction. Volume changes elicited by osmotic forces affect all dimensions of the cell. The effect may not be the same: in guinea pig ventricular myocytes, elongation activated a NSC channel while a hyposmotic challenge stimulated I_{Ks} (842). Because mechanical stability of the cells depends on the integrity of the cytoskeleton, changes in the microscopic architecture may determine the electrical changes. As a whole, our knowledge of stretch-related effects remains deficient.

2. Effect of stretch on channels and exchangers can be direct or indirect

Indirect changes on channels and exchangers are possible secondary to activation of membrane enzymes. It is known that stretch rapidly activates a plethora of second messenger pathways including tyrosine kinases, mitogen-activated protein kinases, PKC, PLC, PLD, and PLA_2 and causes release of substances such as ANG II, which act as auto- or paracrine factors (821).

Mechanical stretch or volume expansion enhances I_{CaL} (657), causes activation of NSC and Cl^- channels, and enhances outward currents through the K1, Ks, KACh, KATP, and KAA channels (821, 1089).

A stretch-activated NSC channel has been described in rat atrial cells (526), in cultured chick hearts (815), and guinea pig ventricular cells (313, 841). The channel is permeable to monovalent cations and Ca^{2+} (526). In atrial cells, activation is followed by an increase in $[Ca^{2+}]_i$, which may trigger atrial natriuretic factor secretion. In neonatal cells of the rat, another channel is activated and has a permeability ratio P_K/P_{Na} of 3.4 but is impermeable to Ca^{2+} (188).

A volume-activated Cl^- channel is present in atrial cells of the rabbit (238, 239, 357), guinea pig (841, 902, 1010, 1012), dog (929, 992), and human (745, 827) and in cultured cells of the chick embryo heart (1176). Activation is slow, and a phosphorylation by stretch-activated tyrosine kinase has been proposed as a possible mechanism (745).

Swelling activates the I_{K1} current in chick embryonic heart (1175) and in feline ventricular cells (334). The delayed K^+ current I_{Ks} is increased in hypotonic solutions in the guinea pig (734, 798, 842) and in chick embryonic heart cells (1175)); inhibition occurs in hypertonic solution (734). Opposite effects occur in the I_{Kr} (798). Pressure applied via the patch electrode enhances the ACh-activated K^+ channel (774), the AA-activated K^+ channel (525), and the KATP channel (1020, 1022). The Na^+/Ca^{2+} exchange current is reduced in hypo- and increased in hyperosmotic conditions (1109).

3. Electrophysiology and arrhythmias upon stretch

From the description of the changes in channel function, it is clear that inward as well as outward currents are activated. At negative potentials, the inward currents prevail, whereas at potentials more positive, outward currents are determining. Stretch or pressure applied to multicellular preparations or total heart chambers elicits depolarization of the resting potential while the action potential is shortened in its early repolarization phase, but lengthened in its final phase (202, 295, 375, 575, 1010, 1069). In atrial tissues in vivo, the effective refractory period is shortened and the vulnerability to arrhythmias increased (795). An explanation

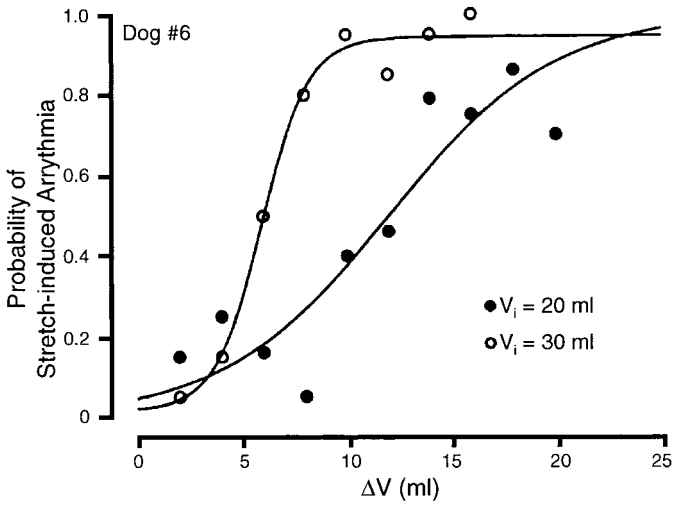


FIG. 22. Probability of stretch-induced arrhythmia by an acute increase in volume in isolated canine ventricle is higher with greater initial volume (V_i). Probability of arrhythmias is also greater larger with acute volume increase (ΔV). [From Hansen et al. (375). Copyright 1990 American Heart Association.]

can be found in the activation of the I_{NSC} and I_{Cl}, which carry outward current at positive E_m values and inward current at negative levels. Shortening of the action potential is facilitated by an increase in outward K^+ currents. The simultaneous opening of K^+ channels and increase of inward current during ischemia leads to increased K^+ loss.

Continuous as well as transient stretch are important (Fig. 22). Continuous distension increases dispersion of ventricular repolarization (1166) and induces ventricular fibrillation in isolated guinea pig hearts in ~20% of the hearts under normal conditions; in hypoxia, the incidence was slightly higher but of importance, the arrhythmia was irreversible on deflation (1181). In acute regional ischemia, the shortening of the action potential upon stretch is more pronounced but varies with time, the greatest effect being seen at 10 min (436). Threshold for extrasystoles is decreased, and susceptibility to arrhythmias in perfused hearts is increased at higher filling volumes (375, 1069). The effect of transient stretch depends on the rate of tension or pressure change, and extrasystoles are easier to elicit at faster rates of stretch (375).

4. Synopsis

Stretch can take two forms depending on whether it is caused by tension (unidimensional change) or by an increase in volume (multidimensional change) consequent to osmotic changes. Stretch causes activation of enzymes and stimulation or opening of stretch-sensitive channels. Inward currents (I_{NSC}, I_{Cl}, I_{CaL}) as well as outward K^+ currents are enhanced. At negative E_m values, inward currents prevail and cause depolarization; threshold for extrasystoles is decreased. At more positive levels, outward current is present; the plateau of the action potential is generally shortened with an increase in dispersion. These modifications make arrhythmias more probable.

IV. ELECTRICAL CHANGES AND ARRHYTHMIAS DURING ISCHEMIA AND UPON REPERFUSION

A. Electrophysiological Changes at the Cellular and Multicellular Levels

1. Description of electrophysiological changes: depolarization, shortening of the action potential, prolongation of the effective refractory period, decrease in conduction velocity, and change in excitability

Arrest of blood flow very quickly, within 2–3 min, results in depolarization from the normal resting potential of −85 mV to −60 mV. The initial fall in diastolic potential is accompanied by a slight increase in excitability. With further depolarization in parallel with the rise in $[K^+]_o$, the amplitude and maximum rate of depolarization during the upstroke of the action potential become reduced and excitability decreases. The upstroke, which normally shows a smooth time course, may now be subdivided into more than one component, and after 2 min, conduction gradually becomes depressed (178). Activation of the ventricles, which is normally complete in 80–100 ms, now requires 200–300 ms. Delayed activation is especially prominent in the subepicardium, whereas activation of the subendocardial layers is relatively unaffected. This may be related to a relatively elevated O_2 tension in the subendocardial layers, due to diffusion from the cavity (1102).

Initially, the duration of the action potential (APD) is slightly prolonged (546, 1039, 1075) but changes rapidly to a shortening. Typical for the ischemic condition is the long effective refractory period (ERP) that follows the shortened action potential. The APD and ERP thus change in opposite directions. Between 7 and 10 min the APD and the ERP may alternate showing quasi-normal and very short durations. Complete unresponsiveness follows very soon. After 15–20 min however, cells in the middle of the infarction may regain temporarily their excitability, although the upstroke and amplitude remain depressed. These changes are correlated with a massive release of catecholamines (865), an opening of the collateral circulation, and a small decrease in $[K^+]_o$. After ~30 min, the cells further depolarize, and conduction is completely blocked (178).

All these changes do not occur uniformly over the infarcted myocardium, and heterogeneity is especially pronounced at the border zone. The border zone is the

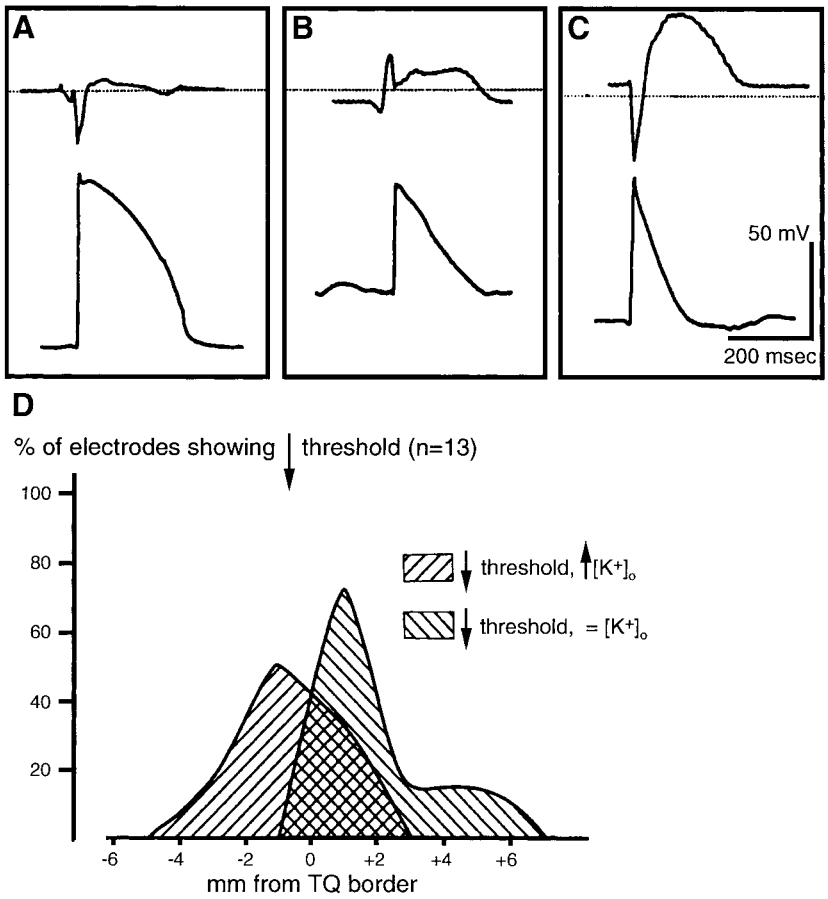


FIG. 23. *A*—*C*: electrocardiograms and action potential recordings in a pig heart at start of an ischemic period (*A*), after 12 min of ischemia (*B*), and upon reperfusion (*C*). Zero potential for electrograms is indicated by dotted line. Note decrease in action potential duration upon reperfusion, associated with a peaked T wave and shift of TQ level in positive direction. These changes are accompanied by a fall of $[K^+]_o$ below control. [Adapted from Coronel et al. (181).] *D*: border zone in acute cardiac ischemia. Spatial distribution of electrical threshold and $[K^+]_o$ over border zone of an ischemic zone (negative to TQ border). Threshold is decreased in a substantial number of sites (electrodes). This increase in excitability may occur with or without concomitant change in $[K^+]_o$. [Adapted from Coronel et al. (182).]

zone that limits the infarct in the plane of the ventricular wall but also in the transmural direction. The existence of marked differences is not unexpected; dispersion exists already under normal conditions and gradients for action potential duration occur from epi- to endocardium (21), between apex, free wall, and septum (332), and between right and left ventricle (1044). The dissociation between APD and ERP is less present or absent in the borderzone of the infarct; in this region both shorten. On both sides of the border, excitability can be increased (Fig. 23*D*). Threshold is decreased at the ischemic side because of a rise in $[K^+]_o$ and at the nonischemic side because of the depolarization caused by injury current (178, 182). A reduction of 20% in threshold has been measured in a 1- to 2-mm width adjacent to the ischemic border (255). At the endocardial border zone, injury current may affect the Purkinje system in such a way that masked spontaneous activity now becomes apparent. A maximum of injury current is obtained when activation of the ischemic zone occurs at the moment the normal zone already repolarizes (182).

In the electrocardiogram recorded with a direct-current amplifier and for an electrode facing the ischemic zone, the fall in resting potential during the first minutes is reflected in an initial depression of the TQ segment (485) (Fig. 23, *A* and *B*). In the classic electrocardiogram, this is seen as a ST elevation. The intrinsic deflection is delayed and the QRS broadened. After ~5 min, the ST segment becomes further elevated because of the shorter action potential in the ischemic part of the heart (477). Later, when activation in the ischemic region is seriously delayed, the ST segment becomes markedly elevated and is followed by a pronounced inverted T wave. When cells in the central zone of an infarct become inexcitable, the electrocardiogram shows monophasic derivations. After 8–15 min, local activation transiently recovers and is often accompanied by T-wave alternans (337).

When reperfusion occurs before 30 min of ischemia, the cells quickly hyperpolarize, whereas the action potential becomes temporarily very short (Fig. 23*C*). This shortening occurs despite the marked hyopotassemia. Because this recovery is not homogeneous, marked heterogeneity may exist in APD and ERP (181). Excitability is depressed, but spontaneous activity may occur, probably as a consequence of DAD. In preparations recovering from simulated ischemia, anoxia, or metabolic inhibition, action potentials are frequently accompanied by DAD, and when later the action potential lengthens, also by EAD (60, 62, 176).

2. Mechanisms underlying the electrophysiological changes during ischemia and reperfusion

A) MECHANISMS UNDERLYING THE CHANGES IN RESTING AND ACTION POTENTIAL DURING ISCHEMIA. Based on the results described in section III, the following picture can be proposed.

The major cause of the large depolarization during ischemia is an increase in $[K^+]_o$ and the simultaneous existence of an inward current (for references, see sect. III*A1*) (scheme 1). The mechanisms responsible for the rise in $[K^+]_o$ are increased K^+ efflux, decreased K^+ influx, and to a certain extent shrinkage of the extracellular space. Inward current as such is responsible for keeping the E_m positive to the K^+ equilibrium potential. It represents the driving force for outward K^+ movement. The possible mechanisms involved in the inward leak are multiple and involve Na^+, Ca^{2+}, and Cl^- channels and exchangers (see sect. III*A1*).

The decrease in upstroke velocity and amplitude of the action potential is caused by a fall in Na^+ influx and the simultaneous increase in outward currents causing a short-circuiting effect. Sodium conductance is decreased secondary to inactivation of Na^+ channels by the fall in resting potential and by the action on the Na^+ channel protein of amphiphiles (LPC, LCAC), FA, oxidative stress, and acidosis (1076, 1177). Inward Na^+ current is further decreased by a fall in the chemical gradient mainly due to an increase in $[Na^+]_i$, caused by an increased passive influx at the resting potential and a decreased active efflux (232). The efficacy of the fast I_{Na} in generating the upstroke of the action potential is further reduced by the short-circuiting effect of the outward current, such as I_{KATP}, I_{KNa}, and I_{KAA}.

Changes in APD consist of an initial lengthening followed by a marked shortening. The transient lengthening of the action potential during the first initial seconds of ischemia can be partly due to a drop in local temperature (>1°C) (802). Other explanations include a reduction in electrogenic pump current, a fall in I_{K1} as a consequence of intracellular acidosis, and an acute inhibition of I_{to} (1039). An initial lengthening also occurs upon exposure to dinitrophenol (122) or upon pump inhibition by ouabain (624). The shortening of the action potential that follows very rapidly and is most prominent is caused by an increase in outward current at plateau potentials; a decrease in inward currents is of minor importance. The augmented outward current is carried by K^+ and Cl^-, of which K^+ is the most important. The K^+ currents involved are not the K^+ currents normally activated under physiological conditions but specific K^+ currents that become activated under ischemic conditions: I_{KATP} (review in Ref. 967), I_{KNa} (497, 625), and I_{KAA} (1050). They carry large time-independent currents and show outward-going rectification in the presence of normal to moderately elevated $[K^+]_o$. Their effect will thus be most marked at depolarized levels, i.e., on the plateau of the action potential. The K^+ currents, activated under physiological conditions, are rather inhibited by ischemic conditions. The voltage-activated, 4-AP-sensitive I_{to} is inhibited during hypoxia (300, 1039), metabolic blockade (768), α_1-receptor stimulation (see Ref. 268), and exposure to oxygen stress (41). The inward rectifier conductance is decreased by LPC (161) and intracellular acidosis (468, 819) but enhanced by the rise in $[K^+]_o$. Simultaneous measurement of $[K^+]_o$ and APD have shown that the shortening was less than anticipated from the rise in $[K^+]_o$ (1075).

A second current source that causes shortening of the action potential is Cl^- channel activation by β-receptor stimulation, osmotic swelling, mechanical stretch, addition of external ATP, and increase of $[Ca^{2+}]_i$. These channels have been mentioned as responsible for an increase in inward leak at the resting potential. Because E_{Cl} is around −30 mV, activation of Cl^- channels will carry outward currents at potential positive to −30 mV and promote repolarization from the peak of the action potential to the plateau.

Changes in inward current are of less importance in causing shortening of the action potential. Whereas most of the fast inward Na^+ current is inactivated by the fall in resting potential and by the acidosis, other factors such as LCAC, LPC, and oxygen radicals induce bursting and persistent activity. The effect is an increase in leak, but the overall effect on APD is difficult to evaluate. The same situation seems to exist for the I_{Ca}. Lactate and protons reduce the current (88, 1000); free FA have the opposite effect (441) via a direct action and secondary to the generation of leukotrienes. The channel may also be stimulated by the release of extracellular ATP (P_2 receptors) (855), but the results are equivocal. Changes in I_{Ca} thus do not seem to play a major role in the shortening of the action potential.

The shortening of the action potential in the border zone is mainly due to "injury current," or short-circuit current, generated by the difference in E_m between ischemic and normoxic myocardium and much less to changes in ionic conductance (507). The injury current that slightly decreases the resting E_m reverses direction during activity and causes enhanced rate of repolarization.

B) MECHANISMS UNDERLYING THE CHANGES IN EXCITABILITY AND CONDUCTION DURING ISCHEMIA. During the first minutes of ischemia, excitability in the central zone of ischemia is increased; threshold potential remains unchanged, but less current is needed to reach it because of the membrane depolarization (231). Later, excitablity gradually decreases mainly because of inactivation of the Na^+ channel by the depolarization. Reduction of excitability is more pronounced in the subepicardial than subendocardial fibers (322). Some cells regain excitability after 15 min of ischemia; this recovery may be caused by some hyperpo-

larization, consequent on washout of extracellular K^+ via collateral circulation and/or stimulation of the Na^+-K^+ pump by the massive release of catecholamines (866) and the rise in intracellular Ca^{2+} (314). Catecholamines in depolarized cells facilitate the occurrence of Ca^{2+}-mediated action potentials by increasing the Ca^{2+} conductance (132). Collateral circulation cannot be the only reason for recovery, since embolization that blocks collateral circulation did not impede the transient recovery (156). Others have related the recovery of excitability to an increase in longitudinal resistance. Experimental measurements (805) as well as theoretical calculations (883) have shown that, although conduction is slowed, the safety factor for generating a conducted action potential can paradoxically increase when the fall in conduction is caused by a reduced gap junction coupling in contrast to the opposite effect when conduction is slowed by a fall in excitability. After 30 min, a new phase of inexcitability starts and is due to a secondary increase in K^+ loss from the cells, concomitant with a fall of [ATP] to very low levels.

In contrast to the effect on APD, ERP is prolonged in the ischemic zone. The prolongation of ERP is caused by slowing of recovery from inactivation of Na^+ channels secondary to the depolarization, block of Na^+ channels by the acidosis (1076), LCAC, LPC (107, 1002), and oxygen radicals (81), and the short-circuiting effect of the opening of K^+ and Cl^- channels. An increase in membrane resting conductance shortens the space constant and hinders propagation of the action potential.

In the border zone, both the ERP and APD are shortened. The effect is due to the existence of injury current. Although the Na^+ channels might be slightly inhibited by a small depolarization, the increase in resting conductance is absent. The total duration of the ERP is short, and recovery after the repolarization is fast (182). A marked dispersion in ERP thus exists over the border zone, a condition which favors the occurrence of reentry arrhythmias. Sympathetic stimulation increases this type of dispersion (741).

Conduction velocity is determined by active properties of the cell (amplitude and upstroke velocity of the action potential) and passive cable properties of the muscle bundle, more specifically, the resistance of the cell membrane, the longitudinal resistance of the cell, the resistance of the extracellular compartment, and, on a larger scale, the tissue architecture (761). In normal conditions, conduction is uniformly anisotropic, i.e., more rapid in the longitudinal than in the transverse direction. All these parameters change early during ischemia and affect conduction velocity in a negative way. At this stage, the decrease in amplitude and in rate of rise of the action potential are most important together with the increased conductance of the cell membrane at rest and the rise of extracellular resistance secondary to blood vessel collapse and cell swelling (545). At a later stage (>15 min), longitudinal cell resistance increases dramatically and results in a marked fall of conduction. This change is due to closing of the gap junctions, following the joint rise in $[H^+]_i$ and $[Ca^{2+}]_i$ (109, 732, 1091), the effect of LCAC (1112, 1124), and fall in [ATP]. The existence of transjunctional potentials may cause additional inactivation of the gap junction conductance (1053). Modifications in gap junctions are not uniform over the whole infarct and thus represent an important factor in the genesis of arrhythmias. Even after isolation of cell pairs from ischemic hearts, this heterogeneity remains (518).

C) MECHANISMS UNDERLYING THE ELECTROPHYSIOLOGICAL CHANGES DURING REPERFUSION. Washout of external K^+ restores quickly the resting potential to normal values. There may even be a transient pronounced hyperpolarization accompanied by a reduction of $[K^+]_o$ below the control value, but the action potential stays very short (181). Both characteristics may be explained by the early and extra stimulation of the Na^+-K^+ pump (1017) secondary to the Na^+ load, producing an outward current and a fall in $[K^+]_o$. This leads to a paradoxical situation where a short action potential is combined with a low $[K^+]_o$. It illustrates nicely that $[Na^+]_i$ is more important than $[K^+]_o$ in determining pump activity. In some cases, removal of the coronary obstruction does not result in an efficient perfusion. The cause is oxidative stress leading to secondary vasoconstriction and the so-called no-reflow phenomenon (6) while a direct effect on myocardial cells persists. Recovery then becomes substantially retarded.

Calcium overload that starts during ischemia may be aggravated during the initial minutes of reperfusion, because of extra Na^+ influx (Na^+/H^+ exchanger) and oxidative stress (578, 905). The hyperpolarization by the activated Na^+-K^+ pump on the other hand is accompanied by an intense Na^+/Ca^{2+} exchange inward current, limiting the Ca^{2+} overload. Calcium oscillations have been measured during the recovery; such oscillations can be translated in EAD, DAD, and arrhythmias. Blockade of the Na^+/H^+ exchanger improves recovery and is antiarrhythmic (28, 29, 1123).

3. Synopsis

Arrest of the coronary circulation causes depolarization of the resting E_m, shortening of the APD, but prolongation of the ERP. Excitability may temporarily be increased but falls after 2–3 min. Changes are nonuniform. At the electrocardiographic level, TQ and ST segments are shifted, and T waves are inverted. The increase in $[K^+]_o$ and the existence of inward current are responsible for the depolarization. The shortening of the action potential is caused by increased outward current through K^+ and Cl^- channels. In the border zone, the shortening is induced by the occurrence of an injury current. Prolongation of the ERP is due to slowed

recovery from inactivation of the Na^+ channels. Increased excitability followed by decrease is related to the extent of depolarization. Partial recovery of excitability has been explained by the release of catecholamines, but a fall in gap junction conductance can also be responsible for an increase in safety factor of conduction. The conduction disturbance is due to a fall in Na^+ conductance and later to an increase in longitudinal resistance. The short APD during reperfusion despite the low $[K^+]_o$ is explained by an excessive stimulation of the Na^+ pump current. Action potential durations show an exaggerated dispersion, and Ca^{2+} overload may become pronounced.

B. Arrhythmias

1. Basic mechanisms of arrhythmias

Before I describe the types of arrhythmia occurring during acute ischemia and reperfusion and analyze their specific mechanisms, it seems appropriate to discuss first the general processes involved in the genesis of arrhythmias. The basic processes include automaticity inside and outside the SAN, triggered activity, and reentry.

A) AUTOMATICITY IN THE SAN. Normal spontaneous activity in the SAN is the result of the interplay between a number of time-dependent currents [I_{Kr}, I_f, I_{CaL}, I_{CaT}, I_{Na} (42)] and time-independent currents [I_{KACh}, I_{KATP} (370), $I_{Na,K}$ pump (828), background I_{Na}, I_{st} (346).] Early diastolic depolarization is mainly determined by activation of the I_f and deactivation of I_K. Deactivation of I_K during the initial phase of diastolic depolarization has been recorded at the whole cell (466, 739) and single-channel levels (1032). Later during diastole, I_{Ca} through the T-type and L-type channels takes over. More to the periphery of the node I_{Na} instead of I_{Ca} plays a major role (553).

Spontaneous activity is modulated by stimulation of adrenergic and muscarinic receptors and by metabolic inhibition. β-Receptor stimulation increases the frequency of beating by shifting the activation curve of I_f in the positive direction (101), increasing the I_{CaL} (101) and the I_{st} current (346), which all directly enhance the rate of depolarization. The increase in I_{Ca} current moves the plateau in the depolarized direction resulting in increased activation of the I_{Kr} and I_{Ks}. The I_{Ks} is furthermore enhanced by β-receptor-dependent phosphorylation (384, 1051, 1149). Combined, these changes result in shortening of the action potential or the systolic period and thus an increase in the frequency.

Muscarinic receptor stimulation has opposite effects on beat frequency: a specific I_{KACh} is activated and most of the currents modulated by β-receptors are changed in the opposite direction. The relative role of a decrease in I_f and increase in I_{KACh} is still a matter of debate (96, 221). The I_f current is more sensitive to ACh than the I_{KACh}. In vitro, the KACh channel is only activated at high concentrations of ACh (221). That the KACh channel plays a role, however, is suggested by the observation that a partial block of the current by Ba^{2+} also reduces the chronotropic effect of ACh on the SAN (96).

Metabolic inhibition antagonizes pacemaker activity by activating I_{KATP}. This current is present in the SAN, although much less than in ventricular cells. However, when the high input resistance of the SAN is taken into account, activation of only a small number of channels may have dramatic effects on the rate of diastolic depolarization and the APD (370).

Pacemaker activity in the SAN can be disturbed and show abnormally slow or excessively elevated rates of depolarization. In ischemia, changes in heart rate often precede the start of arrhythmias. Such changes are mostly due to neurogenic (parasympathetic and sympathetic nervous system) or hormonal stimulation, but a direct modulation of ionic currents by metabolic inhibition is also possible.

B) AUTOMATICITY OUTSIDE THE SAN. Spontaneous depolarization outside the SAN can be caused by activation of I_f, slow release of Ca^{2+} from the SR, activation of the Na^+/Ca^{2+} exchange current, and activation of stretch-sensitive currents, injury, or short-circuit currrents.

Pacemaker activity based on I_f activation occurs in the AVN (555) and Purkinje fibers (115). The I_f is also present in atrial (124, 246, 1184) as well as ventricular cells (139, 1158). In ventricular cells, activation occurs at rather negative potentials (1158); in the presence of catecholamines or after dedifferentiation however, activation is shifted in the positive direction and becomes possible at normal diastolic potentials (267). In Purkinje, atrial, and ventricular cells, diastolic depolarization will eventually lead to activation of the I_{Na} and the generation of a propagated action potential. During the final part of the diastolic depolarization, current through I_{K1} falls because of inward rectification while Na^+ conductance increases in a regenerative way. Both phenomena are responsible for the exponential increase in depolarization.

In Ca^{2+}-overloaded cells, slow spontaneous release of Ca^{2+} from the SR during diastole enhances the level of free cytoplasmic $[Ca^{2+}]$ and stimulates Na^+/Ca^{2+} exchange, I_{NSC}, and I_{Cl}. All these changes cause the cell to slowly depolarize or increase the rate of late diastolic depolarization in subsidiary pacemakers (45, 257, 537).

Depolarization large enough to reach threshold and to generate extra systoles can also be caused by stretching of nonactive cells and activation of stretch-sensitive channels (509).

Depolarization-induced automaticity can further occur in the border zone of an infarct. Injury or short-circuit current (102, 452, 508) may cause diastolic depolarization that is sufficient to reach threshold. Once triggered, sustained rhythmic activity may ensure. The time-dependent diastolic depolarization is due to deactivation of I_{Kr} and

I_{Ks}, decrease in I_{K1} (inward rectification), and activation of I_{Na} (508).

Automatic activity in the AVN, Purkinje, atrial, and ventricular cells may play a role in the initiation of early ischemic arrhythmias (Ia) and in the initiation and maintenance of later arrhythmias (Ib) (see sect. IV*B2*). It is important to note that an ectopic focus will not necessarily stimulate the whole heart. Propagation depends on the critical size of the automatic focus (size and number of cells) and its coupling with neighboring cells (1046). Driving of other cells is only possible within a certain range of coupling conductances; this range shifts with changes in $[K^+]_o$.

C) TRIGGERED ACTIVITY. In contrast to automatism, triggered activity is characterized by the obligatory presence of an external stimulus. Two types of triggered activity can be distinguished: EAD and DAD.

I) EAD. Repolarization during the plateau and phase 3 of the action potential is usually smooth and regular. Early afterdepolarizations are secondary depolarizations that originate during the plateau or final repolarization (417, 566, 684). Two ionic mechanisms are important: a true regenerative increase in conductance of the L-type Ca^{2+} channel or a fast transient activation of the Na^+/Ca^{2+} exchange current following a secondary release of Ca^{2+} from the SR. The two are not mutually exclusive.

1) When repolarization during the plateau is sufficiently slow, the I_{Ca} can reactivate following recovery from voltage-dependent and Ca^{2+}-induced inactivation (414, 684). Recovery from voltage-dependent inactivation and reactivation is possible in a relative narrow voltage range corresponding to the window where activation and inactivation curves overlap. Recovery from Ca^{2+}-induced inactivation is fast, occurs during the plateau of the action potential, and generates directly an inward current (911). The rate of recovery from Ca^{2+}-induced inactivation is determined by activity of the Na^+/Ca^{2+} exchanger in the plasma membrane and of the Ca^{2+}-ATPase in the SR. Because it is not restricted to the window potentials but present over a broad range of potentials, it is potentially more important than voltage-dependent reactivation.

Slowing of the repolarization process is favorable for reactivation of the I_{Ca} and appearance of EAD. Decreasing the rate of repolarization with eventual simultaneous stimulation of I_{Ca} has been the common factor in the choice of conditions to elicit EAD: block of K^+ currents by extracellular Cs^+ (93), drugs (1140), α-receptor stimulation (887), or acidosis (175); increase of inward I_{Na} by veratridine or anthopleurin (93); increase of inward I_{Ca} by BAY K 8644 or isoproterenol (481); and existense of short-circuit current by coupling to depolarized cells (568). The opposite is also valid; increasing the rate of repolarization antagonizes EAD: high frequency of stimulation (782), high $[K^+]_o$, ACh, lidocaine or TTX (93), and Ca^{2+} channel block (481).

2) A second process important in the genesis of EAD is activation of the Na^+/Ca^{2+} exchange current, following the release of Ca^{2+} from the SR. Usually the release of Ca^{2+} is restricted to the initial period of systole. In conditions of Ca^{2+} overload, however, the gain of the system is increased (44, 918), and a second spontaneous release may occur during the later phases of the plateau or the final repolarization. Together with DAD, EAD have been measured during recovery from anoxia (60, 62). The probability of EAD during phase 3 or final repolarization is greater in the presence of Ca^{2+} overload (782, 951). In guinea pig and canine Purkinje fibers (951), the development of EAD elicited by adding $[Cs^+]_o$ was favored by preloading the cell with Ca^{2+}; addition of ryanodine blocked the EAD (782). When EAD occur during phase 3, the extra depolarization may reactivate the T-type Ca^{2+} and/or the fast Na^+ channel and cause a propagated extra systole. At the same time, the inward rectification of the I_{K1} may function as an amplification factor.

II) DAD. Delayed afterdepolarizations are transient depolarizations triggered by a preceding action potential. The underlying current is a composite current and has been called transient inward current. When the depolarization is large enough to reach threshold for a regenerative increase in Na^+ conductance, an extra systole is generated. The situation may become self-replicating, resulting in runs of successive action potentials. Calcium overload favors the occurrence of DAD. Delayed afterdepolarizations were first described in digitalis intoxication (275, 505) but can also be evoked by superfusion with solutions containing elevated $[Ca^{2+}]$ and low $[K^+]$, solutions containing low $[Na^+]$, high concentrations of catecholamines, Ca^{2+} channel agonists, repetitive stimulation (see Ref. 480), or stretch (376, 1069). In all these cases, Ca^{2+} overload results either from block of the active Na^+ pump, reduction of Ca^{2+} efflux, and/or an increase of passive Ca^{2+} and Na^+ influx. When the SR is overloaded, spontaneous release of Ca^{2+} may occur.

The occurrence of DAD is dependent on the level of the E_m upon repolarization. The amplitude of DAD and of the transient inward current is largest at about -60 mV and smaller at more positive or more negative levels (505). Depending on the preparation, species, or experimental conditions, three ionic currents participate: the Na^+/Ca^{2+} exchange current, the intracellular Ca^{2+}-induced or stretch-induced NSC current, and the intracellular Ca^{2+}-induced Cl^- current (121, 368, 369, 579, 747, 912). All three current systems are activated by an increase of $[Ca^{2+}]_i$ and carry inward current at hyperpolarized potentials. The NSC current and the Cl^- current show E_{rev} values close to 0 mV. In the case of the Na^+/Ca^{2+} exchanger, a clear reversal is not seen because the increase in $[Ca^{2+}]_i$ following the release serves as the trigger but acts at the same time as the substrate. A phasic increase in $[Ca^{2+}]_i$ can only promote transient inward

current. In preparations with a high density of exchanger and increased cytosolic $[Ca^{2+}]_i$, the transient inward current may be superimposed on a "creep" current, which signals the increased Na^+/Ca^{2+} exchange upon repolarization. In sheep Purkinje preparations (121), the aftercontraction shows a strong correlation in time with the transient inward current but not with the magnitude or the polarity of the current, demonstrating that Ca^{2+} release from the SR is the primary event.

D) REENTRY. In reentry, an impulse is travelling along a circuitous pathway and instead of dying out like a normal impulse reexcites the previously stimulated cells (see Refs. 480, 1104). The pathway can be constant (ordered reentry) or variable in size and location (random reentry), of small microscopic dimensions, or following a macroscopic length. The pathway can be determined by an anatomical substrate (abnormal junction between auricular and ventricular tissue, orifice of vessels, bifurcation of Purkinje system, and junction with ventricular wall) or entirely determined by functional characteristics (leading circle reentry, anisotropic reentry, spiral wave reentry). Two essential conditions are to be fulfilled: *1*) the existence of a unidirectional block for impulse propagation at a certain site in the pathway and *2*) a combination of slow conduction and short refractory period in the remaining part of the pathway.

The first requirement of unidirectional conduction is necessary to allow the impulse to return and continue its travel. Different ways of how such unidirectional block is generated have been discussed in the literature. It should be realized that each action potential is followed by a critical time (and voltage) window, during which an extra stimulus can induce unidirectional conduction. Stimulation during this vulnerable period results in block of conduction in the anterograde direction, because the cells are still refractory, whereas recovery of the Na^+ conductance has proceeded far enough to allow conduction in the retrograde direction (882). Dispersion in the APD increases the likelihood to generate unidirectional conduction. Dispersion is normally present in the transmural direction (21, 235) but also exists between apex, septum, and free wall (332) and between right and left ventricle (1044). Because of an abnormal increase in I_{to} and/or decrease in I_{Ca}, phase 2 of the subepicardial action potential may be absent, resulting in an early full repolarization and marked transmural dispersion; the type of reentry that may originate in this way has been called phase 2 reentry (626). Dispersion furthermore may change with time: ST-T alternans is symptomatic and associated with a higher tendency to ventricular fibrillation (337).

Conduction is far from uniform even under normal conditions. It is faster in the longitudinal than in the transverse direction; the tissue is anisotropic (see Ref. 931). The existence of anisotropy is responsible for the occurrence of reentry even in the presence of a uniform ERP. Dispersion of refractoriness and anisotropy should be regarded as dynamic parameters. Theoretical as well as experimental studies have shown that transient local inhomogeneity of refractoriness may be sufficient to initiate sustained vortexlike reentry in normal myocardium (spiral waves) (200).

The second condition is related to the length of the reentry pathway; the conduction must be slow enough and the refractory period short enough to avoid the impulse reaching tissue that is still refractory. The product of ERP and conduction velocity or wavelength should be shorter than the length of the pathway. Conduction velocity is determined by the amplitude and the rate of rise of the action potential as well as membrane and gap resistance. Decrease in excitability and cellular uncoupling, however, have different effects on the safety factor of conduction (883). In a theoretical study, it was shown that conduction velocity could be lowered much more before failure occurred, when caused by uncoupling. Under those conditions, the I_{Ca} played a major role in sustaining conduction, stressing the possible role of Ca^{2+}-mediated action potentials in ischemic conditions.

In ischemia, conditions are fulfilled to generate reentry. From the early seconds on, the rise in $[K^+]_o$ and the accompanying depolarization cause inactivation of Na^+ channels and a decrease in the current generated during the upstroke of the action potential. Membrane resistance is decreased as a consequence of the elevated $[K^+]_o$ and decreases the efficiency of the stimulatory current. Conduction velocity falls. Although the action potential is shortened, the effective refractory period is rather prolonged because of the existence of postrepolarization refractoriness. Refractoriness is inhomogeneous, especially in the border zone, where islands of normal and ischemic cells are present, where $[K^+]_o$ concentration may vary considerably, and where in the nonischemic cells a marked shortening may be seen due to the injury current (182). After 15 min, when diastolic $[Ca^{2+}]_i$ rises rapidly, a fall in the gap junction conductance becomes prominent. Cells are uncoupled, making the occurrence of reentry more probable (547).

2. Description of arrhythmias during ischemia and upon reperfusion

Only arrhythmias of the acute stage occurring during the first 30 min of ischemia (phase I) will be analyzed, neglecting the arrhythmias seen between 5 and 72 h (phase II) and arrhythmias of the chronic stage after an infarct (phase III) (see Refs. 480, 1105).

In the dog, pig, sheep, and rat, phase I can be subdivided in Ia and Ib (Fig. 24*A*) (921). In the guinea pig, rabbit, and cat, the distribution of arrhythmias is unimodal with a peak of arrhythmias at 10 min; only the Ib type of disturbances seems to be present (432, 504). In hu-

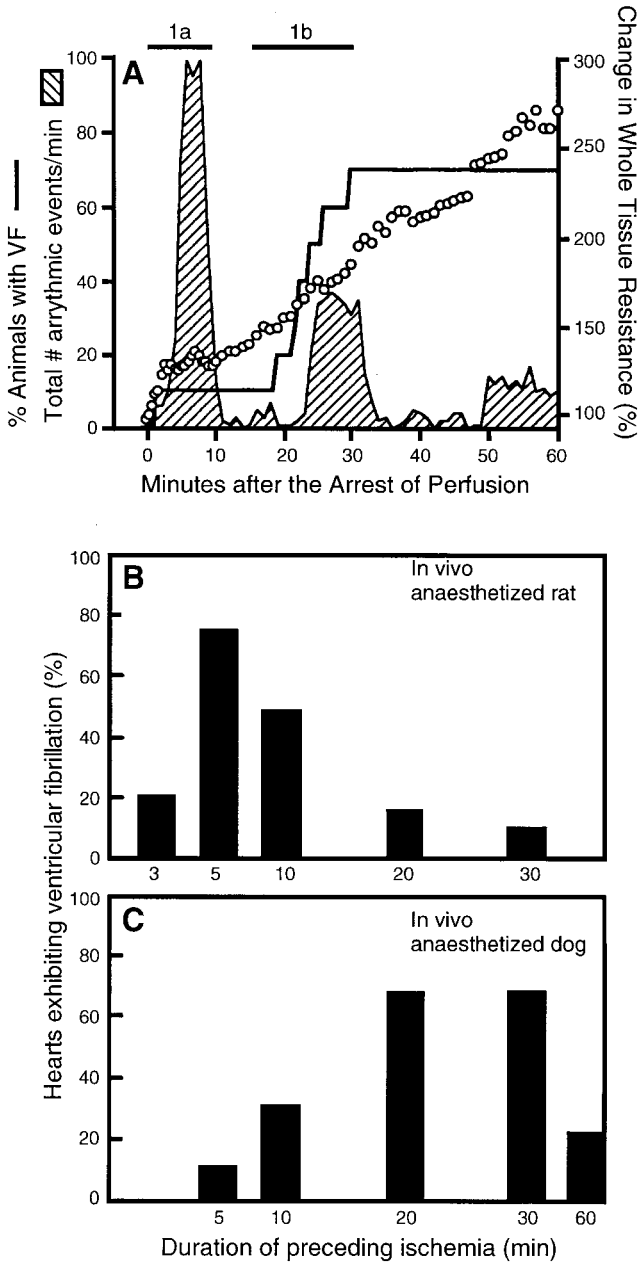


FIG. 24. *A*: 2 phases of arrhythmias (Ia and Ib; solid horizontal bars) can be distinguished during acute cardiac ischemia. Plot shows percentage of pigs with ventricular fibrillation (VF) (solid line, *left axis*) during a 60-min ischemic period; on same axis is shown total number of arrhythmogenic events per 1-min period (hatched areas). Open circles (*right axis*) indicate mean percentage change in tissue resistance. Major increase occurs during Ib period and is accompanied by increased probability of VF. [From Smith et al. (921). Copyright 1995 American Heart Association.] *B* and *C*: percentage of hearts showing VF upon reperfusion following different durations of ischemia. Frequency distribution is bell shaped, but actual maximum occurs for shorter ischemic periods in rat compared with dog. [Adapted from Manning and Hearse (638).]

mans, acute arrhythmias often result in ventricular fibrillation (VF) and sudden cardiac death.

A) PHASE Ia. Between 2 and 10 min of ischemia a first burst of irregular ventricular tachycardia is observed in most of the animals. Evolution into VF is rare (550, 551, 672, 921).

B) PHASE Ib. After a 10 min period of relative quiescence, a second phase of arrhythmias appears between 20 and 30 min. The percentage of animals showing these arrhythmias is less than for phase Ia. The evolution to VF however is more frequent and more animals die.

During reperfusion, the incidence of arrhythmias critically depends on the duration of the preceding ischemic period and can be described by a bell-shaped curve (35, 184, 638, 758). The frequency increases up to 30 min of preceding ischemia and afterward decreases again (Fig. 24, *B* and *C*). The decrease in incidence with prolonged duration of ischemia may explain why complications due to arrhythmias are rare following thrombolysis in hospital conditions; the delay between occlusion and reperfusion may indeed take a few hours.

Arrhythmias occurring after an ischemic period of <10 min, followed by reperfusion, are of the ventricular tachycardia-type (VT) and may degenerate in VF. When reperfusion takes place after 20–30 min of ischemia, the probability of obtaining arrhythmias is maximal and two periods of arrhythmias are seen: an immediate phase developing frequently in VF and, if no VF occurred, a delayed (after 2–7 min) arrhythmia of the tachycardia type, with multiple premature beats (480, 504, 1104).

The occurrence of VF during reperfusion is very variable and depends not only on the duration but also the number of preceding ischemic periods, the heart rate, the size of the ischemic zone, and the presence of drugs (181).

3. Mechanisms of specific arrhythmias during ischemia and upon reperfusion (see scheme 4)

Phase Ia arrhythmias (2–10 min) are most of the tachycardia type and rarely evolve into VF. Mapping stud-

SCHEME 4. Mechanisms of early ventricular arrhythmias

During ischemia

Ia:
- Starting stimulus
  - Location: border zone, propagating into ischemic zone
  - Nature: ¾ intramural reentry; ¼ nonreentry
- Long reentry pathway, evolving into multiple wavelets

Ib:
- Unknown (increase of r_i, intracellular Ca^{2+}, extracellular K^+, catecholamines)

Upon reperfusion

Immediate ventricular fibrillation
- Starting stimulus:
  - location: within reperfused zone
  - nature: nonreentry
- Fast evolution into multiple wavelets

Delayed ventricular tachycardia
- Automatism in Purkinje fibers

ies have demonstrated the reentry nature of these arrhythmias (777), with the abnormal impulse following a fairly long circuit within the ischemic zone or two wavefronts travelling around an area of conduction block in the form of a figure eight. In the period preceding the arrhythmia, activation is slowed especially in the subepicardial region, and much less in the subendocardium. Diastolic bridging or continuous, fractionated electrical activity between two successive QRS complexes is frequent (504, 921). The mechanism is slowed conduction and micro-reentry in the subepicardial region. Tachycardia is often preceded by T-wave alternans and deeply negative T waves, which are generated by the marked dispersion in activation and repolarization. Epicardial delay and VF are more frequent when the coronary obstruction is caused by a thrombus and α-receptor stimulation is pronounced (180). Dispersion is a normal physiological factor (21, 157) but becomes especially prominent in the border zone of an infarct (320, 536) and in hypertrophy (59), with great differences in APD and ERP. The inhomogeneity in the border zone is increased at high frequencies of stimulation and in the presence of sympathetic stimulation (741). β-Receptor stimulation has opposite effects on the action potential and refractory period in normal and ischemic tissue, shortening the ERP in normal but prolonging ERP in the diseased cells.

The initiating stimulus starts close to the ischemic border zone (479). The nature of the starting stimulus has been studied in the cat and found to be intramural reentry in 76% of the cases and premature beats originating in the subendocardium or subepicardium by a nonreentrant or focal mechanism in the remaining 24% (777). Different nonreentrant mechanisms can be involved: triggered DAD (778), spontaneous depolarization due to activation of the I_f current in Purkinje fibers or plain ventricular cells (139, 1156, 1158), spontaneous depolarization consequent to mechanical stretching (935, 1069), or excitation caused by injury current (507). Injury current flows at the border zone between ischemic and nonischemic tissue during diastole because of the difference in resting potential but even more during systole because of differences in APD and delays in activation. Injury current may result in reexcitation of normal cells or induction of spontaneous activity. Reexcitation has directly been measured in guinea pig hearts regionally perfused with a high $[K^+]_o$ solution (507). Model studies in which a normal living cell was coupled to an artificial cell in depolarized condition revealed important decreases in threshold (958) and facilitation of EAD (566). The intensity of the injury current during ischemia is half of the intensity of a propagating impulse (182). During the deep negative T wave, the injury current is much larger than during diastole. Inverted T waves are evidence of marked delays in the activation of the ischemic tissue during which time the voltage difference and thus also the injury current are larger than during diastole (179). It is not surprising that the initiating beats of tachycardia runs were often preceded by deeply negative T waves in the ischemic myocardium.

The mechanism of phase Ib arrhythmias occurring after 20 min of ischemia is less studied. In time they are concomitant to a massive release of catecholamines and the development of contracture, the start of a marked increase in longitudinal resistance (921), a secondary increase in $[K^+]_o$ (544), and a large increase in $[Ca^{2+}]_i$. Such changes favor reentry. In many cases, however, there is no evidence for slowed epicardial conduction or diastolic bridging, as is the case in Ia arrhythmias (504). Before the arrhythmias, action potential characteristics may even show an improvement in amplitude and rate of rise. Conduction delay, which was increased to 80 ms or more in the first period, shortens again to 40 ms (921). At the same time, the TQ depression diminishes (544), corresponding to a less pronounced ST denivellation that may eventually disappear. This phenomenon is not accompanied by a real recovery of E_m ($[K^+]_o$ actually starts to increase again). The rise in longitudinal resistance results in less short-circuit current (921), which in the electrocardiogram is translated in a smaller ST denivellation.

It is a dangerous phase because a certain proportion of the arrhythmias evolves in VF. This second phase is associated with a massive release of catecholamines (865). The release of catecholamines plays an important role as shown by the finding that VF or VT is reduced by surgical or pharmacological denervation, by block of the carrier mechanism and so preventing the release of catecholamines, or by acting postsynaptically and blocking α- and β-receptors (867). α-Receptor agonists, on the other hand (782), facilitate the occurrence of arrhythmias probably by promoting EAD and DAD. Inhibitors of the Na^+/H^+ exchanger exert antiarrhythmic activity (28, 29, 1123). This effect can be explained by a reduction of catecholamine release: less Na^+/H^+ exchange reduces the Na^+ load and avoids reversal of the carrier responsible for the reuptake of norepinephrine in the nerve terminals. It is also possible, however, that the beneficial effect of these inhibitors occurs at the myocyte level, by reducing Na^+ and Ca^{2+} load.

During reperfusion, immediate or early arrhythmias occur within a few seconds after reperfusion. They follow ischemia periods of 10–30 min. They start by an automatic stimulus in the reperfused zone and change afterward in a reentry multiple wavelet type of VT or VF. The underlying factors in the genesis of reentry are *1*) extremely short action potential and refractory period and *2*) slow conduction. Conduction remains subnormal because of the large hyperpolarization together with elevated $[Ca^{2+}]_i$ acting negatively on gap conductance. Unidirectional conduction is favored by the marked heterogeneity in $[K^+]_o$, APD, and ERP (181, 758).

The extra stimulus initiates in the reperfused zone.

The site is the subendocardium (776) at the border of the reperfused zone in 75% of the cases of nonsustained VT. A number of observations suggest EAD or DAD as possible candidates. In cat heart, EAD have been measured during reperfusion, and they were present in 62% of the cases developing reperfusion arrhythmias (783). In isolated cells, reoxygenation after a period of anoxia or metabolic inhibition is accompanied by EAD and DAD and runs of spontaneous action potentials if $[Ca^{2+}]_i$ is not buffered (60, 62). Delayed afterdepolarizations are elicited in guinea pig papillary muscles on reoxygenation after 60-min substrate-free hypoxia (393). Oxygen radicals could play an important role, and scavengers of radicals act as antiarrhythmics (70), although divergent results have been obtained (166). The effect seems to be dependent on species, and protection by radical scavengers is not obtained in the dog (260, 753). There exists a close association between Ca^{2+} overload and ventricular arrhythmias (100, 520). In favor of Ca^{2+} overload translated in DAD is the observation that irregular activity in single rat cells (971), and arrhythmic activity in guinea pig papillary muscles (393) is stopped by treatment with ryanodine. In the same context, one should emphasize the arrhythmogenic influence of sympathetic stimulation, especially the α_1-receptor activation, supposed to promote Ca^{2+} overload via PLC activation (58, 194, 627, 1145). Intracellular IP_3 concentration increases upon reperfusion secondary to α_1-receptor stimulation (17) or thrombus release (474) and is correlated with the occurrence of arrhythmias in the rat. Inhibition was shown by aminoglycoside antibiotics that block the release of IP_3 (236).

Despite this circumstantial evidence in favor of EAD and DAD, the nature of the initiating stimulus remains a matter of debate. In experiments on pig hearts, ryanodine or an increase in $[K^+]_o$ had no antiarrhythmic effect, although these procedures normally antagonize DAD (181). No correlation has been found between the presence of EAD during reperfusion and the development of VF (1036). Early afterdepolarizations dissappeared in 6 of 24 dogs before VF developed, and there were no EAD in 5 dogs that developed VF.

Delayed reperfusion arrhythmias appear as a second period of irregular rhythm when the occlusion period has been longer than 10–20 min. Extra systoles and runs of tachycardia probably originate in surviving Purkinje fibers overlying the ischemic zone. Endocardial mapping (983) of the left ventricular septum following an ischemic period of 30 min has demonstrated excitations during VT always originating in the Purkinje system and expanding in an centrifugal manner through the Purkinje-muscle system. A similar conclusion was reached by Pogwizd and Corr (776) who found that in three-fourths of the cases reentry was not the mechanism.

The underlying mechanism is abnormal automaticity in partially depolarized cells. Upon reperfusion, $[K^+]_o$ is washed rapidly and the extra Ca^{2+} in the cytoplasm is reabsorbed in the SR. Under those conditions, oscillatory release of Ca^{2+} from the SR is highly probable. In multicellular preparations and single cells, DAD have been observed upon reoxygenation (60, 62), or after washout of an "ischemic" solution (165, 176, 392). Oscillatory potentials have been measured in Purkinje fibers isolated from ischemic guinea pig hearts (814). Early afterdepolarizations are possibly mediated by α-receptor stimulation (23, 627). α-Receptor density is increased in ischemic conditions. Because surviving Purkinje fibers are subject to mechanical elongation, stretch-induced depolarizations resulting in extra systoles could be an additional mechanism of inducing arrhythmias (375, 376, 935, 1069).

4. Synopsis

Basic mechanisms in the genesis of arrhythmias include automaticity, triggered activity (EAD and DAD), and reentry. In ischemia, changes in pacemaker activity are mostly due to neurogenic and hormonal stimuli. Automaticity outside the SAN is possible by activation of I_f, present in many atrial and ventricular cells, by the occurrence of EAD and DAD as a consequence of Ca^{2+} overload and catecholamine secretion, and by excessive stretch of border zone cells. Conditions for reentry are equally present: inhomogeneous ERP and slow conduction, caused by slow recovery from inactivation of Na^+ channels and increase of longitudinal resistance.

Early arrhythmias occurring during the first 30 min of ischemia are subdivided in Ia and Ib types, with the Ib type especially dangerous because they frequently evolve in VF. In most cases (three-fourths), phase Ia starts as intramural reentry in the border zone, following initially a long circuitous pathway but evolving rapidly in multiple wavelets. The mechanism of Ib type is less known but is associated with the increase in longitudinal resistance, worsening of Ca^{2+} overload, and secretion of catecholamines. During reperfusion, the incidence of arrhythmias depends on the duration of the preceding ischemia, with a frequency maximum between 10 and 30 min, variable with species. The starting stimulus for VF is of the nonreentry nature and is located within the reperfused zone. It evolves rapidly into multiple-wave reentry.

V. CONCLUDING REMARKS AND PERSPECTIVES

Future research should be directed to fill certain gaps in our knowledge of channel structure and function, about their role at the cellular and tissue level. Recently, the three-dimensional structure of a K^+ channel pore has been elucidated using X-ray analysis of the crystallized channel clone (234). It is evident that similar approaches

are needed to understand the selectivity of Ca^{2+}, Na^+, Cl^-, and other channels. The same approach will lead to a structural analysis of not only the permeation characteristics but also the gating properties and their kinetics. There is no doubt that mutational studies will continue to unravel the details of the biophysical properties of different channels. More information is needed on certain anion and NSC channels, on channels in intracellular organelles, more specifically the SR channel, the mitochondrial VDAC, mega-channel and KATP channel, and nuclear channels. The study of these channels has been restricted to a few groups of researchers and should be expanded. For certain channels, a plethora of molecular isoforms are known, but their integrative function is far from elucidated. It is hoped that the study of these channels will open new avenues for therapeutic interventions, especially for the treatment of Ca^{2+} overload and arrhythmias.

We should strive for a better understanding of the function of different channels at the cellular and tissue level. There is a serious need for integrative physiology, for reopening the study of action potential characteristics and conduction at the tissue level, using the classical microelectrode and the more recently promoted fluorescence methods to measure E_m values. In that context, it is hoped that genetically modulated mice models in which certain channels are knocked out or replaced by mutated channels will lead to useful arrhythmia models. Up to a certain level, arrhythmia mechanisms can be studied at the cellular level, but most, e.g., reentry, requires the study of an interaction of a large number of cells. An understanding of these phenomena will be boosted by integration of the detailed molecular information in quantitative, theoretical models at the organ and organism level. In other words, we have to incorporate molecular biology into physiology.

In the near future, we should direct our attention more to aspects of chronic ischemia. It has become clear that next to acute modulation by ion concentration and phosphorylation-dephosphorylation mechanisms, ion channels undergo changes on a longer time scale. At the present time, atrial fibrillation models (1163) have shown the downregulation of currents by decreasing the number of functional channels. Are permeation or gating also subject to changes on a longer time scale? Heart cells undergo dedifferentiation (hibernation) or may undergo apoptosis and be replaced by fibrocytes, leading to a change in the microarchitecture of the tissue (761). The study of these remodeling processes will further the understanding of the changes occurring during chronic ischemia and help us to plan new therapeutic approaches.

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