Biochemical regulation of developed intraventricular systolic pressure

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The chemical basis of myocardial developed pressure in strong crossbridge interaction, resulting in conformational changes in myosin and thus in shortening of the sarcomere. Regulation of this interaction is directly influenced by [Ca]i and is only indirectly regulated by the phosphorylation potential, i.e., the affinity of adenosine triphosphate (ATP) hydrolysis (1n [(ATP)/(ADP)(Pi) (H^+)]). Intracellular calcium is in the cell in limiting concentrations varying with the cardiac cycle, thus regulating the on/off state of actin-myosin interaction. The rate and amount of calcium entering the cell is in turn regulated by ion gradients, voltage, myofiber length, electrogenicity, hydrodynamics, and factors that in turn regulate these parameters. On the other hand, ATP is in excess concentration in the cell, and end products of ATP hydrolysis (ADP, Pi, and H^+) need to rise excessively high before they influence developed pressure; furthermore, compartmentation of substrate and end products is not regulatory. The high enzymatic activity of creatine kinase favors ATP, allowing for a rapid distribution of the purine nucleotides in the form of ATP. Thus, the phosphorylation potential may only influence developed pressure during severe conditions, such as in heart failure or reperfusion following ischemia. The basis of these observations is the subject of this review.

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REGULATION OF CROSSBRIDGE INTERACTION, MYOFIBRILLAR ATPASE ACTIVITY

Crossbridge interaction. The chemical basis of cardiac mechanics is a strong crossbridge binding state, the shortening phase of the cardiac cycle. Prior to depolarization, when $[Ca^{2+}]i$ is approximately $0.3 \times 10\text{-}6$ M, the troponin-tropomyosin complex blocks myosin head contact with actin in such a way that low calcium prevents a strong binding state.[1] Only a weak or no actin-myosin interaction can occur at low calcium concentrations.[1] With depolarization and greater calcium (two- to fourfold increase) delivered to troponin C, troponin C-calcium binding depresses troponin-tropomyosin interaction. The subsequent troponin-tropomyosin conformational change allows for the active sites of the actin molecule to become available to myosin heads in such a way that a strong actin-myosin binding state occurs. Myosin-ATP (or myosin-ADP) binds to actin. ATP is rapidly hydrolyzed by actomyosin. Following hydrolysis, myosin goes through several conformational changes, while the end products (ADP + Pi) are transferred to different positions in the oligomer. The conformational changes due to transfer of products cause shortening of the sarcomere and contraction, thus resulting in developed (systolic) pressure. The end products are released. If the sarcoplasm binds and sequesters calcium, cycling stops, and myosin goes into the relaxed state. These processes are described in detail and are diagramatically represented in earlier studies.[2,3] Release of crossbridges constitutes the chemical basis of relaxation (diastole), the resting phase of the cardiac cycle. Various factors in the cellular milieu that influence [Ca]i also regulate actin and myosin interaction (crossbridge formation) and the release of actin and myosin crossbridges.

Regulation of myofibrillar ATPase activity. Factors important for regulating myofibrillar ATPase activ-

ity and thus developed pressure, are (1) cofactors, (2) substrate, and (3) end products of myofibrillar ATPase. The cofactor, Ca^{2+}, is in limiting concentration and thus is regulatory. There is a calcium gradient across the cell membrane, an approximate ten- to twentyfold gradient for total [Ca]i (40 to 80×10^{-6} M),[4-6] an approximate one thousandfold gradient for transients (0.5 to 1.0×10^{-6} M),[7,8] and an approximate three thousandfold gradient for the resting level of $[Ca^{2+}]i$ (0.3 to 0.5×10^{-6} M),[8,9] assuming interstitial calcium 1.0 mM. (Total intracellular calcium is about twofold higher in isolated heart cells.[10,11] The steepness of the gradient allows the use of Ca^{2+} as a fast trigger for a series of cytosolic events utilizing channels in the sarcolemma (SL) and sarcoplasmic reticulum (SR) through which Ca^{2+} will flow down the gradient. The calcium concentration required for strong crossbridge interaction, i.e., regulating actomyosin ATPase activity, is between 0.5 and 1.0×10^{-6} M.[8,9,12] This concentration depends on the calcium sensitivity of the cellular milieu. The regulation of intracellular calcium and ion gradients, which are the most influential regulators of formation and release of crossbridges, or going from a weak to a strong actin-myosin binding state.

The substrate for myofibrillar ATPase activity is ATP. ATP is in excess concentration in the cytosol and thus is normally not a regulator of myofibrillar ATPase. The cellular ATP concentration ranges between 7 and 10 mM, depending on the substrate and species.[13-15] Half maximal activation of myofibrillar ATPase activity is 0.05 mM.[16] Thus, ATP is approximately in a one hundredfold excess in the myocardium. The end products, ADP, Pi, and H^+ are in concentrations of 0.02 to 0.05 mM for free ADP, 0.2 to 2.0 mM for free Pi, and 0.1×10^{-6} M for H^+.[13-15] The low concentration of free adenosine diphosphate (ADP) prevents ADP from causing product inhibition, and is continuously buffered by PCr. With exhaustion of phosphocreatine (PCr), ADP rises. With a rise in ADP there is a concurrent increase in adenosine monophosphate (AMP), adenosine, and inosine, i.e., in products of ADP hydrolysis.[14] The latter flow out from the cell and lower the nucleotide pool,[14] thereby keeping ADP in low concentration.[5,14,17] Large concentrations of Pi are required to lower myofibrillar ATPase activity[18,19] as well as high concentrations of H^+.[20] Inorganic phosphate sensitizes the cytosol to calcium.[18,19,21,22] Inorganic phosphate and [pH]i influence calcium sensitivity intracellularly. From the substrate and end products the phosphorylation potential is derived, i.e., 1n [ATP/(ADP)(Pi)]. Standard phosphorylation potential values in mM for rat and hamster range between 4.5 and 6.0, depending on the conditions and substrate.[13,15,20,23] Phosphorylation potential values less than 3.5 predict lack of recovery.[20] The phosphorylation potential is the same as the affinity of ATP hydrolysis ($\Delta\text{-}G^\circ_{ATP} + RT$ 1n [(ATP)/(ADP)(Pi)] during standard conditions, i.e., under constant temperature and atmospheric pressure. Mitochondrial activity and the phosphorylation potential play a role in limiting developed (systolic) pressure,[20,23] but may not cause immediate changes in developed (systolic) pressure.

REGULATION OF INTRACELLULAR CALCIUM

Measurement of intracellular calcium. Advances have been made in the measurement of intracellular calcium. These include measurement of (1) calcium transients, (2) resting levels of calcium, and (3) total intracellular calcium. Calcium transients were first measured by aequorin[24,25] via direct injection of the fluorescent dye into the cell. The dye is sequestered intracellularly, enhancing fluorescence when it interacts with calcium. There are limitations in aequorin use due to its impermeability and relative insensitivity at low calcium concentrations,[24-26] making it difficult to accurately determine fluctuations in the resting level of [Ca]i. [Ca]i has also been studied in tissues by the use of Ca-sensitive microelectrodes.[27,28] Microelectrodes have limitations in that they necessitate piercing a cell membrane, thus channeling the cell and risking extracellular contamination. Recently, a new class of calcium-specific fluorescent indicators, the prototype of which is quin-2,[29,30] have become available. Quin-2 is a fluorescent tetracarboxylic acid that is highly selective for calcium, binding Ca^{2+} with 1:1 stoichiometry. Quin-2, however, requires loading up to 1 mM. At this concentration, it heavily buffers [Ca]i and dampens transients. Quin-2 can detect calcium levels of 10^{-8} M. Introduction of quin-2 intracellularly occurs by the use of an acetoxymethyl ester of quin-2. Intracellularly, the dye is cloven, forming an acid derivative that binds calcium, enhancing fluorescence fivefold as a calcium complex.[29,30] Mean cytosolic calcium activity can be determined through calibration of cellular fluorescence. This technique has been used to measure $[Ca^{2+}]i$ in cell suspensions from several different sources.[31,32] The technique is sensitive and specific. The calcium chelators, Fura-2 and Indo-1, are 30 times brighter than Quni-2.[33] Loading is only necessary up to 1 μm, and thus there is less buffering and less dampening

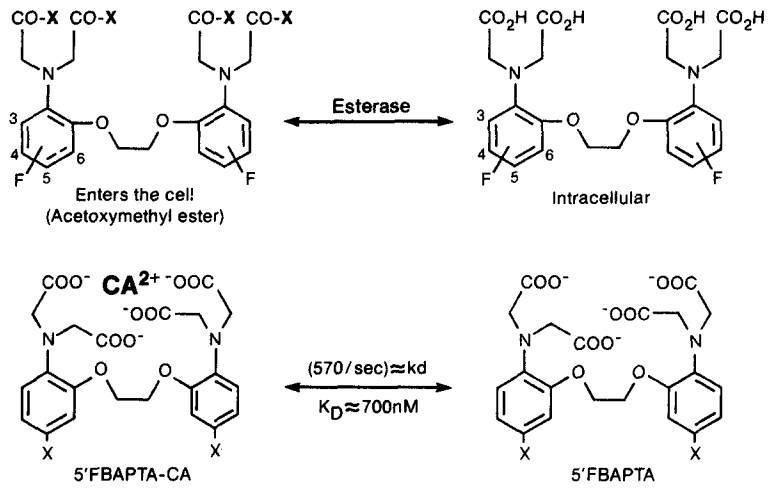

Fig. 1. The acetoxymethyl ester of the chelator FBAPTA freely diffuses into the cell. Inside the cell it is cloven by cellular esterase, forming a tetracarboxylic acid that can bind calcium and can no longer diffuse out of the cell. An equilibrium is set up in the same manner as the chelator EGTA, resulting in a buffering of intracellular calcium. The fluoro groups on FBAPTA allow for detection of the free and bound forms by F-19 NMR (nuclear magnetic resonance imaging), each giving independent peaks.

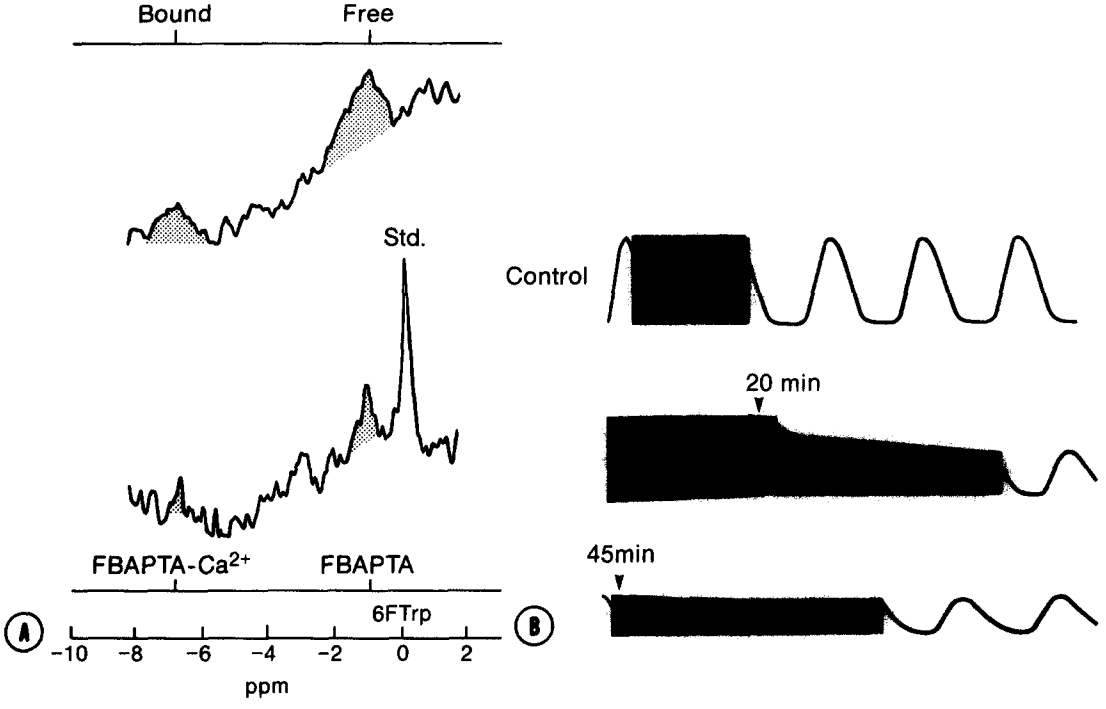

Fig. 2. *A*, F-19 NMR shows peaks for the free and bound forms of FBAPTA. These peaks are identified by their position in relation to the standard, 6F-tryptophan. *B*, FBAPTA is loaded into the cell at 1 mM, thus heavily buffering [Ca]i and thereby lowering developed pressure.

of transients.[8,33] Such dyes work well with single cells but are more difficult to assess fluorometrically with tissues and organs, except for surface fluorometry detection.[34] Fluoro-dyes, e.g., 4-difluoro 1,2-bis (o-aminophenopy)ethane-N,N,N′-tetraacetic acid (4FBAPTA), are detectable by F-19 NMR, allowing the technique to be applied to tissues and organs.[35,36] The cell is loaded as an acetoxymethyl derivative (FBAPTA-AM), and is then cloven intracellularly by an esterase (Fig. 1). The intracellular (free) fluorescent esters and the calcium-bound fluorescent esters are both detectable by F-19 NMR, each

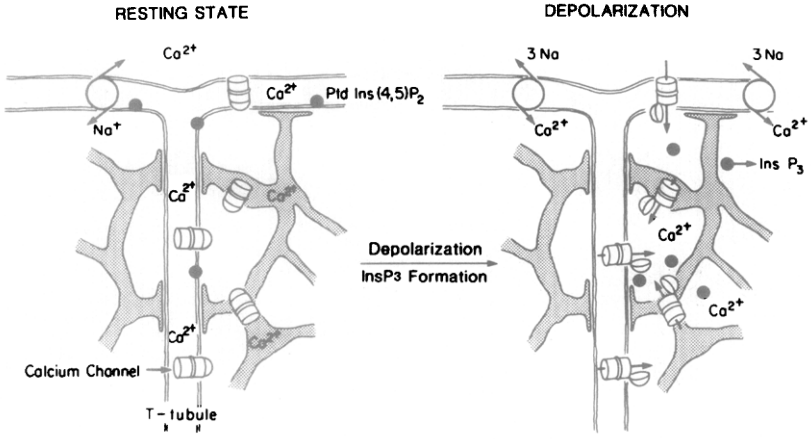

Fig. 3. Diagrammatic representation of the Na/Ca exchange in the sarcolemma and the calcium channels in the t tubules and the lateral cisternae of the sarcoplasmic reticulum. Phosphotidylinositol 4,5 diphosphate is membrane-bound. Following depolarization, it is converted to the soluble inositol triphosphate, which incites the opening of SR calcium channels.

giving distinctive peaks.[35,36] Fluoro-dyes assessed by F-19 NMR work well with relatively large volumes of tissue; however, due to the insensitivity of NMR, the technique is not practical for small quantities of tissue as is Fura-2, which is detected fluorometrically.[37,38] Use of F-19 NMR for detecting $[Ca^{2+}]i$ in the perfused rat heart with the fluoro-dye, FBAPTA, is shown in Fig. 2, *A*. Both FBAPTA and Quin-2 buffer intracellular calcium at about 1 mM, but severely depress cardiac performance (Fig. 2, *B*).[36]

Total intracellular calcium can be measured from acid extracts of tissues in which there is an extracellular marker present [e.g., K(CoEDTA)] to estimate extracellular space.[39] Intracellular calcium includes transients, resting levels, and calcium sequestered by organelles as well as membrane- and protein-bound calcium. The total intracellular calcium is in equilibrium with the resting levels and transients of intracellular calcium; there is a correlation between total intracellular calcium and resting levels of calcium.[40] Since total $[Ca^{2+}]i$ is approximately 8×10^{-6} M, this method can be used to analyze large changes in total $[Ca^{2+}]i$.[5] Limitations in this technique are the ability to determine precisely $[Ca^{2+}]i$, since it is ten- to twentyfold lower than total $[Ca^{2+}]o$, depending on the conditions. In situ and in vivo measurements of [Ca]i are more precise than in vitro measurements of calcium binding, utilizing isolated proteins and other factors. The discrepancy is due to the inability to attain low concentrations of calcium in vitro. Contamination necessitates lowering Ca with chelating agents and using ^{45}Ca to estimate free Ca. Such agents cause anamolies in the interpretation of results.[41]

Intracellular calcium. Ion gradients,[42,43] voltage,[44]

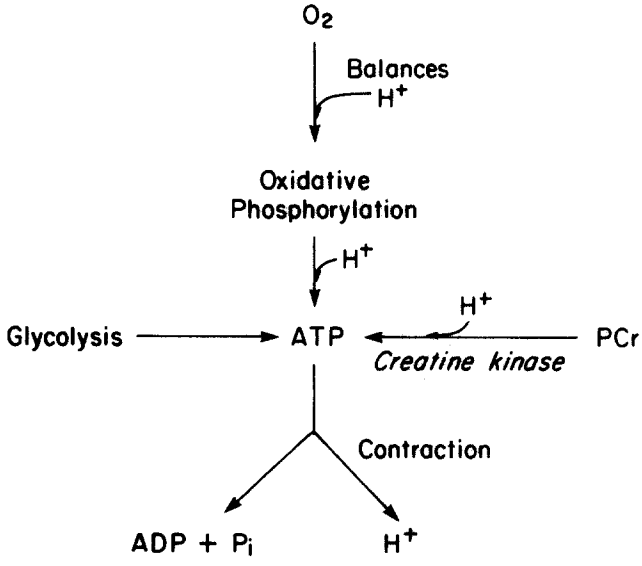

Fig. 4. Protons are utilized by mitochondria in the synthesis of ATP as well as by creatine kinase. A proton is given off with the hydrolysis of ATP via myosin. Protons are neutral with glycolysis. Glyceraldehyde-3-PO_4-dehydrogenase produces one H^+ and lactic dehydrogenase utilizes one H^+. The generation of lactate is associated with acidity. The latter is an indication, however, that glycolysis is prevalent and is not balancing the H^+ generation associated with work.

myofiber length,[45,36] electrogenicity,[47] and hydrodynamics[6] are important for regulation of intracellular calcium. External calcium enters the cell through (1) calcium channels and (2) the Na/Ca exchange. Calcium is sequestered from the cytosol (1) by the CaATPase pump and (2) by Na/Ca exchange.

Calcium channels. A family of diverse calcium channels[48] is regulated by voltage,[49] and by the

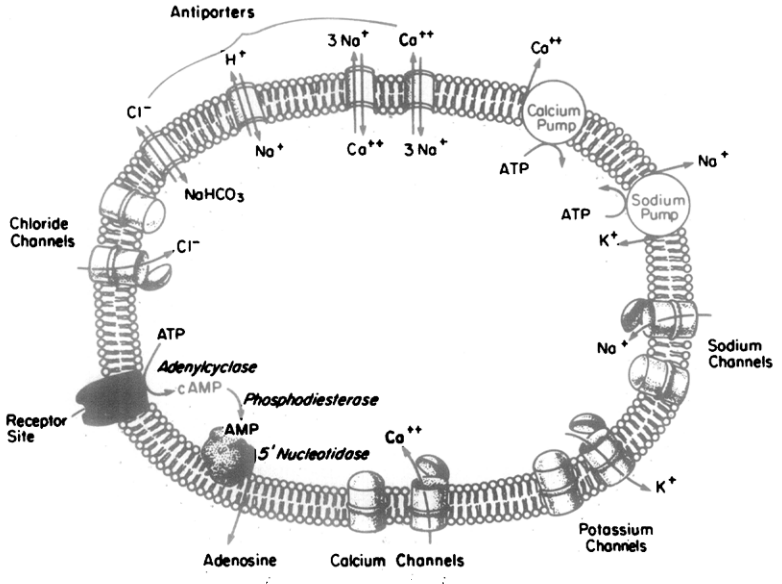

Fig. 5. A diagrammatic representation of antiporters, pumps, channels, and receptor sites associated with the sarcolemma is shown.

calcium gradient across the membrane.[50,51] The calcium channels located in junctional (as compared to free) SR are regulated by inositol 1,4,5 triphosphate.[52-54] The passage of ions through membrane channels has been treated as free electrodiffusion in the past.[55] More recently, evidence indicates the ions interact with channels during their passage.[56] There is a slow voltage-dependent calcium channel (L), and a fast voltage-dependent calcium channel (N), in most species of ventricular fibers. The slow but not the fast calcium channel is inhibited by nifedipine. Depolarization initiates a Ca influx and a subsequent Ca release from the membranes, perhaps through the t tubules that contain calcium channels[57] and that are in a 100 to 200 A proximity to SR.[58] Sarcoplasm also contains calcium channels,[52,59] confined to the junctional cisternae.[60] Close proximity of SR and SL is required, since diffusion of Ca^{2+} intracellularly is slow.[61] There also is a nonuniform spatial distribution of [Ca]i.[62] Mediation between the t tubules and the SR may occur through breakdown of membrane phoshpotidylinositol 4,5-biphosphate [PtdIns(4,5)P_2], releasing the soluble inositol 1,4,5 triphosphate ($InsP_3$). Intracellular receptors for $InsP_3$ exist, and when activated, result in a release of cytosolic Ca^{2+}.[52-54] This process is activated by depolarization. $InsP_3$ has been shown to couple excitation with contraction[52-54,63]; this may occur by opening the calcium channels,[52] as depicted in Fig. 3. Formation of $InsP_3$ is activated by protein kinase C. The latter, in turn, is activated by cAMP, $alpha_1$- activation, and arachadonic acid.

The action potential is the trigger for contraction. Its amplitude and duration exert some control over the force of contraction by modulating the influx of calcium.[64] The amount of calcium entering from extracellular space triggers a second release of calcium from the SR.[65] Ryanodine negates the contribution from SR, allowing for determination of the amount of calcium entering extracellularly.[66] Calcium entry blockers afford a tool to study the family of diverse calcium channels in situ and in vivo.[67] For example, the calcium entry blockers, nifedipine[20] and verapamil,[68] help protect the myocardium against acidosis, presumably by lowering chemical work. Chemical work adds to myocardial intracellular acidity, since one H^+ is produced per ATP hydrolyzed. Protons produced by ATP hydrolysis are balanced by ATP synthesis via oxidative phosphorylation[69] or via creatine kinase[69] (Fig. 4). Glycolysis gives a neutral pH balance. One H^+ is produced during glycolysis by glyceraldehyde 3-PO_4 dehydrogenase and one H^+ is utilized by lactic dehydrogenase in catalysis of pyruvate to lactate.[70] Under acidotic conditions, H^+ produced by crossbridge interaction becomes deleterious without aerobic respiration to balance H^+, by mitochondrial H^+ utilization.[69]

Contraction—Na/Ca exchange. The presence of an Na/Ca exchange mechanism was first demonstrated in the heart in 1968.[71] During the past 5 years, techniques were developed for purification of the Na/Ca exchange from myocardial membranes, allowing for a better understanding of this pro-

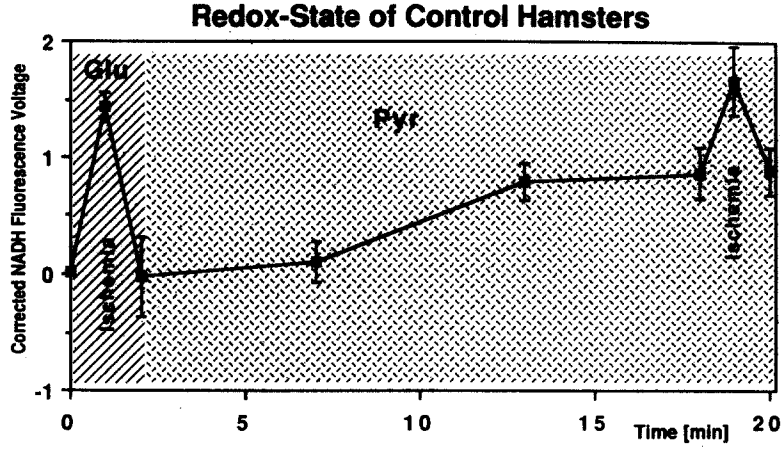

Fig. 6. A surface fluorometry probe was placed in a fixed position next to the left ventricular free wall (hamster). The entire set-up was enclosed in a black chamber. The heart was perfused with glucose and then all flow into the heart was terminated for 30 seconds to observe maximum mitochondrial reduction of NADH. Flow was again resumed. The heart was allowed to return to control levels of NADH. The substrate was then changed from glucose to pyruvate. After 30 minutes, flow into the heart was again terminated to observe maximum change in the redox state. Values are average values for the free wall of the left ventricle of the hamster heart. The probe detects 3 mm depths. The hamster heart is 2 to 3 mm in thickness. The intraventricular pressure is the same whether the heart is perfused in glucose or pyruvate. With glucose as the substrate throughout the experiment, there was no change in the redox state except during ischemia.

tein.[72,73] The Na/Ca exchange is thought to regulate myocardial contractile states by augmenting cytosolic $[Ca^{2+}]i$ following depolarization and sequestering $[Ca^{2+}]i$ during rest.[74] The enzyme is reversible.[75] The influx of sodium during depolarization modifies the kinetics of the Na/Ca exchange in a manner that confers voltage dependence and electrogenicity on the enzyme.[75] Contractile force is dependent on the $[Na^+]i$[76]; during physiologic conditions, there is approximately 10 mM free $[Na^+]i$ and 20 to 30 mM total [Na]i.[77] Calcium enters through an electrogenic coupling, i.e., the Na/Ca exchange located in the sarcolemma and mitochondrial membrane.[78] This cation exchange enzyme is diagrammatically depicted in Fig. 5, along with other membrane proteins regulating the flux of ions. The Na/Ca exchange is regulated by the Na^{2+} and Ca^{2+} gradients across the membrane,[79-81] and perhaps through a cAMP-dependent phosphorylation of the Na/Ca exchange enzyme.[79] Thus, factors such as H^+,[82] depolarization,[83] K^+,[84] catecholamines,[85,86] etc., which influence intracellular Na^{2+}, also influence intracellular Ca^{2+}. Agents that increase [Na]i also increase [Ca]i, e.g., ouabain through inhibition of Na/K ATPase, and amiloride through activation of the Na/H exchange. The Na/Ca exchange affords an electric current to the cell, since there is an uneven exchange of cations, three Na^+ for one Ca^{2+}.[87] This number is influenced by the Na concentration.[88] The Na/Ca exchange produces a membrane current that affects the membrane potential and indirectly influences all potential-dependent cellular processes.[89] Transport of ions by the Na/Ca exchange depends on the membrane potential. Inhibition of the Na/Ca exchange causes cells to lose their electrical current.[90]

Since the cardiomyopathic Syrian hamster UM X7.1 has both a calcium overload[91] and a paritial inhibition of the Na/Ca exchange,[92] it is a good model to study the importance of the Na/Ca exchange and factors that activate the Na/Ca exchange, such as verapamil,[93] prenylamine,[94] and cAMP.[79] Verapamil activates the Na/Ca exchange[95] while inhibiting the Ca channels,[96] and diltiazem inhibits the Na/Ca exchange.[97] Treatment of the cardiomyopathic hamster UM X7.1 with verapamil[98] (but not with diltiazem or nifedipine[99]) results in improvement of cardiac performance[100] and alleviation of a [Ca]i overload.[101] This may be due to the ability of verapamil but not diltiazem to augment [Ca]i efflux via Na/Ca exchange. The antimitotic agent, doxorubicin, has been shown to inhibit the Na/Ca exchange.[102] There is protection against the depressive affects of doxorubicin by pretreatment of animals with verapamil,[103] an agent that protects the Na/Ca exchange.[104] In alcohol-induced cardiac dysfunction, ingestion of alcohol is associated with inhibition of Na conductance[105] and intracellular dehydration.[4] The depressed state and high total

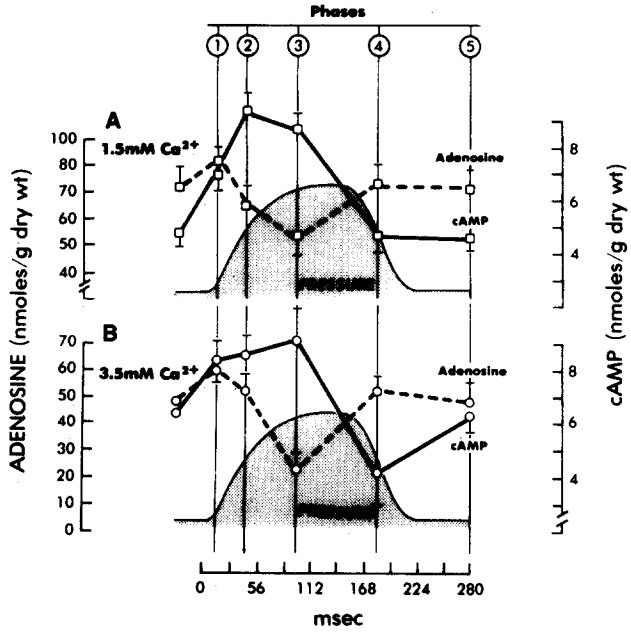

Fig. 7. Oscillations in cAMP and adenosine fluxing with the cardiac cycle are shown for the rat heart. Series of hearts were freeze-clamped at five phases of the cardiac cycle as shown (N = 5). In *A* the hearts were perfused with 1.5 mM calcium and in *B* they were perfused with 3.5 mM calcium. Alteration in calcium concentration did not significantly change cAMP or adenosine fluxes. Analyses were carried out as described previously.[156]

[Ca]i[106] are alleviated with verapamil but are not alleviated with diltiazem.[107]

Membrane Ca^{2+}-activated ATPase. Relaxation is initiated by SR and SL sequestering Ca through an ATP-dependent calcium pump[108] activated by catecholamine-induced phosphorylation.[109] This is an electrogenic ion pump, currents of which have been measured.[110] The SR contains a Ca^{2+}-specific ATPase that constitutes 50% of membrane dry weight.[11] This enzyme transports Ca^{2+} from sarcoplasm into vesicles of the SR with high velocity and affinity.[112] The reaction proceeds both in the SL and SR, with a stoichiometry of two Ca^{2+} transported per ATP hydrolyzed.[113] Ca^{2+}-ATPase has a high affinity for Ca (0.1 to 0.5 μM).[114] Pi and H^+ decrease the calcium sensitivity of the SR ATPase.[115] The existence of high and low affinity systems for the regulation of Ca^{2+} is in keeping with the different demands of the signaling function. The sequestering of calcium by the Ca-activated ATPase is required for the transient inward current in the action potential. The Na/Ca exchange of the SL can also transport Ca^{2+} out of the cytosol; however, the affinity for Ca^{2+} export is low (2 to 20 μM).[116] This may be due to inherent problems in purification and reconstitution of the enzyme, or the Na/Ca exchange may only be active for efflux of $[Ca^{2+}]$i in Ca-overloaded systems, such as during heart failure.

Intracellular calcium overload. Calcium overload, i.e., an excessive increase in total [Ca]i, does not result in a positive inotropic effect because the Ca is sequestered and/or bound. Calcium overload has been observed in the cardiomyoapthic Syrian hamster,[117] in the calcium paradox,[118] and during ingestion of large amounts of alcohol.[107] An increase in free or total [Ca]i does not augment cAMP[119] (Fig. 6). Calcium overload is often associated with a small increase in intracellular acidity[73] and inhibition of the Na/Ca exchange.[5] The increase in H^+ may be due to [Ca]i displacement of $[H^+]$i from protein sites.[121] $[H^+]$i competes with $[Ca^{2+}]$i for myofibrillar protein binding sites and sarcoplasm sites.[121] Such competition will shift the concentration curve for calcium vs calcium binding proteins toward higher Ca concentration, so that a given concentration of Ca^{2+} will have less effect e.g., less pressure will be generated for a given calcium release but more calcium will remain free in the myoplasm. Calcium overload resulting from energy deficiency is associated with a large increase in Pi.[17] The increase in Pi may immobilize Ca.[122] High Pi concentration has been shown to cause a depressive effect on tension development[22] and calcium sensitivity of the myocardium.[23,24] An increase in Pi from 1 to 20 mM can reduce tension by 50% at a given Ca concentration.[22] Ca overload can also cause an excessive Ca uptake by the mitochondria, resulting in inhibition of enzymes essential to the Citric Acid Cycle, thereby depressing mitochondrial function.[123] In cases of Ca overload, the free calcium may be normal, not influencing the inotropic state, while large amounts of calcium may be immobilized.[124] On the other hand, free [Ca]i may be high but so may [Pi]i and $[H^+]$i, competing for Ca binding sites.[125] Such competition occurs with membrane-bound ATPase sites that sequester Ca, as well as those of myosin[126] and troponin.[127]

Regulation of intracellular calcium

Beta-adrenergic activation. Activation of beta-adrenergic sites, and the subsequent increase in cAMP, leads to an increase in total intracellular calcium,[128] resting levels of calcium,[129] and calcium transients.[130] A cascade of events occurs with an elevation in cAMP, leading to protein phosphorylation and enzyme activation. Cyclic AMP may regulate the slow inward current[130] via the L channels in cardiac muscle. Fig. 7 demonstrates how beta-adrenergic activation, via isoproterenol, augments cAMP. Beta activation resulting in cAMP synthesis is a stronger positive inotropic agent than adminis-

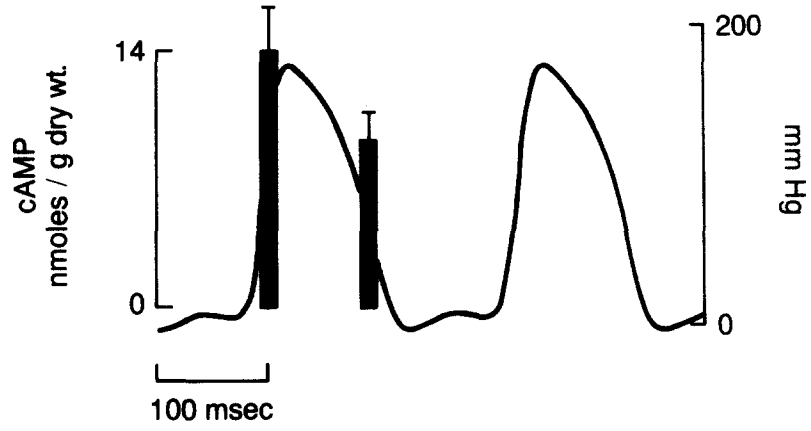

Fig. 8. Similar to analysis described in Fig. 7, hearts were analyzed for cAMP after addition of isoproterenol (10^{-7} M). Isoproterenol raised both the resting level and the transients in cAMP.

tration of pure dibutyrl cAMP. This may be due to the advantageous compartmentation of cAMP when synthesis is evoked by beta activation. Fig. 8 demonstrate how addition of dibutyrl cAMP, which is taken up by the cell, causes an increase in the rate of pressure development, peak systolic pressure, and the rate of relaxation. Activation of calcium channels occurs by an increased time open, density, and/or conductance as demonstrated with administration of dihydropyridine. Phospholamban, an important coenzyme (Ca^{2+}-activated ATPase) in the SR for sequestering calcium, is activated by a cAMP-dependent phosphorylation.[131] With depolarization, there is an increase in cAMP. Isoproterenol increases both the resting level and the transient flux of cAMP. The flux of cAMP is not essential to contraction, since the flux does not occur when a beta blocker, e.g., propranolol, is added to the perfusate (Fig. 9). Beta-adrenergic activation, and the subsequent increase in cyclic AMP, increase total intracellular calcium,[129] calcium transients,[27,130] and the resting level of calcium.[131] Cyclic AMP also lowers the resting level of calcium during heart failure of the cardiomyopathic hamster.[132] This may occur due to cAMP-dependent phosphorylation of the Na/Ca exchange, activating the efflux as well as the influx of calcium.[133] The direction of the Ca exchange is influenced by both the Na and calcium gradients.[134] Thus, cAMP augments ventricular pressure by increasing and regulating the flux of calcium, as well as increasing the ATPase activity of regulated actomyosin.[135]

Hydrodynamics. Water movement also influences the distribution of calcium. Rate of flow and volume in the coronaries is influenced by perfusion pressure and vasodilation. Perfusion pressure is the head pressure that regulates the rate of water exchange throughout the heart. Starling[136] has demonstrated that an increase in perfusion pressure augments filtration of coronary fluid at the arterial end and increases reabsorption at the venous end of capillaries.[136] Hydrostatic pressure forces more fluid out of the capillary walls,[137] causing an increase in interstitial volume with an increase in hydrostatic pressure.[138] An increase in interstitial volume causes an increase in intracellular volume, due to the dynamic diffusion of water between interstitial tissue and intracellular space.[139,140] This is diagrammatically depicted in Fig. 10. Such an increase in hydrostatic pressure and a subsequent increase in intracellular volume have been shown to increase cation exchange, especially calcium, in cells such as red blood cells,[141,142] endothelium of the vasculature,[143] and the myocardium.[6] There is an interchange of water between the vasculature and interstitial space within microseconds,[138] and between the interstitial space and the intracellular space within milliseconds.[143] Water is forced out of the stretched endothelial cells of the vasculature[143] as well as through clefts.[143] Water then passively diffuses into cells through water channels.[144] It is believed that these water channels may be the same as ionic channels.[144] Thus, a decrease in hydrostatic pressure causes an immediate and rapid decrease in both intracellular and extracellular water, along with a decrease in cations such as calicum. This can be demonstrated by a decrease in hydrostatic pressure to 0 cm H_2O, where there continues to be an efflux but no influx of water.[6]

Cardiac ventricular muscle is a multicellular preparation in which the electrical source strength is strongly influenced by the interstitial potential.[145] The interstitial potential is regulated by the interstitial volume and the latter is regulated by hydrostatic

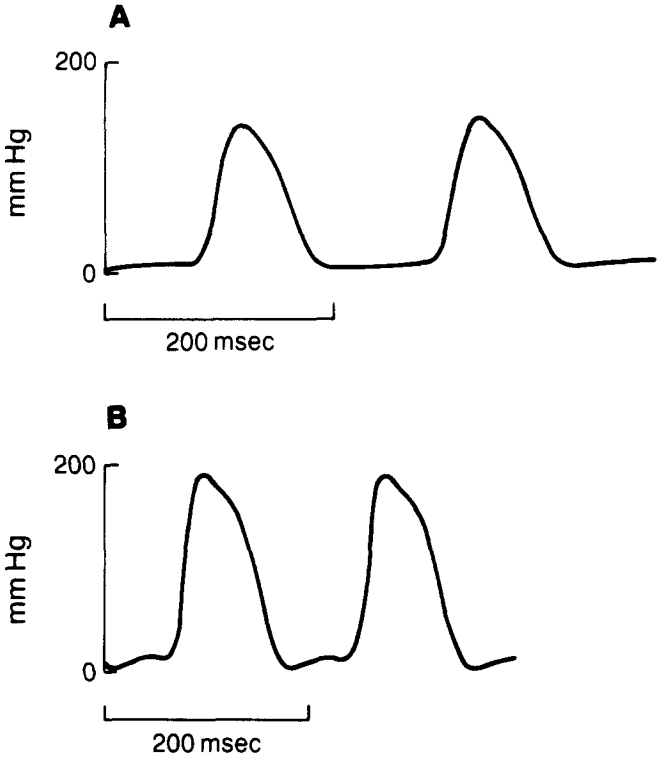

Fig. 9. Dibutyrl cAMP (10^{-7} M) was added to the perfusate *(B)*. Within seconds, the physiologic parameters changed. *(A)*, Control hearts with no dibutyrl cAMP added.

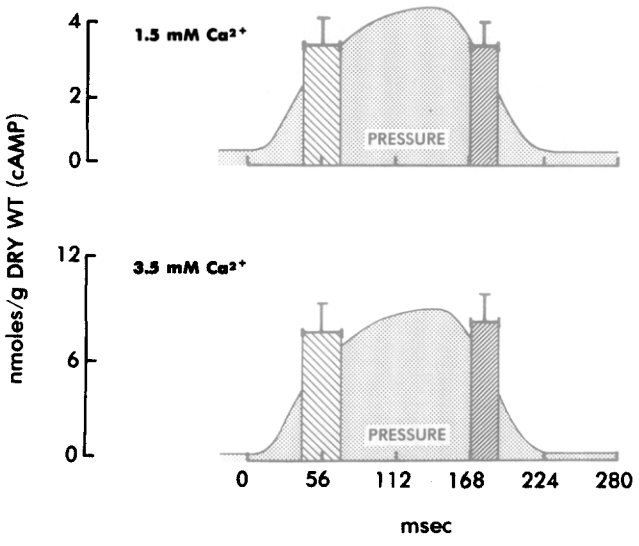

Fig. 10. Addition of propranolol (10^{-5} M) obviated transients in cAMP, indicating that fluxes of cAMP are not essential to contraction.

pressure, i.e., perfusion pressure.[146] A decrease in interstitial space depresses capillary filtration,[147] cell volume,[6] electrical potential,[148] and cation exchange,[149] factors that influence ventricular pressure.[6] With a decrease in perfusion pressure, there is a decrease in coronary flow, a narrowing of the capillary bed, a decrease in interstitial space, a decrease in cell volume, and a thinning of the ventricular free wall.[150] These changes occur in 10 to 30 seconds. After 20 minutes, energy levels fall and edema ensues. Changes in myocardial water content can be studied by H-1 NMR in the presence and absence of shift reagents, two-dimensional echocardiography, and total myocardial water measurement, combined with use of extracellular markers.[150]

*Ion gradients.*Sodium enters the cell by voltage-dependent channels, such as the fast Na channel, and by exchangers such as Na/H and Na/Ca exchange. The exchangers are potentially reversible, the direction of operation depending on the gradients for each ion, the stoichiometry, and the membrane potential.[72] Enzyme reactions and exchange mechanisms, which influence the Na gradient, also modulate $[Ca^{2+}]i$. For example, cardiac glycosides inhibit Na-KATPase and thus elevate [Na]i. The increase in [Na]i is partially allevated by activating the Na/Ca exchange, thereby increasing [Ca]i.[72] As [Ca]i increases, it binds to intracellular protein sites, releasing H^+ and causing an increase in $[H^+]i$.[151] This leads to a further increase in [Na]i via the Na/H exchange.[152] The Na/H exchange is an important mechanism for pHi homeostasis. Activation of the Na/H exchange results in a further increase in [Ca]i due to an extended activation of the Na/Ca exchange.

SUBSTRATE AND END PRODUCTS OF MYOFIBRILLAR ATPASE

ATP. The substrate for crossbridge interaction, i.e., ATP, is in excess concentration in myocardial cytosol, approximately 8 mM in rat heart and 10 mM in hamster heart, for example.[15] The concentration at which enzymes work at half maximum rate is 0.18 mM ATP for Ca^{2+}-ATPase[153] and 0.05 mM for crossbridge interaction.[16] Two millimolar ATP is required for the transient inward current. Thus, ATP is usually not a rate-limiting factor during physiologic conditions. The pools of ATP in cytosol vs that of the mitochondria are distinct, but are bridged by the creatine kinase shuttle.[154] Inhibition of mitochondrial enzymes, such as mitochondrial ADP-ATP translocase (inner membrane), may regulate the ATP pool.[155]

ADP. Only free-ADP reacts with creatine kinase, thus bound ADP is not considered in relation to the

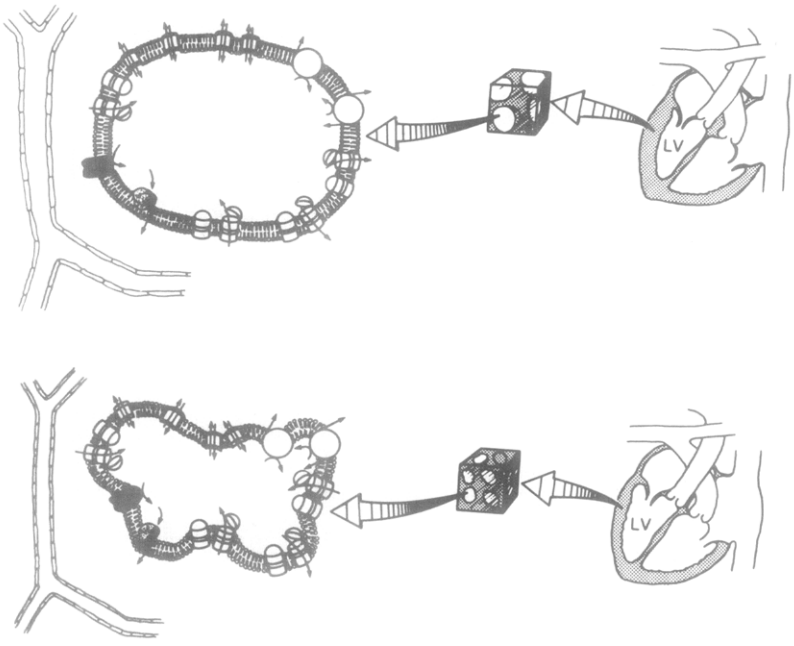

Fig. 11. Diagrammatic representation of the heart perfused at 140 cm H_2O *(upper figure)* and at 80 cm H_2O *(lower figure)*. As determined by two-dimensional echocardiography, there is a thinning of the left ventricular wall with a decrease in perfusion pressure,[6] and a decrease in intracellular and extracellular water as determined by H-NMR with the shift reagent Dy(TTHA-3). With a decrease in perfusion pressure, there is less volume in the capillaries[136-138] as well as in the myocardial cells.[6]

energy potential. For this reason, free-ADP is calculated from the creatine kinase equilibrium and is only a theoretical value: $[ADP_{free} = (ATP)\ (Cr)/(KEq)\ (PCr)\ (H^+)]$. The KEq is that for creatine kinase. Because the KEq is large, then the free-ADP value becomes very small, approximately 0.02 mM cytosolic,[156] whereas total ADP is approximately 1.0 mM cytosolic.[156] Since ADP is bound during the shortening phase of the cardiac cycle,[156, 157] and since ADP is utilized by creatine kinase for production of ATP, resynthesis of ATP occurs during the resting phase of the cardiac cycle.[156] In cases where ATP and total ADP remain relatively constant, free-ADP can increase, principally due to the rapid changes in Cr/PCr. Since cytosolic changes can take place that cause alterations in free- and bound ADP, calculated free-ADP may not always be precise.

PCr. Phosphocreatine is present in rat myocardial cytosol at about 16 mM, and total creatine is approximately 26 mM.[4] During maximum working conditions, the heart utilizes approximately 0.4 mM phosphocreatine per beat. Fluxes in phosphocreatine can be directly observed in the perfused heart[158] and in situ[159] during maximum working conditions with glucose as the substrate. The heart needs to consume at least 0.32 μmol O_2/gm dry weight/beat to observe such cyclical changes.[158] With glucose as the substrate (as compared to pyruvate), mitochondrial activity is limiting. This is observed by differences in the redox state of isolated perfused hearts between the two substrates (Fig. 11). Surface fluormetry utilized in these experiments detects mitochondrial reduced nicotinamide adenine dinucleotide (NADH).[160] Cytosolic NADH is 99% bound and is not detected by surface fluorometry.[161] There is a phosphocreatine shuttle between the mitochondria and the cytosol.[162] Creatine kinase is bound to myosin and immediately replenishes ATP after it is hydrolyzed, utilizing the released ADP as substrate. Because the creatine pool is considerably higher (PCr, 16 mM) (Cr, 10 mM) than ADP (0.02 MM), and since ADP binds to several proteins, this buffering system is needed for rapid replenishing rather than depending on the diffusion of ADP. Because of the large concentration of PCr, the rapid rate of creatine kinase, and the binding of creatine kinase to myosin, ATP remains relatively constant during working conditions. When oxygen consumption is high and with glucose as the substrate, ATP fluctuates between 0.2 and 0.4 mM/cardiac cycle during systole, when ADP is bound.[156]

Phosphorylation potential and oxygen consumption. The free energy of ATP hydrolysis ($\Delta G°_{KEq}$ + RT 1n [ADP] [Pi]/[ATP]) is a differential negative quanti-

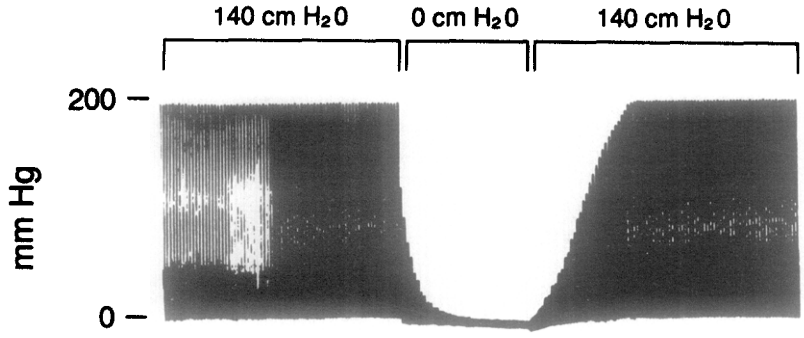

Fig. 12. With a decrease in perfusion pressure from 140 to 0 cm H_2O, there is an immediate decrease in developed systolic pressure.

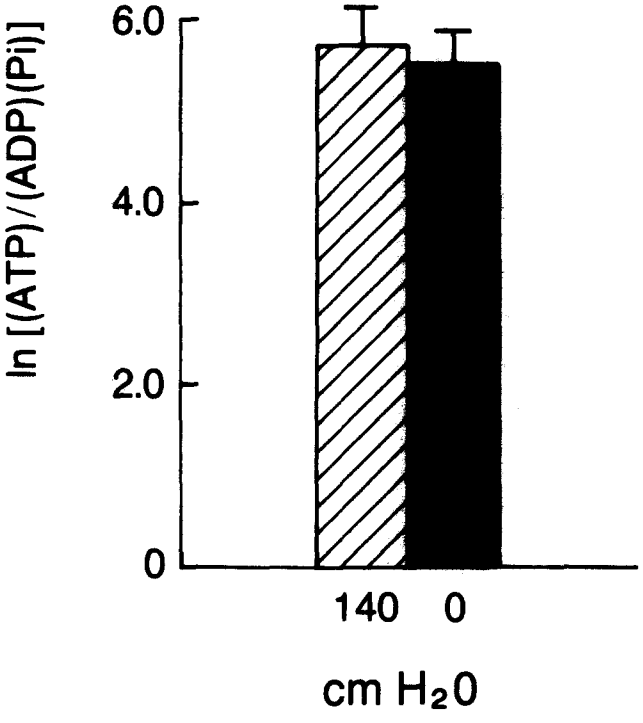

Fig. 13. With an abrupt decrease in perfusion pressure from 140 to 0 cm H_2O (10 seconds), there is no change in the phosphorylation potential.

ty whose magnitude depends on the KEq of ATP hydrolysis. An alternate form is the positive thermodynamic affinity $\Delta G°_{ATP}$, i.e., the extent of ATP hydrolysis,[162] $(-\Delta G°_{ATP} + RT \ln [ATP]/[ADP][Pi])$. This can be reduced to the ln phosphorylation potential (ln [ATP]/[ADP] [Pi]) during standard conditions, i.e., constant temperature, pH, and atomospheric pressure. With this equation, even when there is no decrease in ATP but a 10% decrease in PCr (e.g., rat heart-control concentration 16 mM), there is a corresponding 160% increase in Pi (control concentration 1 mM) and a large increase in ADP (control concentration 0.02 mM).[4] However, even under severe conditions such as stopping all flow to the heart, there is an immediate decrease in developed pressure (Fig. 12) at a time where there is no decrease in the phosphorylation potential (Fig. 13). Control values are not the same for hearts of all species. For example, in the hamster the control value for ATP is 10 mM, Pi is 7 mM, PCr is 12 mM, and creatine pools is 24 mM,[26,117] thus making the control phosphorylation potential smaller than that of the rat.[26,117] Representative P-31 NMR spectra of rat and hamster hearts are shown in Fig. 14. During conditions where the hydrostatic pressure no longer augments coronary flow, there is no further increase in the phosphorylation potential. Under these conditions, if physical work is further increased such as increasing balloon volume in the isovolumic heart,[163] or after increasing afterload pressure in the apex ejecting heart,[164] oxygen consumption further augments and there is a discrepancy between energy supply and demand.

[pH]i. The intracellular pH influences the activity of several important cytosolic enzymes,[165] including glycolytic enzymes,[166] membrane ATPase activity,[167] and mitochondrial enzymes,[168] and competes with Ca^{2+} for SR binding sites, thus influencing relaxation.[169] Protons (between pH 6.6 and 7.15) also compete for Ca sites,[165] thus influencing contractility, since Ca bound to SL proteins plays a critical role in Ca uptake and amplitude of contraction.[165] At high H^+ concentration, higher Ca is required to obtain half maximum ATP fluxes.[20,170,171] The Na/Ca exchange in the direction of Ca influx is linearly related to [pH]i between pH 6.0 and 7.0, with maximum activity at pH 7.4. The enzyme is directed toward Ca efflux at the lower pH.

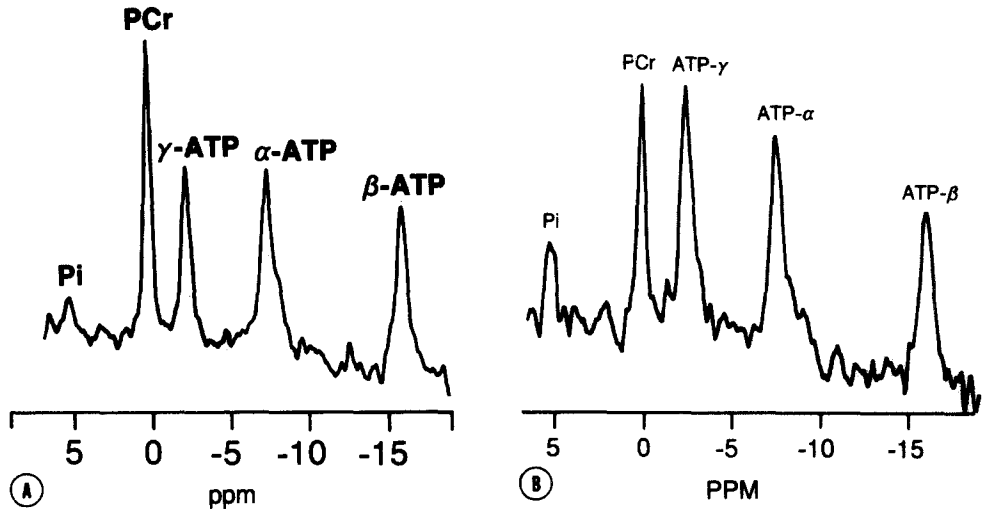

Fig. 14. *A*, P-31 NMR spectra of rat heart showing the values for high energy phosphates and intracellular inorganic phosphate. *B*, P-31 NMR spectra of hamster heart showing the values for high energy phosphates and intracellular inorganic phosphate. There is 10 mM ATP in hamster hearts and 8 mM in rat hearts, whereas there is 16 mM PCr in rat hearts and 12 mM in hamster hearts. [Pi]i is 1 mM in rat hearts and 4 mM in hamster hearts.

CONCLUSIONS

Mechanics, hydrodynamics, cell volume, electrogenicity, ion gradients, stretch of myofibers, beta-adrenergic activation, and the action potential may be the most important regulators of systolic pressure in the heart by control of [Ca]i transients. For example, perfusion pressure directly regulates developed pressure by causing an increase in cell volume.[6] An increase in cell volume is associated with a concurrent increase in [Na]i.[172] By the Na/Ca exchange there is a corresponding increase in [Ca]i, with an increase in cell volume and [Na]i,[173] except during acidotic conditions.[173] During acidotic conditions, the Na/H exchange is activated,[174] interchanging [Na]i for [H^+]o, resulting in an increase in an increase in [H^+]i with an increase in volume.[173] In another example of [Ca]i regulation, cAMP increases [Ca]i by causing an increase in functional calcium channels,[175] as well as increasing the kinetics in opening and closing of calcium channels.[175] On the other hand, cAMP also increases the efflux of [Ca]i by activation of the Na/Ca exchange and Ca-activated ATPase, as described above. Stretch of myofibers may increase [Ca]i[45] by making more calcium channels available at the intercalated discs. There are stretch-activated calcium channels in most myocardial fibers. Total [Ca]i, for example, increases when the endocardium is stretched by large increments of balloon volume in an isovolumic heart (Auffermann et al, submitted for publication). Transients of calcium that oscillate with the cardiac cycle also regulate matrix free-Ca^{2+} in the mitochondria,[176] thereby controlling Ca-dependent matrix dehydrogenases and setting limits to NADH production during high workloads. Intracellular [pH]i and [Pi]i may be important in regulating Ca sensitivity of cytosolic proteins by competing for Ca sites and shifting the myocardial requirements of contractility toward higher [Ca]i concentrations. The latter may result in higher resting levels of [Ca]i by delaying relaxation. Under these conditions, an increase in heart rate would lead to calcium loading.[171] The phosphorylation potential, which is a sensitive index of the energy state of the heart, may set limits to contractility and developed intraventricular pressure as well as recovery from a depressed state by influencing the thermodynamics of various enzymes.

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