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Review

Structure and function of cardiac potassium channels

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Abstract

Recent advances in molecular biology have had a major impact on our understanding of the biophysical and molecular properties of ion channels. This review is focused on cardiac potassium channels which, in general, serve to control and limit cardiac excitability. Approximately 60 K^+ channel subunits have been cloned to date. The (evolutionary) oldest potassium channel subunits consist of two transmembrane (Tm) segments with an intervening pore-loop (P). Channels formed by four 2Tm–1P subunits generally function as inwardly rectifying K^+-selective channels (*KirX.Y*): they conduct substantial current near the resting potential but carry little or no current at depolarized potentials. The inward rectifier I_{K1} and the ligand-gated K_{ATP} and K_{ACh} channels are composed of such subunits. The second major class of K^+ channel subunits consists of six transmembrane segments (S1–S6). The S5–P–S6 section resembles the 2Tm–1P subunit, and the additional membrane-spanning segments (especially the charged S4 segment) endow these 6Tm–1P channels with voltage-dependent gating. For both major families, four subunits assemble into a homo- or heterotetrameric channel, subject to specific subunit–subunit interactions. The 6Tm–1P channels are closed at the resting potential, but activate at different rates upon depolarization to carry sustained or transient outward currents (the latter due to inactivation by different mechanisms). Cardiac cells typically display at least one transient outward current and several delayed rectifiers to control the duration of the action potential. The molecular basis for each of these currents is formed by subunits that belong to different *Kvx.y* subfamilies and alternative splicing can contribute further to the diversity in native cells. These subunits display distinct pharmacological properties and drug-binding sites have been identified. Additional subunits have evolved by concatenation of two 2Tm–1P subunits (4Tm–2P); dimers of such subunits yield voltage-independent leak channels. A special class of 6Tm–1P subunits encodes the 'funny' pacemaker current which activates upon hyperpolarization and carries both Na^+ and K^+ ions. The regional heterogeneity of K^+ currents and action potential duration is explained by the heterogeneity of subunit expression, and significant changes in expression occur in cardiac disease, most frequently a reduction. This electrical remodelling may also be important for novel antiarrhythmic therapeutic strategies. The recent crystallization of a 2Tm–1P channel enhances the outlook for more refined molecular approaches.

1. Introduction

Cardiac K^+-selective currents carry outward currents in the physiological range of potentials. Therefore, they act either to set the resting potential near the K^+ equilibrium potential or to repolarize the action potential. The long duration of the cardiac action potential is necessary to control contraction and to prevent premature excitation. This waveform is the net result of a finely tuned coordinated action of multiple ionic conductances that activate and inactivate on different time scales (Fig. 1); potassium currents are involved in nearly all phases.

The upstroke of the action potential – caused by inward sodium current – is followed by a partial early repolarization caused by outward potassium flux through rapidly activating and inactivating K^+ channels. The extent of this early repolarization (notch) affects the time course of the other voltage-gated currents and, therefore, controls indirectly the action potential duration (APD). The plateau phase depends on a delicate balance of inward (depolarizing) and outward (repolarizing) currents. The depolarizing force is mainly a Ca^{2+} influx which slowly declines as L-type Ca^{2+} channels inactivate, but also non-inactivating Na^+ current can support the plateau phase. The repolarizing action depends on K^+ efflux due to activation of

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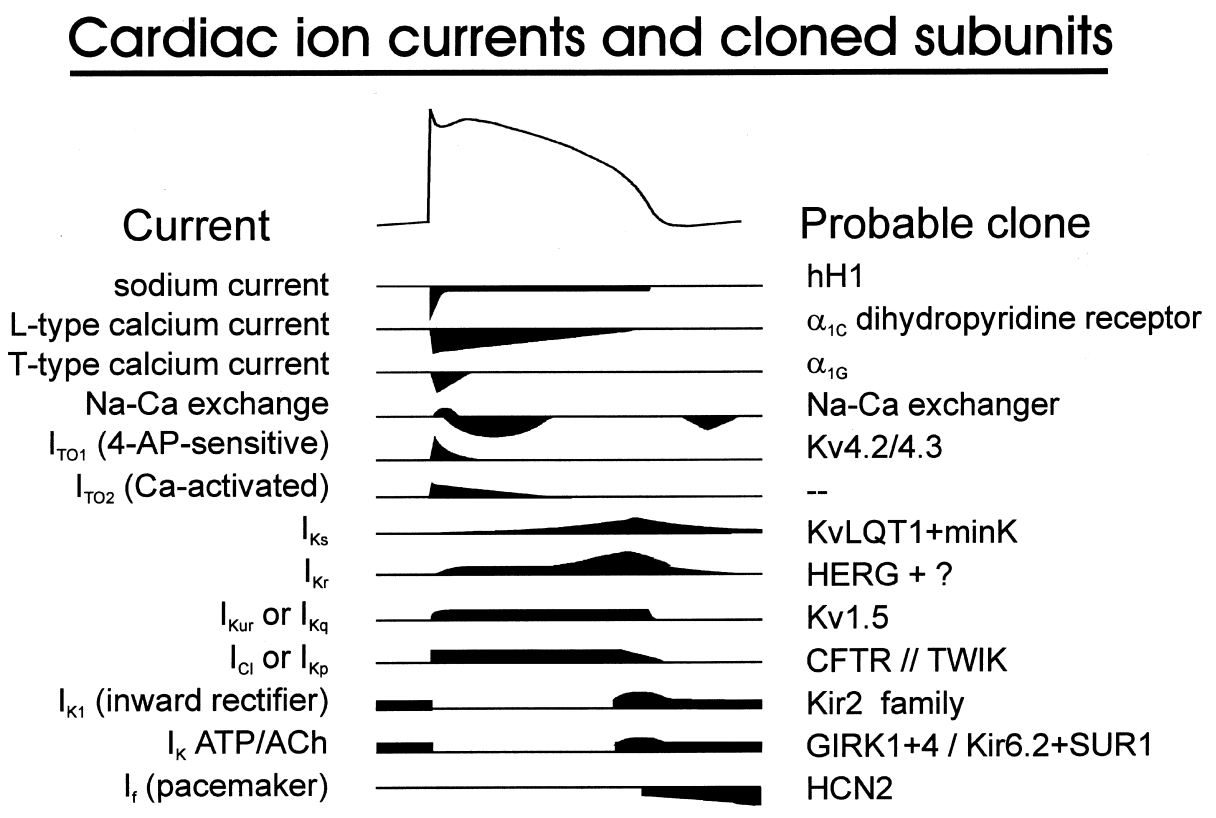

Fig. 1. Ionic and molecular basis of the cardiac action potential. Schematic indication of the time course of depolarizing inward currents (downward) and repolarizing outward currents (upward). The established or most probable corresponding clones are indicated. Abbreviations: see text.

several voltage-gated potassium channels. The sequential activation of distinct channels over different time frames represents a system with built-in redundancy to control and ensure the repolarization of the cell. Several other conductances maintain or modulate the resting potential, most of which are inwardly rectifying K^+ channels which carry (almost) no current during the plateau phase.

Separation of these K^+ currents was not trivial and relied in part on distinct physiological and pharmacological properties. The molecular cloning of K^+ channel subunits raised initially the hope of rapidly establishing the molecular nature of the various currents. However, progress over the past decade has revealed an impressive molecular diversity in K^+ channel subunits (more than 60 cloned to date). These new tools have led to an increased appreciation of the complex heterogeneity of expression of cardiac K^+ currents and underlying subunits (e.g. regional, developmental and pathological heterogeneity). The potentially heteromeric assembly of multiple α- and/or accessory β-subunits has presented a considerable challenge to establish the molecular architecture of channels identified in native myocytes. This is an important problem since these channels represent a major molecular target for class III agents (antiarrhythmic drugs that act by prolonging APD). Correct identification of the molecular architecture should help in drug development in the face of the emerging molecular view of drug binding and the recent crystallization of a K^+-channel. In this review we will address (1) the molecular structure and function of pore-forming α-subunits and accessory β-subunits, (2) the molecular architecture of the native cardiac K^+ currents in terms of these subunits, and (3) some factors underlying heterogeneity in expression.

2. Molecular structure and function of cloned K^+ channel subunits

Ion channels are membrane-spanning proteins with three essential functional properties: (1) a central tunnel or pore through which ions flow down their electrochemical gradient, (2) a selectivity filter that dictates which ion(s) are allowed to cross the pore, and (3) a gating structure that controls switching between open and closed conformations and thus determines whether permeation occurs (for review see Hille, [1]). These proteins are more efficient than enzymes since a single conformational change allows permeation of up to 10^8 ions/s. Ion channels may be gated by electrical, chemical or mechanical forces which are detected by a sensor that is linked to the access gate. The behavior of ion channel can be modified by intracellular processes, toxins or drugs; the latter makes them potential molecular targets for therapeutic drugs. The molecular cloning of channel subunits over the past decade has demonstrated a large number of genes with a remarkably preserved basic blueprint.

2.1. Voltage-gated potassium channels (6Tm–1P)

2.1.1. Molecular diversity of gene products

The successful cloning and expression of the first voltage-gated K^+ channel from the *Drosophila Shaker* mutant [2] was the starting point for investigations to identify many subfamilies, and to identify the pore, voltage sensor, gates and drug/toxin binding sites at the molecular level. Initially, four *Drosophila* subfamilies were described (*Shaker, Shab, Shal, Shaw*), and the first cloned mammalian K^+ channels were related to these subfamilies, including those cloned from rat and human heart [3,4]. The mammalian *KvX.Y* nomenclature is shown in Fig. 2, in which Kv reflects K channel, voltage-gated, *X* represents the subfamily and *Y* the number of the gene within the subfamily. The encoded protein contains six membrane spanning segments (S1–S6, Fig. 2), and the amino- and carboxy-terminal ends are located intracellularly. Assembly of four α-subunits into a tetrameric structure is needed to create a functional K^+ channel (Fig. 2). Other K^+ channel subunits with the same molecular structure have been cloned in part on the basis of their involvement in congenital arrhythmias: *KvLQT1* and *HERG*. Unfortunately they have not been assigned formal numbers in *Kv.XY* convention, resulting in the present hybrid nomenclature. The following sections summarize mutagenesis studies from many laboratories that resulted in the identification of the molecular domains involved in specific channel functions as postulated from classical biophysics.

2.1.2. Conduction pathway and selectivity filter

The ion conduction pathway consists of a sequence of approximately 20 amino acids (P-region) between the S5 and S6 segments, with contributions of S6 and the S4–S5 linker (Fig. 2). The subunits are oriented such that the S5–P–S6 sections face each other creating the central pore. Consistent with the functional view, mutagenesis has revealed that the selectivity filter forms an essential part of the overall permeation pathway. The narrow P-region dictates the K^+ selectivity: its amino acid sequence motif (TxTTx)GYG is now considered the K^+-selectivity signature motif (for review see [5]). The extracellular access to the pore consists of the exposed part of the P-loop and adjacent residues between S5 and S6 and forms the binding site for many toxins. The intracellular sections of S5 and S6 presumably line a wider internal mouth of the channel: the S4–S5 linker forms (part of) the receptor for the inactivation 'ball', and mutations in S6 affect properties of the open pore such as single channel conductance and block by internal TEA or antiarrhythmic drugs such as quinidine [6,7].

2.1.3. Voltage sensor

The S4 segment contains a basic residue at approximately every third position, resulting in a regularly spaced array of 5–7 positive charges. This segment represents the major component of the voltage sensor for gating, although negative charges in S2 and S3 also contribute [8]. The accessibility of S4 amino acid side-chains differs between the closed and open channel conformation, and most of the transmembrane voltage drop occurs over a few S4 residues (for review see [8]). Thus, S4 is not heavily buried inside the protein, but moves within a water-filled hourglass-like channel or crevice ('canaliculus', Fig. 2) between the other helices. Depolarization of the membrane causes a physical (outward) movement of S4 which then induces further conformational changes that open the channel and permit selective K^+ permeation. This movement can been monitored electrically as the gating current [9], or by means of fluorescence [10,11]. The nature of the gate that effectively opens the pore has not been fully established. However, several residues in S6 are inaccessible in the closed state, but become accessible upon depolarization which may indicate that this pore-lining segment also contributes to the activation 'gate' [6].

2.1.4. Inactivation gate(s)

After initial activation some K^+ channels inactivate, i.e. enter a non-conducting state during maintained depolarization. At least two types of inactivation have been identified (N- and C-type) which are associated with distinct molecular domains. The N-terminus is involved in the fast 'ball and chain' (N-type) inactivation [12] of *Shaker* K^+ channels: once the channel has opened, this N-terminal domain moves into the internal mouth and occludes the pathway (Fig. 2). After removal of this region, inactivation can be restored by the corresponding synthetic peptide, and an NMR structure of this domain has been obtained [11]. The second inactivation mechanism (C-type inactivation) is modified by mutations in the S6 and P regions [13]. This inactivation process is usually slow and incomplete, exists in almost all K^+ channels and may reflect a slow constriction of the pore [14].

2.1.5. Subunit interactions and assembly domains

The explosive growth of K^+ channel subfamilies and genes raised the problem of an almost unlimited number of potential tetrameric subunit assemblies. However, each subunit appears to contain 'identity tags' that mediate subunit–subunit interactions and restrict coassembly. Highly conserved domains immediately preceding the S1 segment are involved in subunit assembly for the Kv1 through Kv4 subfamilies (Fig. 2). These domains (called T- or Nab-domain) prevent heteromeric assembly of subunits belonging to different *Shaker*-like subfamilies [15]. For the more distantly related *HERG* and *KvLQT1* subfamilies assembly may primarily involve C-terminal domains [16], as is the case for the *Drosophila eag* and plant ATK1 K^+ channels [17,18].

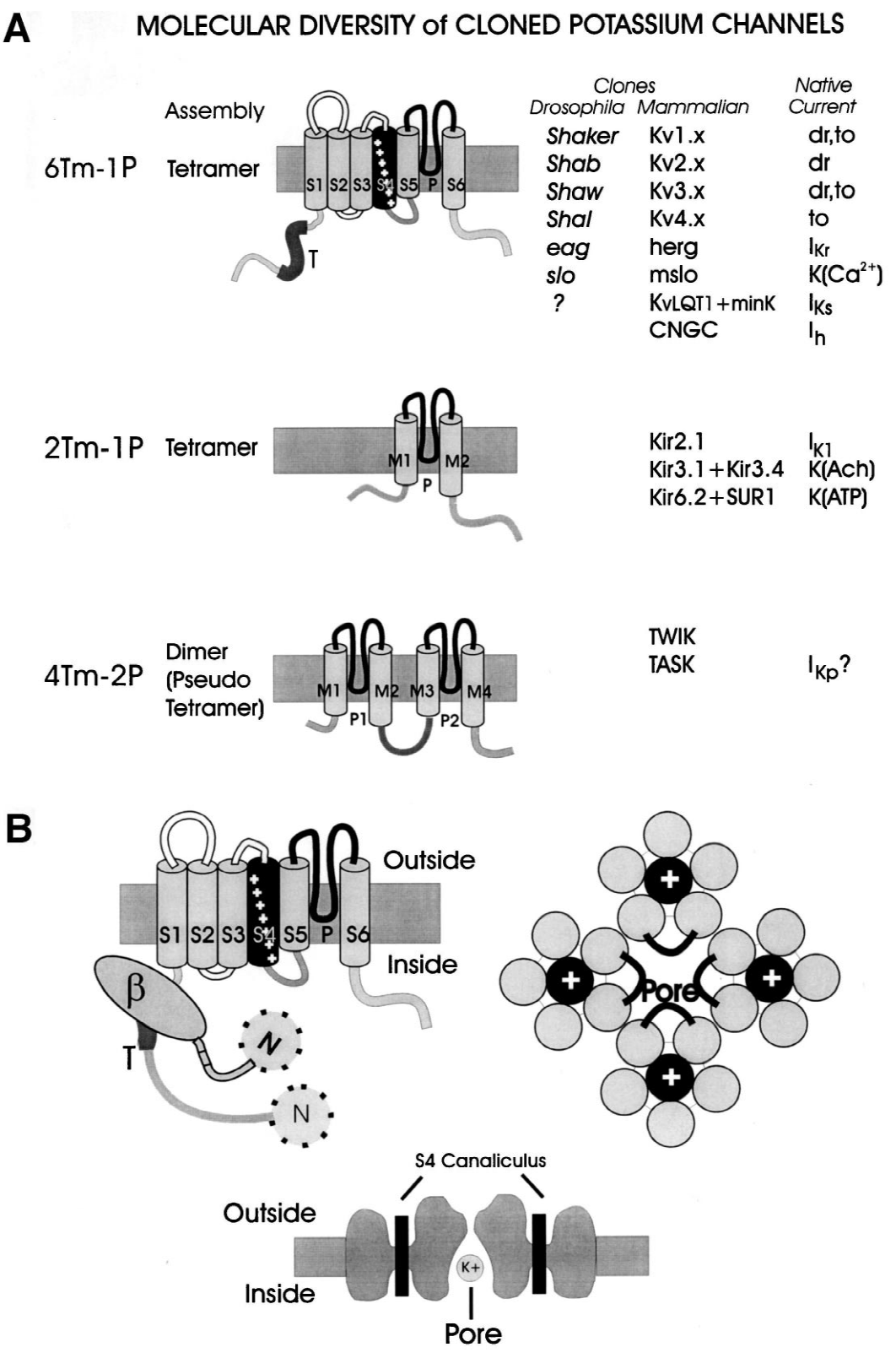

Fig. 2. Molecular structure of K^+ channel subunits. (A) Schematic representation of the putative membrane folding of cloned K^+ channel subunits. Top: subunits with six transmembrane segments (S1–S6) encode voltage–gated channels. P represents the P-loop. Middle: subunits with two transmembrane segments (M1, M2) encode inward rectifiers, and correspond to the S5–P–S6 section of 6Tm–1P subunits (top). Bottom: duplication of this core structure in subunits encoding tandem pore domains (P1, P2). Mammalian *Kv1–Kv4* families encode delayed rectifier (dr) or A-type transient outward (to) currents. Where possible, corresponding native currents are indicated. (B) The bottom diagrams indicate the assembly of the voltage gated channels with a central pore between the four subunits as well as the N-terminal inactivation 'ball' of Kvα and Kvβ subunits (dashed since not all have this structure).

2.2. Inward rectifier potassium channels (2Tm–1P)

A second major class is formed by of K^+-selective channel subunits that contain only two membrane-spanning segments (M1, M2) with an intervening P-loop (Fig. 2), homologous to the S5–P–S6 pore section of *Shaker*-like channels [2]. Channels formed from these subunits lack typical voltage-dependent gating and generally display K^+-selective inwardly rectifying currents, hence the Kir*X.Y* nomenclature. The K^+-selectivity signature sequence GYG from the *Shaker* K^+ channels is remarkably preserved in the P-loop of the Kir subunits. The striking degree of conservation of the overall ion conducting part (S5–P–S6) of *Shaker* channels and the M1–P–M2 section of the Kir channels suggests that the voltage-gated channels evolved from the simpler Kir structure by incorporating additional domains needed to acquire voltage sensing and gating (e.g. S4).

We will designate the six-transmembrane *Shaker* channels with a single pore domain as 6Tm–1P, and the Kir subunits as 2Tm–1P. Recently, additional variants on these basic themes have been recognized: subunits with tandem M1–P–M2 domains (4Tm–2P), e.g. TWIK [19] and *Shaker*-like subunits with a second pore domain (8Tm–2P), e.g. TOK1 [20]. Interestingly, the GYG K^+-selectivity motif is preserved in the first pore domain of most 4Tm–2P subunits, but replaced by GFG or GLG in the second pore. Thus, the fourfold symmetry of homomeric Kv and Kir channels is reduced to a twofold symmetry in the channels with tandem pore subunits.

2.3. First crystal structure of the potassium channel pore

The molecular biology of K^+ channels indicates that the pore structure for selective permeation is well preserved but structural implications remained speculative despite the combined power of biophysical and molecular approaches including mutagenesis and molecular modeling. The conserved 2Tm–1P blueprint with the GYG K^+-selectivity signature facilitated cloning of a homologous bacterial K^+ channel (KcsA) from *Streptomyces lividans* enabling MacKinnon and colleagues to obtain a crystal structure of this 2Tm–1P channel with a 3.2 Å resolution [21]. The X-ray analysis confirms that the channel contains four identical subunits that each have two transmembrane α-helices and an intervening P-domain (Fig. 3). The subunits create an inverted cone (teepee), in which the α-helices resemble the poles of the teepee. The selectivity filter fills the wider base on the extracellular face. The overall length of the pore is 45 Å. It starts from the inside with a tunnel (18 Å long, ~6 Å wide) which opens into a 10 Å wide cavity followed by the narrow selectivity filter (12 Å). Part of the P-segment is α-helical; these four helices point their electronegative carboxyl end toward the central cavity which helps stabilizing the potassium ions. The selectivity filter is lined by the carbonyl oxygen atoms of the GYG signature sequence. The tyrosine (Y) points away from the pore and interacts with other conserved aromatic residues of the pore helices. Thus a fairly rigid donut of 12 interlocking aromatic amino acids is formed around the selectivity filter that holds the GYG backbone at the optimal distance from the center to achieve potassium selectivity: the four backbones form a pore with the carbonyl oxygens at the proper distance to compensate for cost of dehydration of a K^+ ion (~3 Å). Although the smaller Na^+ ions can fit physically in this pore, the diameter is too wide to substitute for the hydration energy of the Na^+ ions, i.e. the geometry cannot accommodate energetically the Na^+ ions. Interestingly, the inner tunnel

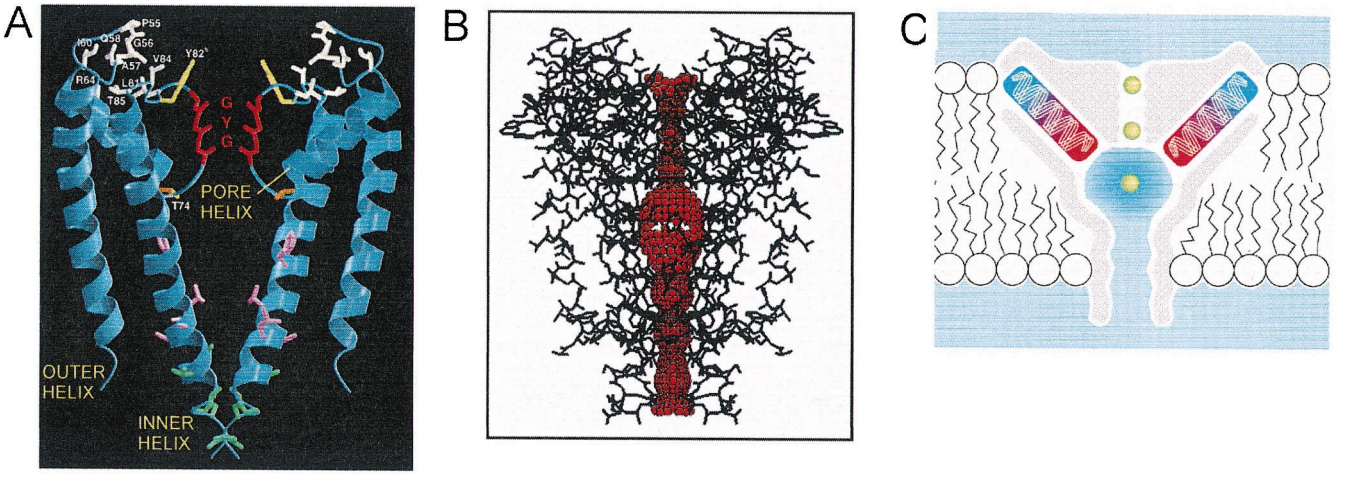

Fig. 3. Crystal structure of a 2Tm–1P potassium channel, KcsA. (A) Side view of the structure in flat ribbon display. The subunits to the front and back have been omitted for clarity. The outer and inner transmembrane helices cradle the selectivity with its pore helix and the GYG signature sequence. (B) Stick model of the full KcsA channel. The red surface represents the width of the central pore. (C) Diagram of the pore. K^+ ions (green) are stabilized deep into the pore by the aqueous central cavity and the electronegative C-terminal end of the pore helix. Reproduced with permission from Doyle et al., Science 280:69–77, 1998. Copyright 1998 American Association for the Advancement of Science.

is largely coated with hydrophobic side chains, which explains why binding of open channels blockers such as TEA (derivatives) or quinidine are stabilized by hydrophobic interactions [6,7]. The structure also underscores the non-linearity of the transmembrane electrical field: When these open channel blockers enter the pore from the cellular side, they get 'stuck' in the central cavity (>50% of the physical distance) but traverse only 15–20% of the voltage difference; the remaining 80% of the transmembrane drops over the 12 Å selectivity filter. The overall structure of the pore of voltage-gated K^+ channels is generally expected to be similar, given the impressive evolutionary conservation of the 2Tm–1P structure, although differences are to be expected given the conserved proline residues in the S6 helix of Kv channels.

2.4. Potassium channel β-subunits

Heterologous expression of the pore-forming α-subunits discussed above is sufficient to generate functional K^+ channels. However, an expanding family of function-altering β-subunits has been identified. The first β-subunits for Kv channels (Kvβ1.1 and Kvβ2.1) belonged to a family of cytoplasmic subunits that encode proteins of 360–400 amino acids [3,22] with ~85% homology between the ~330 C-terminal residues. The molecular and functional differences are due to the distinct N-terminal segments: the N-terminal sequence of Kvβ1.1 inactivates the delayed rectifier Kv1.1 with a ball-and-chain mechanism, similar to N-type inactivation in Kvα subunits (Fig. 2). Several splice variants of the Kvβ1.1 isoform have been cloned from heart [3,23–26]. They all induce inactivation in delayed rectifier channels, and display 100% identity with Kvβ1.1 in the C-terminal section, but <25% identity in the inactivating N-terminus. The Kvβ2.1 subunit does not induce fast inactivation but induces more subtle kinetic changes [27]. It is possible that Kvβ subunits also modulate functional expression and several groups are addressing the molecular nature of the interaction between the Kvα and Kvβ subunits [28,29].

The subunits of Kvβ1–Kvβ3 families associate with Kv1 α-subunits, while Kvβ4 associates with Kv2 family member(s) [30]. Additional K^+ channel associated proteins are being identified, e.g. KChAP which may act as a chaperone [31].

Other β-subunits are transmembrane proteins, e.g. the β-subunits of Maxi-K channels have two membrane-spanning segments [32]. A special case is formed by *minK*: this protein contains a single membrane-spanning segment that interacts with specific α-subunits as discussed below. Finally, some 6Tm–1P Kvα subunits do not sustain functional current when they form a homotetrameric channel, but they modify (in a heterotetrameric assembly) the current induced by other Kvα subunits. Thus, these non-functional α-subunits act as function-altering subunits: e.g. Kvα9.1/9.2 specifically modulates Kvα2.1/2.2 currents [33].

3. Molecular architecture of native cardiac currents

3.1. Criteria for assigning cloned channel subunits to endogenous cardiac currents

Since many cDNAs have been cloned from mammalian heart, the challenge is to establish in which cells these proteins are expressed. Aside from those clones that are positively implicated in the congenital LQT syndrome, this is not a trivial problem. Most cDNAs were cloned from tissue samples that include cells other than myocytes. Thus the crude assignment from whole tissue RNA, or the mere resemblance between some properties of a cloned channel with a native current is insufficient to assign a cloned K^+ channel subunit to a specific endogenous current. Furthermore, a native inactivating current could result from heteromeric assembly of delayed rectifier and inactivating α-subunits or from the association of a delayed rectifier α subunit with an inactivating β-subunit.

To test the hypothesis that a specific clone encodes the channel responsible for a specific myocyte current, the following criteria should be evaluated [3].

(1) The basic biophysical properties (kinetics and their voltage-dependence, conductance, rectification, ion selectivity) should be in reasonable agreement.

(2) The pharmacology of compounds known to interact directly with the channel pore, e.g. dendrotoxin, charybdotoxin, TEA, and quinidine, should be similar.

(3) Immunohistochemistry with isoform-specific antibodies made against the cloned subunits should confirm that the channel protein is present in the cardiac myocytes.

(4) Affinity-purification from native tissue should confirm the protein composition of the native channel in terms of accessory subunits and heterotetramer formation.

(5) Deletion of the cloned channel using in vitro antisense approaches or in vivo suppression should further confirm the identity of the current that this gene supports.

(6) Refined versions of these techniques should be used to identify the exact splice variant(s) of relevant K^+ channel subunits involved in the native channel.

Among the more than 12 cardiac K^+ currents (Fig. 1), two types of voltage-gated channels play a major role in determining repolarization: transient outward (I_{TO}) and delayed rectifier (I_K) currents [3,4]. I_{TO} activates rapidly and subsequently inactivates. The delayed rectifier I_K has several components [34,35]. The rapidly activating current (I_{Kr}) displays inward rectification and is sensitive to block by La^{3+}, flecainide, E-4031 and other methanesulfonanilides, while the slowly activating component (I_{Ks}) is insensitive to these drugs. A time-independent (background) K^+ channel has been described and designated as I_{Kp} [36]. Finally, a very rapidly activating K^+ current

(I_{Kur}), has been described in several species including human [4,37]. Establishing the molecular architecture and subunit composition of the protein complexes that constitute these native currents has been more challenging than initially anticipated, but leading candidates have emerged as discussed below.

3.2. Voltage-gated currents

3.2.1. Transient outward current (I_{TO}): Kv4.2/4.3

Activation of the 4-aminopyridine (4-AP) sensitive transient outward current (I_{TO}) causes the early rapid repolarization and notch of the cardiac action potential. As such, I_{TO} may influence the time course currents flowing during the plateau phase and modulate APD. Several K^+ channel subunits cloned from cardiac tissue including *Kv1.4*, *Kv4.2* and *Kv4.3* generate transient outward currents similar to I_{TO} [38–40].

Kv1.4 was an early candidate for I_{TO}, but many properties of the *Kv1.4* currents differ significantly from those of adult rat ventricular I_{TO}, most notably its very slow recovery from inactivation. Moreover, Western blot and immunohistochemical studies have failed to detect the *Kv1.4* protein in rat ventricular myocytes [41] making it unlikely that *Kv1.4* plays a major role in (rat) I_{TO}. In the endocardium of human heart, a small I_{TO} component with slow recovery kinetics has been described which could correspond to *Kv1.4* [42].

In contrast, several lines of evidence suggest that Kv4.x subunits underlie the molecular architecture of cardiac I_{TO} (Fig. 4). Most functional and pharmacological properties of *Kv4.2* and *Kv4.3* [39,40,43] correspond to those of I_{TO} recorded in rat and human myocytes, including sensitivity to block by 4-AP, quinidine and flecainide [44,45]. *Kv4.2* is abundantly expressed in rat myocytes [3,41,46] and the transmural gradient for mRNA expression matches the gradient in I_{TO} density across the left ventricular wall [46,47]. A related isoform (*Kv4.3*) is expressed at high levels in rat, canine and human hearts [38,39]. The correspondence issue is further confounded by the existence of alternatively spliced variants [48,49]. Nevertheless, the emerging picture is that both *Kv4.2* and *Kv4.3* contribute to the I_{TO} in rat heart; differential expression of these subunits may be the molecular basis for the functional heterogeneity of the I_{TO} in different regions of the rat ventricle [38,39]. Dominant negative suppression by truncated or non-conducting *Kv4.2* subunits has further confirmed the role of the Kv4 gene family as the basis of cardiac I_{TO} [50]. However, the rate of recovery from inactivation of both *Kv4.2* and *Kv4.3* is still 3–5 times slower than the native current [51]. Additional subunits could be associated since unidentified low molecular mRNA products from brain accelerate recovery from inactivation, as do non-functional Kvα subunits in jellyfish [52,53]. In conclusion, the subunits of the Kv4 family are major contributors to the cardiac transient outward current and are probably associated with function-altering subunits.

3.2.2. 'Ultra-rapid' delayed rectifier (I_{Kur}): human Kv1.5

The mRNA for *Kv1.5* is expressed in the earliest stages of the rat embryonic cardiac development and it is abundantly present in adult human atrium [3,54]. h*Kv1.5* is an outwardly rectifying and highly selective K^+ channel with a rapid and sigmoidal time course of activation (time constants <2 ms at $+60$ mV) [55]. The voltage range for activation (midpoint at -14 mV) indicates that this channel would be active in the plateau range of the cardiac action potential. Like many delayed rectifiers, h*Kv1.5* displays slow and partial inactivation (Fig. 4). The h*Kv1.5* kinetics display a marked temperature dependence: the faster activation and inactivation give h*Kv1.5* a transient outward appearance above 30°C. *Kv1.5* is sensitive to quinidine in clinically relevant concentrations ($EC_{50}=6$ μM) [56] and molecular analysis has shown that quinidine

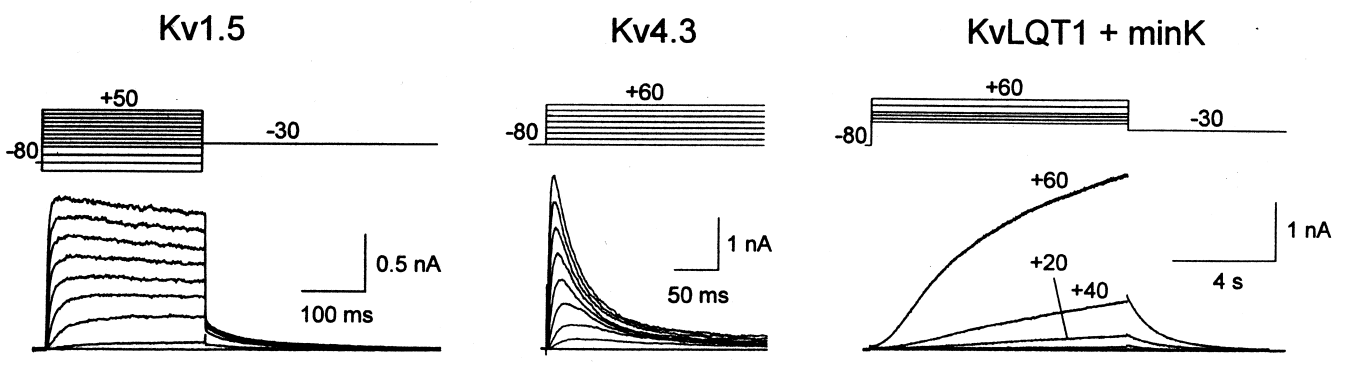

Fig. 4. Voltage-gated subunits encoding cardiac currents. Currents elicited by step depolarizations from -80 mV to potentials as indicated. Recordings after heterologous expression of the cloned subunits (h*Kv1.5*, *Kv4.3* and *KvLQT1+minK*) in mammalian cells (L-cells or HEK293). *Kv4.3* exhibits a 'transient outward' phenotype (the channels inactivate during the step); the other currents are 'delayed rectifiers': they remain open during the step, and channel closure is visible as the decaying current upon repolarization. Note that the delayed rectifiers activate over different time frames (compare also with Fig. 5).

and bupivacaine act as an open channel blockers and that residues in S6 form part of the binding site [7,57].

The close biophysical correspondence between *Kv1.5* and the I_{Kur} current identified in human atrial myocytes [37] suggests that this channel may be present in human atrium as a homotetrameric channel. The native current has the same pharmacological profile as *Kv1.5*: sensitive to quinidine and 4-AP and resistant to TEA and DTX [37,45]. Furthermore, the *Kv1.5* protein has been detected in human atrial and ventricular tissue [58], and *Kv1.5*-specific anti-sense oligonucleotides suppress the native I_{Kur} current in atrial cells [59]. The immunolocalization of the *Kv1.5* protein in human and rat heart has revealed that the protein is indeed expressed in the myocytes [58]. Interestingly, the channel is not evenly distributed over the myocyte surface but is localized in high density at the intercalated disks. Finally, specific *Kv1.5* expression was more diffuse in newborn tissue and the increasing organization with age was similar to that observed for connexin. *Kv1.5* contains two SH3 domains (see below) and the C-terminal amino acids ETDL might constitute a PDZ binding motif, which would provide two mechanisms for channel clustering [60].

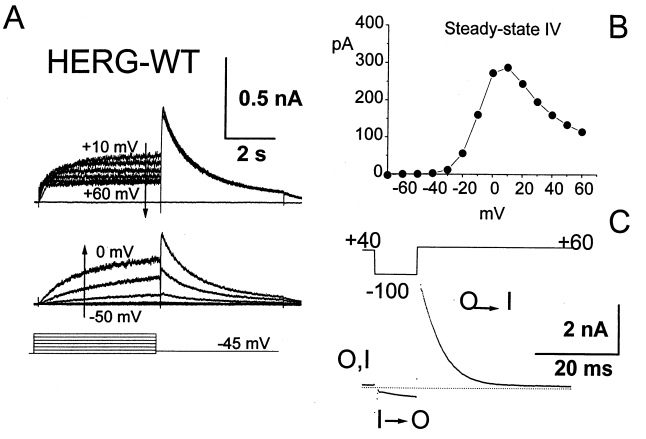

Fig. 5. Fast inactivation underlies inward rectification in *HERG*. (A) Outward currents elicited by step depolarizations from −80 mV increase up to 0 mV and become smaller again at more positive potentials. (B) The steady-state current–voltage relationship displays this 'inward rectification'. (C) Pulse protocol and tracing to illustrate that fast inactivation is responsible for the apparent rectification. During the first step channels convert from the rested (R) to the open (O) and in activated (I) state. Brief hyperpolarization allows for recovery from inactivation; the rapidly declining current during the third pulse reveals the rate and extent (>80%) of fast inactivation at positive potentials.

3.2.3. Methanesulfoanilide sensitive component I_{Kr}: HERG

The 'rapid' component of delayed rectification (I_{Kr}) has several unique features: inward rectification, block by micromolar La^{3+} and specific block by the methanesulfonanilide antiarrhythmic agents as E4031 and dofetilide [35,61]. The methanesulfonanilide sensitivity represents a defining pharmacological signature of the I_{Kr} current. The molecular basis of I_{Kr} was elucidated when *HERG* (human *eag*-related gene) was linked to a congenital form of the long QT syndrome [62]. The current encoded by *HERG* (Fig. 5) displays indeed the major functional and pharmacological properties of I_{Kr} [63–67]. Detailed kinetic studies of *HERG* gating revealed that the inward rectification is due to fast inactivation [65,68–70] (Fig. 5) as originally proposed for I_{Kr} by Shibasaki [71]. However, compared to native I_{Kr} the expressed current has slower kinetics and block by methanesulfonanilide drugs is less reversible Therefore, the initial clone (now called *HERG*1) is probably not the major subunit underlying cardiac I_{Kr}. Indeed, new members of the *erg* K^+ channel gene family have been cloned but may not exist in heart (*erg*2, *erg*3) [72]. More importantly, the genomic structure of *erg* displays at least 15 exons, and several splice variants of *(h)erg*1 have been identified in human and mouse heart [73,74]. The *erg*1b isoform has a distinctively short N-terminus and the kinetics of the expressed current (alone or with *erg*1) more closely resemble cardiac I_{Kr}.

Interestingly, C-terminal truncated subunits do not form functional *HERG* channels [16,69] and a C-terminal splice variant in exon 10 ($HERG_{USO}$) is non-functional when expressed by itself, but modifies *HERG*1 current if coexpressed [16]. These results indicate that (hetero)tetrameric assembly of *HERG* subunits require interactions in the C-terminal domain (last 100 amino acids), in contrast to the case for *Shaker* channels. [16]

3.2.4. Slowly activating current I_{Ks}: KvLQT1 with the minK β-subunit

Since the recognition that the delayed rectifier in guinea-pig myocytes consists of at least two components [34,35], the slowly activating component has been designated I_{Ks}. The molecular basis of this current has been controversial over the past decade. Initially it was suggested that this current was created by *minK* (for review see [75]). This gene encodes a protein that contains only 130 amino acids and a single membrane-spanning domain with an extracellular N-terminus. *MinK* mRNA has been found in mouse and neonatal rat heart, and the protein has been detected immunohistochemically in guinea pig ventricular myocytes.

Expression of *minK* protein in oocytes resulted in a current that resembled the slow delayed rectifier I_{Ks}. Altered ionic selectivity and regulation of the expressed *minK* current after mutagenesis of the transmembrane segment and the PKC consensus site seemed to support the idea that *minK* encoded a channel [76,77], but other studies suggested that *minK* activates both endogenous K^+ and Cl^- currents in the *Xenopus* oocytes [78].

Positional cloning identified a novel 6Tm–1P subunit responsible for the chromosome 11 associated form of the congenital LQT syndrome: *KvLQT1.* However, the expressed *KvLQT1* current displayed a delayed rectifier phenotype unlike any current previously identified in

cardiac preparations, which was surprising since mutations in *KvLQT1* cause the LQT syndrome [79,80]. Both Sanguinetti and Barhanin showed that coexpression of *KvLQT1* and *minK* yielded a current that corresponds to the native slow I_{Ks} component (Fig. 4) [79,80]. Thus, the *minK* controversy was ended by their demonstration that *minK* acts as β-subunit that alters the intrinsic gating of *KvLQT1*; the *minK* current in oocytes was due to inadvertent association with an endogenous *Xenopus XKvLQT1* subunit. *MinK* may also interact with *HERG* (and possibly other) K^+-channel subunits [81]. The molecular mechanism by which *minK* modifies the gating and pharmacology of *KvLQT1* remains to be elucidated.

These observations also explain the finding that mutations in either the *minK* or the *KvLQT1* gene can cause the congenital long QT syndrome [82]. Furthermore, heterologous suppression ('knock-out') of *minK* leads to inner ear abnormalities and deafness as seen in the Jervell–Lange–Nielsen syndrome [83], a rare disease in which the congenital LQT syndrome is associated with deafness. In vivo suppression of *minK* has also revealed that *minK* expression is largely restricted to the conduction system in mice [84]. Other *KvLQT1* related subunits have been cloned indicating the existence of an extended subfamily as is the case for the other K^+ channel subunits. The genomic structure of *KvLQT1* reveals at least six exons that give rise to several alternative spliced mature isoforms. The native current may represent a heterotetramer of *KvLQT1* isoforms 1 and 2, together with *minK* [85]. It remains to be established whether additional *minK* isoforms exist.

3.3. Inward rectifier currents

3.3.1. Inward rectifier I_{K1}: Kir2.x subunits.

Several inwardly rectifying currents exist in the mammalian heart. These include the quasi-instantaneous rectifier I_{K1}, the ATP inhibited I_{KATP} and the muscarinic receptor stimulated I_{KACh}. The inward rectification is very marked for I_{K1}. This allows I_{K1} to carry a substantial current at negative potentials and set a stable resting potential. Upon depolarization this large conductance is virtually shut down by the rectification, allowing other K^+-currents to control the plateau phase. Subunits from the 2Tm–1P class, especially the *Kir2.x* family underlie this current (Fig. 2). The expressed *Kir2.1* currents show strong inward rectification and are blocked by extracellular Ba^{2+} and Cs^+ [2]. The inward rectification is due to block of outward current by cytoplasmic Mg^{2+} or polyamines [86,87]. The functional channel is assumed to be assembled from four subunits similar to the basic stoichiometry of the KcsA channel [21].

3.3.2. G-protein-regulated K^+ channels (K_{ACH}): heteromeric Kir3.1+Kir3.4 complexes

Parasympathetic stimulation slows heart rate by activation of muscarinic receptors. This reduces the hyperpolarization activated cation current (I_f) in pacemaking tissue, and opens muscarinic K^+ channels (K_{ACh}) in the sinoatrial node and atrium. The latter is an inwardly rectifying K^+ channel coupled directly to a G protein. A cDNA for a G-protein-coupled inwardly rectifying K^+ channel was isolated from rat heart and designated GIRK1 [2]. In the Kir nomenclature, GIRK1 corresponds to *Kir3.1*. However, significant functional discrepancies between the homomeric *Kir3.1* current and the native current, and the discovery that a second protein consistently co-purified, led to the identification of another 2Tm–1P (Kir) subunit: GIRK4 or *Kir3.4* [88]. This led to the current view that the native K_{ACh} current results form a heterotetrameric complex consisting of two GIRK1 (*Kir3.1*) and two GIRK4 (*Kir3.4*) subunits [89]. Native and reconstituted K_{ACh} channels are activated directly by Gβγ dimers [90] and the kinetics of the G-protein mediated transduction pathway are modulated by RGS proteins (regulators of G protein signaling) [91]. The K_{ACh} channel is inhibited by atrial stretch, a property conferred largely by the *Kir3.4* subunit [92]. Observations in *Kir3.4* knock-out mice indicate that K_{ACh} mediates approximately 50% of the negative chronotropic effects of vagal stimulation in vivo [93].

3.3.3. ATP-regulated K^+ channels: heteromeric Kir6.2+SUR1 complexes

ATP-sensitive K^+ channels (K_{ATP}) link the membrane potential to the metabolic status of the cell: K_{ATP} channels are inhibited by physiological intracellular ATP levels, but open when the ATP levels fall. K_{ATP} channels play a role in myocardial ischemia, are important regulators of smooth muscle tone and are involved in insulin secretion in pancreatic β-cells. It is now generally accepted that the ATP-inhibited channel is a heteromeric complex that contains of four pore-forming subunits of the 2Tm–1P family (*Kir6.2*) and the ATP-binding cassette (ABC) protein SUR1. The latter represents the sulfonylurea receptor which endows the complex with its specific pharmacology [94,95].

3.3.4. I_{Kp}: a channel with twin pore subunits?

A small and time-independent potassium conductance has been described in guinea-pig cardiac myocytes [36,96]. None of the cloned 6Tm–1P or 2Tm–1P channels encode such current, but several recently cloned tandem pore subunits (4Tm–2P) encode currents with this 'leak' current behavior, e.g. the fairly ubiquitous TWIK subunit [19]. A related subunit (TASK) is highly expressed in heart [97], is highly sensitive to pH variations in the physiological range, and contains a C-terminal PDZ binding motif. This relatively new family is rapidly expanding and the exact subunits responsible for the cardiac I_{Kp} component remain to be established. Presumably a dimer of these tandem subunits yields a pseudo tetrameric channel.

3.4. Pacemaker current: related to CNCG channels

The pacemaker current was one of the last major cardiac ion channels to be cloned. The slow activation of this non-selective cation current (reversal potential around −30 mV) at the end of the action potential contributes to the diastolic depolarization and spontaneous pacemaking activity in the SA node [98]. Functional data indicated that cAMP directly modulates this current and enhances heart rates by shifting the activation of this current in the depolarizing direction. As might be anticipated from its functional properties, the cloned channel belongs to the family of cyclic nucleotide gated channels (CNGC) [99–101]. At least five isoforms have already been described, indicating the existence of another extended family of 6Tm−1P channel subunits. The expressed currents display the major functional properties of the I_h/I_f current, including modulation by cyclic nucleotides. Two interesting biophysical questions are raised by the molecular structure of these newly cloned channels.

First, a surprising problem is posed by its P-loop signature. The native I_f/I_h current and its cloned counterparts do not discriminate between Na^+ and K^+ ions. Previously cloned CNCG channels are non-selective cations channels and indeed lack in their P-loop the critical YG amino acids of the signature GYG motif for K^+-selectivity. Yet, the newly cloned pacemaker channels are non-selective despite containing the full GYG motif. The crystal structure of the bacterial *S. Lividans* K^+ channel suggests that the S5/S6 segments act as a scaffold on which the P-structure rests, and which keeps the carbonyl backbone of the GYG sequence at the critical distance to act as a K^+-selective filter [21]. It is conceivable that the scaffolding provided by the S5 and S6 segments in HCN channels changes this geometry resulting in a loss of the K^+-selectivity.

Second, the channel opens upon hyperpolarization, a 'funny' kinetic behavior and opposite to most 'regular' K^+ channels which are closed at the resting potential and open upon depolarization. However, the S4 sequences of the cloned pacemaker subunits are strikingly similar to those of other voltage-gated channels with positive charged residues at approximately every third position. Thus the S4 segment should move outward upon depolarization in all these channels, but somehow results in an opposite gating pattern. Resolving this paradox of 'funny' activation may provide further insight in the linkage between S4 movement and activation gating in general.

4. Regulation of K^+ channels

4.1. Physiological regulation

Many voltage gated K^+ channels contain multiple consensus sites for PKA and PKC mediated phosphorylation. Functional effects of PKC stimulation have convincingly been shown for *Kv4.2*, the cloned counterpart of cardiac I_{TO} [102]. *HERG* contains a putative cyclic nucleotide binding motif but its relevance is unclear [63]. Interestingly, human *Kv1.5* contains two repeats of a SH3 binding sequence. Direct interaction between *Kv1.5* and *Src* tyrosine kinase has been observed and *Kv1.5* is tyrosine phosphorylated in human heart [103]. Thus, *Kv1.5* appears to be part of a signaling complex that would enhance channel phosphorylation after kinase activation.

K^+ channels can also be regulated at the transcriptional and/or translational level. Reduction of glucocorticoids decreased *Kv1.5* mRNA and protein in rat ventricle, while injection of dexamethasone increased both more than 20-fold [104]. Interestingly, the effect was specific for ventricular *Kv1.5*, since *Kv1.4* and *Kv2.1* mRNA levels were unchanged as was atrial *Kv1.5* mRNA expression [104]. Similarly, thyroid hormone levels appear to alter channel expression, although it is not fully established whether this represents a nuclear effect of thyroid hormone itself or whether it is a secondary response to the increased heart rate due to autonomic effects of thyroid hormone [105,106].

4.2. Potassium channels are largely down-regulated in cardiac disease: electrical remodelling

The *HERG* and *KvLQT1* subunits were identified based on the genetic study of patients with the congenital long QT syndrome and numerous mutations have been identified [82]. Incorporation of the abnormal subunits in the channel tetramer causes mostly a dominant negative effect. Severely disrupted subunits (e.g. truncations) may not associate with normal subunits at all leading to haploinsufficiency [82]. In either case, the currents are typically reduced by 50% or more, leading to prolonged action potentials predisposing to arrhythmias.

While the long QT syndrome is a rare disorder, prolongation of APD is a consistent electrophysiological abnormality in myocardium and myocytes from hypertrophied and failing hearts. The resulting delay of repolarization predisposes to early after-depolarizations and to fatal arrhythmias [107]. It is now well established that repolarizing outward currents are reduced in human atrial and ventricular myocytes in a variety of pathological states (for a detailed review see [108]). Significant reduction of I_{K1} and I_{TO} was observed in myocytes from patients with severe heart failure [109,110]. Reduction of I_{TO} is the most marked effect in the canine pacing-induced heart failure model [111], and decreased expression of *Kv4.2* and *Kv4.3* mRNA was observed in the hypertrophied hearts of rats with renovascular hypertension [49]. Similarly, outward currents and *Kv1.5* mRNA and protein are reduced in human atrial fibrillation [112]. Heart failure and atrial fibrillation are common clinical problems. Therefore further detailed analysis of the molecular basis of the electri-

cal remodelling has important practical implications, especially since these outward currents are the molecular target for class III antiarrhythmic drugs. First, the reduction of currents can exacerbate the arrhythmogenic effect of predisposing factors (e.g. hypokalemia due to diuretics). Second, the rationale of using class III antiarrhythmic drugs needs to be re-evaluated since their intended target may be down-regulated or absent. Indeed, therapeutic strategies efforts aimed at enhancing or restoring the currents seem warranted [113,114].

5. Conclusion

Subunits have now been cloned for almost all important K^+ channels in the mammalian heart, and progress is being made toward the elucidation of the molecular architecture of the channels in the native environment. This molecular analysis has revealed an even greater diversity than previously anticipated from the native myocyte studies. The molecular approach now allows both the detailed characterization of human cardiac potassium channels, and elucidation of drug–channel interaction at the molecular level. The atrial localization of human cardiac *Kv1.5* appears important in relation to atrial arrhythmias. Despite the homology among the voltage-gated K^+ channel subunits, the major components of repolarizing K^+ currents are encoded by subunits that belong to distinct families (*Kv4.2/3*, *Kv1.5*, *HERG*, *KvLQT1*, *TWIK*, *Kir*). This greatly facilitates (pharmacological) targeting of a specific component, although the expression of these subunits in other tissues needs to be considered (e.g. for side effects). Arrhythmias are frequently treated with pore blocking drugs, but the observation that subunits are down-regulated in disease indicates that novel therapeutic interventions should be contemplated. Analysis of promoter regions will undoubtedly foster further understanding of this electrical remodeling process. Another aspect highlighted by the molecular diversity is that no single species can serve as 'the' animal model for the human heart. Indeed, a similar K^+ current can be carried in different species either by different subunits (e.g. *Kv1.2* vs. *Kv1.5*), or by different splice variants of the same subunits (e.g. *HERG*). In summary, the molecular analysis of subunits underlying the native K^+ currents in heart has provided us with a number of tools to further elucidate the molecular basis of cardiac excitability and the electrical remodeling in disease.

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