Pharmacology of cardiac potassium channels

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Abstract

Cardiac K⁺ channels are membrane-spanning proteins that allow the passive movement of K⁺ ions across the cell membrane along its electrochemical gradient. They regulate the resting membrane potential, the frequency of pacemaker cells and the shape and duration of the cardiac action potential. Additionally, they have been recognized as potential targets for the actions of neurotransmitters and hormones and class III antiarrhythmic drugs that prolong the action potential duration (APD) and refractoriness and have been found effective to prevent/suppress cardiac arrhythmias. In the human heart, K⁺ channels include voltage-gated channels, such as the rapidly activating and inactivating transient outward current (I_{to1}), the ultrarapid (I_{Kur}), rapid (I_{Kr}) and slow (I_{Ks}) components of the delayed rectifier current and the inward rectifier current (I_{K1}), the ligand-gated channels, including the adenosine triphosphate-sensitive (I_{KATP}) and the acetylcholine-activated (I_{KAC}) currents and the leak channels. Changes in the expression of K⁺ channels explain the regional variations in the morphology and duration of the cardiac action potential among different cardiac regions and are influenced by heart rate, intracellular signalling pathways, drugs and cardiovascular disorders. A progressive number of cardiac and noncardiac drugs block cardiac K⁺ channels and can cause a marked prolongation of the action potential duration (i.e. an acquired long QT syndrome, LQTS) and a distinct polymorphic ventricular tachycardia termed torsades de pointes. In addition, mutations in the genes encoding I_{Kr} (KCNH2/KCNE2) and I_{Ks} (KCNQ1/KCNE1) channels have been identified in some types of the congenital long QT syndrome. This review concentrates on the function, molecular determinants, regulation and, particularly, on the mechanism of action of drugs modulating the K⁺ channels present in the sarcolemma of human cardiac myocytes that contribute to the different phases of the cardiac action potential under physiological and pathological conditions.

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Keywords: K⁺ channels; Cardiomyocytes; Pharmacology; Cardiac arrhythmias; Antiarrhythmic agents; Torsades de pointes

1. Introduction

Cardiac K⁺ channels determine the resting membrane potential, the heart rate, the shape and duration of the action potential and are important targets for the actions of neurotransmitters, hormones, drugs and toxins known to modulate cardiac function [1–3]. Blockers of certain or most K⁺ channels prolong the cardiac action potential duration (APD) and refractoriness without slowing impulse conduction, i.e. they exhibit Class III antiarrhythmic actions, being effective in preventing/suppressing re-entrant arrhythmias. Unfortunately, drugs that delay the repolarization prolong the QT interval of the electrocardiogram and represent a major cause of acquired long QT syndrome (LQTS) [4–6].

Cardiac K⁺ currents can be distinguished on the basis of differences in their functional and pharmacological properties. In mammalian cardiac cells, K⁺ channels can be categorized as voltage-gated (Kv) and ligand-gated channels [1–4]. The first category includes the rapidly activating and inactivating transient outward current (I_{to1}), the ultrarapid (I_{Kur}), rapid (I_{Kr}) and slow (I_{Ks}) components of the delayed rectifier and the inward rectifier (I_{K1}), whereas the ligand-gated channels include those activated by a decrease in the intracellular concentration of adenosine triphosphate (K_{ATP}) or activated by acetylcholine (K_{AC}) [1–4]. The configuration and duration of the cardiac action potentials vary considerably among species and different cardiac regions (atria vs. ventricle) and specific areas within those regions (epicardium vs. endocardium). This heterogeneity mainly reflects differences in the type and/or expression patterns of the K⁺ channels that participate in the genesis of the cardiac action potential. Moreover, the expression and properties of...
K+ channels are not static but are influenced by heart rate, neurohumoral state, pharmacological agents, cardiovascular diseases (cardiac hypertrophy and failure, myocardial infarction) and arrhythmias (atrial fibrillation-AF) [1,7–10].

The cardiac action potential reflects a balance between inward and outward currents. Fig. 1 shows the relationship between a cardiac action potential and the time course of individual ionic currents that participate in its genesis. The initial upstroke of the atrial and ventricular cells (phase 0) is due to the activation of the fast inward Na+ current (I_Na). Initial rapid repolarization (phase 1) is a consequence of the rapid voltage-dependent inactivation of I_Na and the activation of I_to1 and the I_Kur. During phase 2 inward depolarizing currents through Na+ (slowly inactivated) and L-type Ca+2+ channels (I_CaL) are balanced by the different components of the delayed rectifier K+ current (I_Kur, I_Kr and I_Ks). The terminal phase 3 of repolarization is due to the increasing conductance of the I_Kr, I_Ks and I_K1. I_K1 is also responsible for the maintenance of the resting potential.

In this review we shall attempt to address (1) the function and molecular basis of cardiac K+ channels, (2) the pharmacological modulation and its cellular consequences, and (3) the signaling pathways involved in the regulation of channel function and expression under pathophysiological conditions. This information should provide fundamental understanding of the role of K+ channels in modulating cardiac function, in the genesis and maintenance of cardiac arrhythmias and as potential targets of potentially useful therapeutic agents. For more detailed review of structure–function relationship, distribution and pharmacological modulation of K+ channels, the reader is referred to recent reviews [11–16].

2. K+ channel diversity and classification

K+ channels are formed by coassembly of pore-forming α-subunits which surround a water-filled pore and accessory β-subunits. Over 80 human K+ channel-related genes have been cloned and characterized [1–4,11]. The genes encoding the α- and β-subunits of cardiac K+ channels are shown in Table 1. Key features of α-subunits are: (a) a pore region through which K+ ions flow across the plasma membrane, (b) a selectivity filter that allows K+ but not other ions to cross the pore, and (c) a gating mechanism that controls switching between open-conducting and closed-nonconducting states and determines whether permeation occurs in response to either changes in membrane potential or a ligand. The recent identification of the atomic structures of several K+ channels (KcsA, KvAP and MthK) provides the basis for understanding, at a molecular level, the mechanisms that control ion selectivity and conduction [15]. K+ channels have been classified into three major families on the basis of the primary amino acid sequence of the α-subunit [1–4,11]:

1. Channels containing six transmembrane segments and one pore-forming region (Fig. 2A). This architecture is typical of Kv channels, whose members include Shaker-related channels (Kv1–4), ether-a-go-go-related channels (ERG) and KCNQ channels. Immediately after depolarization Kv channels move from the closed to the open state (activation) and then many channels enter into a nonconducting state (inactivation), leading to a decline in activated macroscopic current. When the membrane is repolarized channels recover from the inactivated state and are once again capable of opening in response to membrane depolarization. There are two major types of inactivation [16]. N-type inactivation results from the occlusion of the intracellular mouth of the pore by a “ball and chain” mechanism when the channel opens. C-type inactivation appears to involve a rearrangement of residues in the external mouth of the channel that becomes occluded.

Kv channel α subunits have six transmembrane-spanning segments (S1–S6) with cytoplasmic N- and C-terminal domains and a pore loop between S5 and S6 bearing the K+ selectivity filter signature TxGYG [1,4,11]. The pore region, formed by S5 and S6 segments and the S5–S6 linker, is responsible for K+ ion conduction and selectivity. The S4 segment contains positively charged residues (R/K) at approximately every third position and serves as the voltage sensor. Most Kv channels contain a Pro-X-Pro sequence (PXP) that induces a sharp bend in the S6 helices near the activation gate and reduces the inner vestibule of
the pore [16]. Kv channels can be formed from four identical $\alpha$-subunits (homomultimers) or from combinations of different $\alpha$-subunits from the same subfamily (heteromultimers). In the Kv1 subfamily, tetramerization appears to be controlled to some extent by the N-terminal intracellular T1 domain (T1) that immediately precedes S1. However, in HERG and KCNQ1 channel subfamilies, C-terminal domains appear to be important for heterotetramerization [13,16].

(2) Inward rectifier K$^+$ channels (Kir$s$) contain two transmembrane domains (M1 and M2) connected by a pore containing the G(Y/F)G sequence and intracellular N- and C-termini (Fig. 2B). This architecture is typical of K$^+$,K ATP and K$^+$,ACh channels [1–3,17–19]. They conduct K$^+$ currents more in the inward direction than the outward and play an important role in setting the resting potential close to the equilibrium potential for K$^+$ ($E_{K}$, approximately $–90$ mV for [K$^+$]$_o$= $5$ mM) and in repolarization [1,11,17–19]. Kir channels form either homo- or heterotetramers.

(3) Four transmembrane segments and two pore channels (K2P) have intracellular N- and C-termini and exist as homodimers or heterodimers (Fig. 2C) [20–22]. The conventional G(Y/F)G of K$^+$-selective motif is preserved in the first pore loop, but it is replaced by G(F/L)G in the second. K2P currents display little time- or voltage-dependence and I/V curves can be described by the Goldman–Hodgkin–Katz (GHK) equation, thus regulating resting membrane potential and excitability. There are four classes of cardiac K2P: TASK, TWIK, TREK and THIK.

The diversity of K$^+$ currents in native tissues exceeds the number of K$^+$ channel genes identified. The explanations for this diversity include alternative splicing of gene products, post-translational modification, heterologous assembly of $\alpha$-subunits within the same family and assembly with accessory $\beta$-subunits that modulate channel properties (see below) [1–4,11,23].

### 2.1. Auxiliary subunits

Recapitulation of the physiological features of the native K$^+$ current frequently requires accessory $\beta$-subunits. Most Kv$\beta$ subunits assemble with $\alpha$-subunits giving rise to an $\alpha$4$\beta$4 complex. K$^+$ channel $\beta$-subunits represent a diverse molecular group, which includes cytoplasmic proteins (Kv$\beta$1-3, KChIP and KChAP) that interact with the intracellular domains of Kv channels, single transmembrane spanning proteins, such as minK and minK-related proteins (MiRPs) encoded by the KCNE gene family, and large ATP-binding cassette (ABC) transport-related proteins, such as the sulfonylurea receptors (SUR) for the inward rectifiers Kir6.1–6.2 (Fig. 2) [2–4,23–25].

### Table 1

<table>
<thead>
<tr>
<th>Current</th>
<th>$\alpha$-Subunit</th>
<th>$\beta$-Subunit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{Ks}$</td>
<td>Kv7.1 (KVLQT1)</td>
<td>minK</td>
</tr>
<tr>
<td>$I_{Kr}$</td>
<td>Kv11.1 (HERG)</td>
<td>KCNE1</td>
</tr>
<tr>
<td>$I_{Kur}$</td>
<td>Kv1.5 (HK2)</td>
<td>K$\beta$1 (Kv$\beta$3)</td>
</tr>
<tr>
<td>$I_{KCh}$</td>
<td>Kir2.1 (IRK1)</td>
<td>K$\beta$2</td>
</tr>
<tr>
<td>$I_{KATP}$</td>
<td>Kir2.2 (IRK2)</td>
<td>K$\beta$A</td>
</tr>
<tr>
<td>$I_{K1}$</td>
<td>Kir3.1 (GIRK1)</td>
<td>K$\beta$1 (Kv$\beta$3)</td>
</tr>
<tr>
<td>$I_{K2P}$</td>
<td>Kir4.1 (KIR4)</td>
<td>K$\beta$B</td>
</tr>
<tr>
<td>$I_{K3}$</td>
<td>Kir6.2 (BIR)</td>
<td>K$\beta$1 (Kv$\beta$3)</td>
</tr>
<tr>
<td>$I_{K4}$</td>
<td>Kv4.3</td>
<td>K$\beta$A</td>
</tr>
<tr>
<td>$I_{K5}$</td>
<td>Kv1.4</td>
<td>K$\beta$A</td>
</tr>
<tr>
<td>$I_{K6}$</td>
<td>Kv4.1</td>
<td>K$\beta$A</td>
</tr>
<tr>
<td>$I_{K7}$</td>
<td>Kv4.2</td>
<td>K$\beta$A</td>
</tr>
<tr>
<td>$I_{K8}$</td>
<td>K$\beta$1.1 (TWIK-1)</td>
<td>SUR2A</td>
</tr>
<tr>
<td>$I_{I1}$</td>
<td>K$\beta$2.1 (TREK1)</td>
<td>ABCC9</td>
</tr>
<tr>
<td>$I_{I2}$</td>
<td>K$\beta$3.1 (TASK-1)</td>
<td>KChIP2</td>
</tr>
<tr>
<td>$I_{I3}$</td>
<td>K$\beta$5.1 (K$\beta$-TASK-2)</td>
<td>KChIP2</td>
</tr>
<tr>
<td>$I_{I4}$</td>
<td>K$\beta$6.1 (TWIK-2)</td>
<td>KChIP2</td>
</tr>
<tr>
<td>$I_{I5}$</td>
<td>K$\beta$9.1 (TASK-3)</td>
<td>KChIP2</td>
</tr>
<tr>
<td>$I_{I6}$</td>
<td>K$\beta$10.1 (TREK-2)</td>
<td>KChIP2</td>
</tr>
<tr>
<td>$I_{I7}$</td>
<td>K$\beta$13.1 (THIK-1)</td>
<td>KChIP2</td>
</tr>
<tr>
<td>$I_{I8}$</td>
<td>K$\beta$17.1 (TASK-4)</td>
<td>KChIP2</td>
</tr>
</tbody>
</table>

*Synonyms are those approved by the Human Genome Nomenclature-HGNC database (http://www.gene.ucl.ac.uk/nomenclature) and the International Union of Pharmacology (http://www.iuphar-db.org/iuphar-ic/).*
domain at the N-terminus similar to the N-terminal inactivation ball of the α-subunit and when coassembled can produce a N-type inactivation [26]. Kvα2.1 and Kvα4.1 behave as chaperone proteins, promoting proper protein folding and/or subunit coassembly, channel trafficking and cell surface expression of coexpressed subunits [26]. KChIPs are cytosolic Ca²⁺-binding subunits that interact with the N-terminus of the Kv4.3 α-subunits and enhance their expression in the cell membrane [24,26]. Coexpression of KChAP with Kv channels also enhances total current density, suggesting a true chaperone function [23].

3. Cardiac voltage-gated channels

3.1. The transient outward current, I_{to1}

I_{to} is rapidly activated and inactivated in response to depolarization [3,27]. I_{to} is the sum of a voltage-dependent, 4-aminopyridine (4-AP)-sensitive, calcium-independent K⁺ current (I_{to1}) and a 4-AP-insensitive, Ca²⁺-activated Cl⁻ or K⁺ current (I_{to2}). In human atrial and ventricular myocytes the presence of I_{to2} has not been clearly demonstrated, so it will be not discussed further. I_{to1} is responsible for early rapid repolarization (phase 1) and determines the height of the early plateau, thus influencing activation of other currents that control repolarization, mainly I_{CaL} and the delayed rectifier K⁺ currents (I_K). Furthermore, variations in cardiac repolarization associated with I_{to1} differences strongly influence intracellular Ca²⁺ transient by modulating Ca²⁺ entry via I_{CaL} and Na⁺–Ca²⁺ exchange, potentially exacerbating impaired Ca²⁺ cycling in heart disease [28].

I_{to1} density is 4- to 6-fold higher in atrial tissue, Purkinje fibers, epicardial and midmyocardial (M) cells than in the endocardial cells [29,30]. In human subendocardial cells I_{to1} is lower and recovers more slowly than in epicardial or M cells [29]. In canine right atria, I_{to1} density is lower in the appendage than in the crista terminalis, pectinate muscle and AV ring area cells [31]. I_{to1} density is higher in the right than in the left canine ventricular M cells [32] and differences in I_{to1} density across the ventricular wall are causally linked to the J wave of the ECG [30]. The prominent epicardial I_{to1} contributes to the selective electrical depression of the epicardium during ischemia and to the development of a

Fig. 2. Side view of the topology of three classes of K⁺ channels subunits. (A) Schematic representation of a voltage-gated K channel α subunit composed of six membrane-spanning α helices (S1 to S6) and one pore. The C-terminal cytoplasmic domain is drawn off to the side of the T1 domain, but its precise relative location is until yet unknown. The schematic representation of single transmembrane spanning proteins, such as minK and minK-related proteins (MiRPs) is also shown. NBD: nucleotid binding domain (B) Schematic representation of the α subunit of an inward rectifier K⁺ channel (Kir6.x) formed only by two transmembrane domains (M1 and M2) connected by a pore. The topology of the sulfonylurea receptor (SUR) that interacts with Kir6.x α subunits is also shown. SUR has been proposed to have three transmembrane domains (TMDO, TMD1A and TMD2), each of which consists of five, five and six membrane spanning regions. (C) Schematic representation of the α subunit of “background” channels formed by four transmembrane segments and two pores. SID: self-interacting domain. Abbreviations: see text.
### Table 2
Pharmacological blockade of voltage-gated potassium channels

<table>
<thead>
<tr>
<th>Drug</th>
<th>$I_{h0}$</th>
<th>$I_{k10}$</th>
<th>$I_{Kv1.5}$</th>
<th>$I_{Kr}$</th>
<th>$I_{Ks}$</th>
<th>KCNQ1/KCNE1</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-AP</td>
<td>1.15 mM</td>
<td>50 μM</td>
<td>180 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[38,76–78]</td>
</tr>
<tr>
<td>Almokalant</td>
<td>23 μM</td>
<td>34–45 μM</td>
<td>50 nM</td>
<td>5.6 μM</td>
<td>32 μM</td>
<td></td>
<td></td>
<td>[5,12]</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>4.9 μM</td>
<td>7%</td>
<td>2.8 μM</td>
<td>9.8–38 μM</td>
<td>68%</td>
<td>10 μM</td>
<td></td>
<td>[39,40,80]</td>
</tr>
<tr>
<td>Azimilide</td>
<td>60%, 100 μM</td>
<td>39%, 100 μM</td>
<td>0.3–7 μM</td>
<td>0.7–5 μM</td>
<td>1–3 μM</td>
<td>5.6 μM</td>
<td></td>
<td>[41,81,130,131]</td>
</tr>
<tr>
<td>Bepridil</td>
<td>6.6 μM</td>
<td>0.55 μM</td>
<td>10 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[42,128,132,194]</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>22 μM</td>
<td>18 μM</td>
<td>39%, 5.6 μM</td>
<td>13%</td>
<td>39%, 100 μM</td>
<td>1 μM</td>
<td></td>
<td>[43,83,134]</td>
</tr>
<tr>
<td>Candesatarn</td>
<td>100%, 30 μM</td>
<td>30 μM</td>
<td>20 μM</td>
<td>30 μM</td>
<td></td>
<td></td>
<td></td>
<td>[44,137]</td>
</tr>
<tr>
<td>Cisapride</td>
<td>21.2 μM</td>
<td>15 nM</td>
<td>1 nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[85,138]</td>
</tr>
<tr>
<td>Clonolium</td>
<td>0.5 μM</td>
<td>15 nM</td>
<td>1 μM</td>
<td>50 μM</td>
<td>&gt;10 μM</td>
<td>&gt;200 μM</td>
<td>HEK,GPV, CHO</td>
<td>[5,47,76]</td>
</tr>
<tr>
<td>Cocaine</td>
<td>115 μM</td>
<td>17.3 μM</td>
<td>130 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[5]</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>30 μM</td>
<td>27 μM</td>
<td>130 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[48,140]</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>3.9–31 nM</td>
<td>10–110 nM</td>
<td>&gt;10 μM</td>
<td>1 μM</td>
<td>1 μM</td>
<td>&gt;10 μM</td>
<td>GPV, CHO, CHO</td>
<td>[142]</td>
</tr>
<tr>
<td>Dronedaron</td>
<td>3 μM</td>
<td>1 μM</td>
<td>10 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[86]</td>
</tr>
<tr>
<td>E-3174</td>
<td>&gt;50 μM</td>
<td>&gt;10 μM</td>
<td>&gt;10 μM</td>
<td>1 μM</td>
<td>1 μM</td>
<td>&gt;10 μM</td>
<td>AT-1, HAM, GPV, TXO</td>
<td>[39,71,123]</td>
</tr>
<tr>
<td>Eprosartan</td>
<td>14%, 3 μM</td>
<td>25%, 1 μM</td>
<td>1 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[44,87]</td>
</tr>
<tr>
<td>Eptifibatin</td>
<td>&gt;10 μM</td>
<td>&gt;10 μM</td>
<td>&gt;10 μM</td>
<td>1 μM</td>
<td>1 μM</td>
<td>&gt;10 μM</td>
<td>GPV, AT-1, CHO</td>
<td>[38,78,80,137]</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>25%, 10 μM</td>
<td>36%, 10 μM</td>
<td>&gt;10 μM</td>
<td>&gt;10 μM</td>
<td>34 nM</td>
<td>120 nM</td>
<td>CHO, Ltk, CHO</td>
<td>[144]</td>
</tr>
<tr>
<td>Ibutiline</td>
<td>3.7 μM</td>
<td>16 nM</td>
<td>20 nM</td>
<td>15%, 1 μM</td>
<td></td>
<td></td>
<td></td>
<td>[49]</td>
</tr>
<tr>
<td>Indapamidine</td>
<td>98 μM</td>
<td>20 nM</td>
<td>193 μM</td>
<td>314 μM</td>
<td></td>
<td></td>
<td></td>
<td>[51,188]</td>
</tr>
<tr>
<td>Loratadine</td>
<td>1.2 μM</td>
<td>173 nM</td>
<td>49 nM</td>
<td>100%, 37 nM</td>
<td>100 nM</td>
<td></td>
<td></td>
<td>[89,149]</td>
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<td>Mexiletine</td>
<td>0.9 μM</td>
<td>19 nM</td>
<td>1 μM</td>
<td>1 μM</td>
<td></td>
<td></td>
<td></td>
<td>[86]</td>
</tr>
<tr>
<td>Nicotine</td>
<td>40 nM</td>
<td>34 nM</td>
<td>1.3 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[52]</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>&gt;30 μM</td>
<td>81 μM</td>
<td>4.75 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[53,78]</td>
</tr>
</tbody>
</table>

(continued on next page)
marked dispersion of repolarization between normal and ischemic epicardium and between epicardium and endocardium, thereby providing the substrate for reentrant arrhythmias [33]. Furthermore, flecainide-induced abolition of the action potential dome in epicardial but not in endocardial cells leads to a marked dispersion of repolarization across the ventricular wall that, when accompanied by prominent conduction delays related to Na\(^+\) channel blockade, results in extrasystolic activity through a phase 2 reentrant mechanisms [34].

3.1.1. Molecular basis of I_{to1}

Kv4.3 channels are the leading candidate for encoding human I_{to1} [35,36]. Kv4.3 mRNA decreases in failing hearts and this reduction correlates with the reduction in I_{to1} density, indicating that part of the downregulation of this current may be transcriptionally regulated [3,9,35]. However, Kv1.4 channels possibly represent the I_{to1} in the human endocardium [3,29]. Heterologous expression of hKv4.3, Kv1.4 or both does not reproduce the properties of the native I_{to1} [3,27,29]. KChIP2, when coexpressed with hKv4.3, increases surface channel density and current amplitude, slows the inactivation, accelerates the recovery from inactivation and shifts the half-maximal inactivation to more positive potentials [23,24]. Thus, features of Kv4.2/KChIP2 currents closely resemble those of I_{to1}. In human ventricle KChIP2 mRNA is 25-fold more abundant in the epicardium than in the endocardium, and this gradient parallels the gradient in I_{to1} expression, while Kv4.3 mRNA is expressed at equal levels across the ventricular wall. Thus, transcriptional regulation of the KChIP2 gene is the primary determinant of I_{to1} expression in the ventricular wall [37].

3.1.2. Pharmacology of I_{to1}

I_{to1} is blocked by 4-AP in the mM range in a fashion suggesting a preferential interaction with the closed state [38]. Table 2 shows the blocking potencies, expressed as IC_{50} values (i.e., concentrations producing 50% inhibition of the current), of several drugs on native I_{to1} in human atrial myocytes [38–59]. 4-AP, quinidine and propafenone produce an open channel blockade, results in extrasystolic activity through a phase 2 reentrant mechanisms [40].

![Table 2 (continued)](https://academic.oup.com/cardiovascres/article-abstract/62/1/9/373105)

<table>
<thead>
<tr>
<th>Drug</th>
<th>I_{to1}</th>
<th>I_{Kur}</th>
<th>Kv1.5</th>
<th>I_{Kr}</th>
<th>HERG</th>
<th>I_{Ks}</th>
<th>KCNQ1/ KCNE1</th>
<th>Species</th>
<th>References</th>
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<tbody>
<tr>
<td>Papaverine</td>
<td>&gt;100 μM</td>
<td>43 μM</td>
<td></td>
<td>2.75 mM</td>
<td>1.6 mM</td>
<td>0.2 mM</td>
<td>0.2 mM 27%, 10 μM</td>
<td>HAM,Ltk, GPV,TXO, HEK,CHO</td>
<td>[91]</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td></td>
<td></td>
<td></td>
<td>2.5 mM</td>
<td>18 nM</td>
<td>0.2 mM</td>
<td></td>
<td></td>
<td>[151]</td>
</tr>
<tr>
<td>Pimozide</td>
<td>29%, 10 μM</td>
<td>4.4 μM</td>
<td>0.76 μM (I_{Kc}), 20%, 5 μM</td>
<td>0.5 μM</td>
<td>43%, 5 μM</td>
<td></td>
<td></td>
<td>[92]</td>
<td></td>
</tr>
<tr>
<td>Propafenone</td>
<td>4.8 μM</td>
<td>&gt;10 μM</td>
<td>4.4 μM</td>
<td>0.76 μM (I_{Kc}), 20%, 5 μM</td>
<td>0.5 μM</td>
<td>43%, 5 μM</td>
<td></td>
<td></td>
<td>[54,55,93,152,153]</td>
</tr>
<tr>
<td>Quinidine</td>
<td>5–10 μM</td>
<td>5 μM</td>
<td>6 μM</td>
<td>9 nM–2 μM</td>
<td>0.3–1 μM</td>
<td>∼ 50 μM</td>
<td></td>
<td>CHO,GPV, AT-1,HAM, TXO,Ltk, CNA,HEK</td>
<td>[12,38,80,94,122,143,154]</td>
</tr>
<tr>
<td>Risperidone</td>
<td>&gt;30 μM</td>
<td></td>
<td></td>
<td>30–260 nM</td>
<td>167 nM</td>
<td>20.3 μM</td>
<td>2.2 μM</td>
<td>RVM,CHO, Ltk, CHO</td>
<td>[56,155]</td>
</tr>
<tr>
<td>Ropivacaine</td>
<td>80 μM</td>
<td></td>
<td></td>
<td>7 μM (I_{Kc})</td>
<td>22 nM</td>
<td>0.2 μM</td>
<td>50 nM</td>
<td>Ltk,GPV,CNV, GPV</td>
<td>[72,76,78]</td>
</tr>
<tr>
<td>RP58866</td>
<td>2.3 μM</td>
<td></td>
<td></td>
<td>7 μM (I_{Kc})</td>
<td>12 μM</td>
<td>14 μM</td>
<td>&gt;100 μM</td>
<td>GPV,HAM,MEL, CHO</td>
<td>[39,71,78,141]</td>
</tr>
<tr>
<td>Serradilide</td>
<td>&gt;0.5 μM</td>
<td>0.5–1 mM</td>
<td>2.1 μM</td>
<td>1 μM</td>
<td>88 μM</td>
<td>23 μM</td>
<td></td>
<td>CNA,Ltk,MEL</td>
<td>[135]</td>
</tr>
<tr>
<td>Spirolocalonate</td>
<td>&gt;10 μM</td>
<td>330 μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GPV,RTV, CHO</td>
<td>[72,76,78]</td>
</tr>
<tr>
<td>Tetrabenazine</td>
<td>&gt;2 μM</td>
<td></td>
<td></td>
<td>2.5 μM (I_{Kc})</td>
<td>50 nM</td>
<td>9–350 μM</td>
<td>10 μM</td>
<td>RVM,CHO, GPV, TXO,RTV</td>
<td>[5,54,44]</td>
</tr>
<tr>
<td>Tegaserod</td>
<td>&gt;30 μM</td>
<td>2.2 μM</td>
<td>45 μM</td>
<td>0.14–0.83 μM</td>
<td>&gt;100 μM</td>
<td></td>
<td></td>
<td>RTV,HEK, COS,CNA</td>
<td>[53,80,133,158]</td>
</tr>
</tbody>
</table>

Data are expressed as IC_{50} values (concentrations producing 50% inhibition of the current).

3.1.3. Pathological role

Heart failure, cardiac hypertrophy and myocardial ischaemia and infarction are associated with prolongation of the APD, an effect that has been attributed, in part, to a down-regulation of $I_{\text{to1}}$ [8,9,27]. The decrease in $I_{\text{to1}}$ in heart failure may be adaptive in the short-term because increased depolarization during the cardiac cycle means that more time is available for excitation–contraction coupling, which mitigates the decrease in cardiac output, but it becomes maladaptive in the long-term, since a prolongation of the APD may contribute to arrhythmogenesis, either by causing inhomogeneous repolarization or by increasing the likelihood of early afterdepolarizations [27]. However, there is also evidence of an up-regulation of $I_{\text{to1}}$ in hypertrophied cardiac myocytes [9,65] and in cardiac myocytes after induced myocardial infarction [8,66]. It can be speculated that up-regulation of $I_{\text{to1}}$ may represent a protection mechanism counteracting the excessive prolongation of the APD and Ca$^{2+}$ influx, in order to minimize the incidence of ventricular proarrhythmia. Chronic AF reduces $I_{\text{to1}}$ density and Kv4.3 mRNA levels [10]. Hypothyroidism reduces the expression of KCND2 (Kv4.2) genes [67].

3.1.4. Regulation of $I_{\text{to1}}$

The phosphorylation, mediated by protein kinase A (PKA) and C (PKC), alters channel properties by modifying the kinetics and/or the expression of active channels present on the cell surface. Phorbol 12-myristate 13-acetate (PMA), an unspecific activator of serine/threonine and tyrosine protein kinases, suppresses both Kv4.2 and Kv4.3 currents and native $I_{\text{to1}}$ but not after preincubation with PKC inhibitors (chelerythrine) and staurosporine, a nonspecific inhibitor of serine/threonine protein kinases [68]. PKC increases the inactivation time constants and slows the time course of recovery from inactivation of $I_{\text{to1}}$, α-adrenergic agonists (phenylephrine and methoxamine) reduce $I_{\text{to1}}$ in rat ventricular myocytes [63], whereas β-adrenergic agonists have no effects on $I_{\text{to1}}$. Furthermore, phenylephrine and carbachol inhibit Kv4.3 currents only when coexpressed, respectively, with α1-adrenoceptors or M1 receptors and this effect is also prevented by chelerythrine, suggesting that it may be mediated by activation of PKC [68]. In hKv4.1 channels, PMA causes an initial increase followed by a reduction in current amplitude and these effects are prevented by staurosporine or chelerythrine [69]. Angiotensin II inhibits $I_{\text{to1}}$ in rat ventricular myocytes, an effect prevented by PD123391, a selective antagonist of AT2 receptors. This inhibition is abolished by okadaic acid, an inhibitor of protein phosphatases 1 and 2A, suggesting that the stimulation of AT2 receptors activates the serine–threonine protein phosphatases to reduce $I_{\text{to1}}$ density [70].

3.2. The delayed rectifier K$^+$ currents

In the human heart, $I_{K}$ can be separated into at least three different components, the ultrarapid ($I_{\text{Kur}}$), rapid ($I_{\text{Kr}}$), and slow ($I_{\text{Ks}}$) components. These currents exhibit different kinetics and pharmacological properties, are regulated by different intracellular signaling pathways and encoded by separate genes [1–4,71–75].

3.2.1. The ultrarapid component of the delayed rectifier K$^+$ current, $I_{\text{Kur}}$

Atrial cells possess a K$^+$ current that activates rapidly in the plateau range ($\tau<10$ ms), displays outward rectification and inactivates very slowly during the time course of the action potential [72,76,77]. $I_{\text{Kur}}$ has been recorded in human atria but not in human ventricular tissue, so that it is the predominant delayed rectifier current responsible for human atrial repolarization [72,77]. $I_{\text{Kur}}$ density is similar in cells isolated from different regions of the canine right atrium [31]. Kv1.5 protein is located at the intercalated disks, a pattern that is disrupted after ischemic damage.

3.2.1.1. Molecular basis of $I_{\text{Kur}}$. Kv1.5 (613 amino acids) encodes the α-subunit of the $I_{\text{Kur}}$ channel (10–14 pS). Heterologous expression of Kv1.5 results in a delayed rectifier current with the biophysical and pharmacological characteristics of $I_{\text{Kur}}$ and exposure to Kv1.5 antisense oligodeoxynucleotides-AsODN specifically inhibits $I_{\text{Kur}}$ density in human atrial myocytes [72,77]. Kv1.5 subunits coassemble with Kvβ1.2 subunits to form the $I_{\text{Kur}}$ in human atrium [26].

3.2.1.2. Pharmacology of $I_{\text{Kur}}$. $I_{\text{Kur}}$ is relatively insensitive to TEA [76,78], Ba$^{2+}$ and class III antiarrhythmics of the methanesulfonanilide group [39,78], but is highly sensitive to 4-AP [38,76–78]. Selective inhibition of $I_{\text{Kur}}$ by 4-AP prolongs the human atrial APD [77] and the atrial but not the ventricular refractoriness in the dog [84]. The potency of various drugs to block $I_{\text{Kur}}$ and heterologously expressed Kv1.5 channels is summarized in Table 2 [47,58,76–95].

3.2.1.3. State specific block of $I_{\text{Kur}}$. Antiarrhythmic drugs and local anesthetics are weak bases that predominate in their cationic form at physiological pH. The cationic form enters into the permeation pathway only after channel opening and blocks the current by binding at a site in the internal mouth of the ionic pore. The binding of these drugs is determined by an electrostatic component reflecting the electrical binding distance (about 20% of the transmembrane electrical field) and a hydrophobic component that
largely determines the intrinsic stability of the drug–receptor complex, and thus, the affinity (Fig. 3A and B). It has been proposed that most of the critical residues for hydrophobic binding of quinidine in the hKv1.5 channel are aligned on one side of the putative S6, so that it is hypothesized that this region is the pore-lining region and is important in determining drug affinity and specificity [96]. Stereoselective bupivacaine block of hKv1.5 channels is determined by a polar interaction with T507 and two hydrophobic interactions at positions L510 and V514 [97]. The length of the alkyl substituent at position 1 of bupivacaine-related local anesthetics determine their potency and stereoselectivity, possibly because this alkyl chain interacts with a hydrophobic residue (L510) in the S6 of the channel [98]. In contrast, at low concentrations uncharged-neutral (benzocaine [82,99], nifedipine [100], loratadine [89]) and negatively charged drugs (angiotensin II type 1 receptor antagonists [84,86,88]) affect the gating properties of hKv1.5 channels without obvious block (Fig. 3C–E), and, frequently, they increase the current amplitude at voltages at which activation of channels reaches saturation (Fig. 3C) [82,86]. Moreover, these drugs produce a voltage-dependent block, but their voltage-dependence is exactly a mirror image of that observed in the presence of cationic drugs (Fig. 3D) [84,93]. Furthermore, the residues in the S6 segment that determine the binding of quinidine and bupivacaine also determine the binding of neutral and acid drugs (benzocaine [99]) suggesting the existence of a common receptor site at the channel level. Very recently ala-scanning mutagenesis within the pore helix and the S6 segment identifies the residues (T479, T480, V505, I508 and V512) that determine the binding of S0100176, a new selective open I\(_{\text{Kur}}\) blocker [101]. The implicated residues face towards the central cavity and overlap with putative binding sites for other blockers and Kv channels, like HERG and KCNQ1, indicating a conservation of drug binding sites among different K\(^+\) channel families.

The external mouth of the channel pore formed by the P loop and adjacent S5–S6 segments is the binding site for some drugs [3,12]. Long-chain polyunsaturated fatty acids (arachidonic and docosahexaenoic acids) bind to an external site and block Kv1.5 channels [102] and \(R(+)-N\)-methylbupivacaine, a membrane-impermeant permanently charged drug, produces different effects on Kv1.5 channels when

Fig. 3. Effects produced by weak bases (bupivacaine and IQB-9302) and weak acids (losartan) on hKv1.5 channels. (A) Current traces of hKv1.5 currents obtained by applying 250 ms depolarizing pulses from \(-80\) to \(+60\) mV in 10 mV steps in control conditions and in the presence of bupivacaine (20 \(\mu\)M). Tail currents were elicited upon repolarization to \(-40\) mV. (B) Relative hKv1.5 current obtained in the presence and in the absence of bupivacaine and IQB-9302 (20 \(\mu\)M), plotted together with the activation curve (dotted line). At membrane potentials positive to 0 mV, a shallower increase in block was observed. This voltage-dependence was fitted (continuous line) following a Woodhull equation (\(f\approx[D]/([D]+K_{D}\times\exp(-zFE/RT))\)) and yielded \(z\)-values of 0.20 and 0.17 for bupivacaine and IQB-9302, respectively. Modified from Ref. [83]. (C) Effects of losartan (1 \(\mu\)M) 4 min after beginning the infusion (left) and after steady-state was reached (right). hKv1.5 currents traces were elicited by 500 ms pulses to \(+60\) mV and tail currents upon return to \(-40\) mV. (D) Relative hKv1.5 current obtained in the presence and in the absence of losartan, plotted together with the mean activation curve (dotted line). At membrane potentials positive to 0 mV, a shallower decrease in block was observed. This voltage-dependence was fitted (continuous line) following a Woodhull equation and yielded \(z\)-values of \(-0.27\). (E) Effects of losartan on the voltage-dependence of hKv1.5 channels activation. Activation curve in control conditions was fitted with a single Boltzmann component (\(y=1/(1+\exp((Vh-Tm)/\delta))\)) (continuous line). Dashed line shows that this approach was not optimal for data in the presence of losartan, whereas best fit was obtained with a sum of two Boltzmann components. Modified from Ref. [86].
applied from the intra or the extracellular side of the membrane, thus confirming the existence of an external binding site for bupivacaine-like local anesthetics [103].

3.2.1.4. Clinical consequences of \( I_{Kur} \) block. \( I_{Kur} \) is a promising target for the development of new safer antiarrhythmic drugs to prevent atrial fibrillation and/or flutter without a risk of ventricular proarrhythmia [104]. However, in patients with chronic AF, \( I_{Cal} \) is inhibited which limits the increase in the plateau height produced by \( I_{Kur} \) blockade resulting in a significative lengthening of the atrial APD. However, \( I_{Kur} \) density and \( K_{1.5} \) expression are reduced in these patients [10], so that the degree of APD prolongation produced by selective \( I_{Kur} \) blockers under these conditions is unknown. In a rat model, rapid atrial pacing immediately and transiently increases \( K_{1.5} \) mRNA levels (Kv4.3 mRNA levels decreased, while mRNA levels of Kv2.1, erg, KCNQ1 and KCNE1 remained unaltered), which might contribute, at least in part, to the rapid shortening of the atrial refractoriness at the onset of AF [105]. If so, selective \( I_{Kur} \) blockers might antagonize the shortening of the atrial APD produced at rapid atrial rates and prevent episodes of paroxysmal AF. Selective \( I_{Kur} \) blockers (NIP-142, S9947, AVE0118, S0100176 and S20951) have no effect on the QT interval and markedly prolong left vs. right atrial refractoriness, decrease left atrial vulnerability and reverse AF to sinus rhythm [90,104,106]. Unfortunately, there are few clinical data on the efficacy and safety of these drugs.

3.2.1.5. Regulation of \( I_{Kur} \). Membrane depolarization and elevated \( [K^{+}]_{o} \), reduce \( K_{1.5} \) expression, while dexamethasone increases ventricular \( K_{1.5} \) mRNA [72]. Cyclic adenosine 3’5’-monophosphate (cAMP), mechanical stretch and hyperthyroidism increase, whereas extracellular acidosis, phenylephrine, and hypothyroidism decrease \( K_{1.5} \) expression [67,72,107]. \( K_{1.5} \) mRNA levels decrease in hypertensive hypertrophied rat ventricle [108] and in the epicardial border zone of the infarcted canine ventricle [8].

\( hK_{1.5} \) has one consensus site for phosphorylation by PKC located on the extracellular S4–S5 linker and 4 consensus sites for PKA located in the N- and C-terminal domains. Isoproterenol increases \( I_{Kur} \) in human atrial myocytes and this effect is mimicked by direct stimulation of adenylate cyclase and suppressed by a PKA inhibitor peptide [109]. In the presence of propranolol, phenylephrine inhibits \( I_{Kur} \) an effect that is prevented by the PKC inhibitor bisindolylmaleimide. These results indicate that \( \beta \)-adrenergic stimulation enhances, whereas \( \alpha \)-adrenergic stimulation inhibits \( I_{Kur} \) and suggest that these actions are mediated by PKA and PKC, respectively.

Coexpression of \( K_{1.5} \) channels and human thrombin or rat 5-HT1c receptors, two receptors that increase phospholipase C (PLC) activity, inhibits \( K_{1.5} \) current amplitude without altering the kinetics or voltage sensitivity of activation [110]. Simultaneous injection of inositol 1,4,5-tri-phosphate and superfusion of PMA reproduces the modulation of the \( K_{1.5} \) current suggesting that these receptors can modulate \( K_{1.5} \) channels by increasing PLC activity [110]. The \( K_{1.5} \) channel can associate via its N-terminus-located proline-rich sequences with the SH3 domain of the Src tyrosine kinase [111] and coexpression of \( K_{1.5} \) with \( \nu \)-Src leads to tyrosine phosphorylation of the channel and inhibition of the current.

Coexpression of \( K_{\nu 1.3} \) with \( K_{1.5} \) induces a fast inactivation and a hyperpolarizing shift in the activation curve, i.e. \( K_{\nu 1.3} \) subunit converts \( K_{1.5} \) from a delayed rectifier with a modest degree of slow inactivation to a channel with both fast and slow components of inactivation [26]. These effects do not occur when \( K_{1.5} \) is coexpressed with either \( K_{\nu 1.2} \) or \( K_{\nu 1.3} \) lacking the N-terminus, after removal of a consensus PKA phosphorylation site on the \( K_{\nu 1.3} \) N-terminus (S24) or following preincubation with calphostin C, a PKC inhibitor [26]. Moreover, okadaic acid blunts the effects of calphostin as predicted if protein dephosphorylation be required to remove the \( \beta \)-induced inactivation. These data suggest that \( K_{\nu 1.2} \) and \( K_{\nu 1.3} \) subunit modification of \( K_{1.5} \) currents require phosphorylation by PKC or a related kinase. PMA has minimal effect on \( K_{1.5} \) channels, but markedly reduces \( K_{1.5} \)/\( K_{\nu 1.2} \) currents and this effect is inhibited by chelerythrine, indicating that \( K_{\nu 1.2} \) enhances the response of \( K_{1.5} \) to PKC activation [112]. \( K_{1.5} \)/\( K_{\nu 1.3} \) channels are less sensitive to quinidine- and bupivacaine-induced block than homoergic \( K_{1.5} \) channels [113]. These results suggest that the \( K_{\nu 1.3} \) subunit markedly reduce the affinity of these drugs for their internal receptor site in \( hK_{1.5} \) channels.

3.2.2. The rapidly activating component of the delayed rectifier \( K^{+} \) current, \( I_{Kr} \).

\( I_{Kr} \) is characterized by rapid activation at \(-30\) mV, rapid inactivation and strong inward rectification at positive potentials which is due to rapid voltage-dependent C-type inactivation [16,71,73]. Inward rectification results from the fact that channel inactivation develops faster than channel activation at positive potentials and limits the amount of time that channels spend in the open state [73,114]. On repolarization the rate of recovery from inactivation through the open state is much more rapid than deactivation, which at voltages negative to 0 mV results in a large outward current and promotes phase 3 of repolarization. Thus, \( I_{Kr} \) plays an important role in governing the cardiac APD and refractoriness.

\( I_{Kr} \) has been identified in human atrial and ventricular myocytes, rabbit sinoatrial (SA) and atrioventricular (AV) nodal cells and Purkinje cells [3,14]. In rabbit SA myocytes HERG channels play an important role in the pacemaker activity and \( I_{Kr} \) blockers decrease the maximum rate of diastolic depolarization [115]. In rats \( I_{Kr} \) density is higher in atria than in ventricles, while in humans HERG expression is higher in the ventricles [116]. In canine atria \( I_{Kr} \) density is larger in the AV ring region and the left atrial wall than in crista terminalis, pectinate muscles, appendage cells and right
atrial wall, which may account for the shorter APD of the left atria [14,31]. However, in the canine ventricle \( I_{Kr} \) is distributed homogeneously [117], while in guinea pig left ventricle, \( I_{Kr} \) is smaller in subendocardial than in midmyocardial or epicardial myocytes [118]. In rabbit left ventricle, \( I_{Kr} \) is greater in the apex than in the basal regions and \( I_{Kr} \) blockers cause more significant APD prolongation in the apex than in the base, increasing the regional dispersion of APD [119].

3.2.2.1. Channel gating. The S4–S5 linker and the C-terminal half of the S6 are crucial components of the activation gate and point mutations at these regions have marked effects on the voltage-dependence and kinetics of channel activation and deactivation [73,74]. The N-termini of HERG is involved in the slow deactivation process and some mutations in this region linked with type 2 (LQT2) of the Romano–Ward variant of the congenital long QT syndrome (cLQTS) reduce outward currents through the HERG channel by accelerating channel deactivation [74]. Deletion of amino acids 2 to 354 or 2 to 373 markedly increase HERG deactivation rates [73]. HERG channels also contain a PAS (Per–Arnt–Sim) domain on their cytoplasmic N-terminus that may interact with other regions of HERG such as the S4–S5 linker to affect channel deactivation [73,74,114]. C-type inactivation of HERG channels is slowed by extracellular TEA and elevated \([K^+]_o\) and can be removed by mutations in the outer mouth of the pore or the N-terminal half of S6 [73,74]. It involves a rearrangement of the outer pore region of the channel that either constricts the pore and prevents ion fluxes, or alters the ion selectivity in such a way that the channel cannot conduct \( K^+ \) currents.

3.2.2.2. Molecular basis of \( I_{Kr} \). HERG \( \alpha \)-subunits (1159 amino acids) encoded by the KCNH2 gene coassemble to form heterotetrameric channels [120]. However, native \( I_{Kr} \) and HERG channels expressed in heterologous systems differ in terms of gating, regulation by external \( K^+ \) and single channel conductance [73,74]. These findings suggest the presence of a modulating subunit that co-assembles with HERG in order to reconstitute native \( I_{Kr} \) currents. A likely candidate is MiRP1 (123 amino acids) encoded by the KCNE2 gene. When coexpressed with HERG, KCNE2 shifts the HERG activation curve in the positive direction, accelerates the rate of deactivation, decreases single channel conductance (from 13 to 8 pS) and mediates the direct stimulatory effect of cAMP on HERG/KCNH2 channels [120,121]. KCNE2 reduces channel sensitivity to \([K^+]_o\) and enhances sensitivity to clarithromycin [120] but not to dofetilide, E-4031 or quinidine [122]. KCNE2 can also modulate KCNQ1, Kv4.2 and even the distinctly related hyperpolarization-activated cation (HCN) pacemaker channels [74]. HERG can coassemble with other \( \beta \)-subunits like minK encoded by KCNE1 gene (Table 1). Treating AT-1 cells with AsODNs against KCNE1 reduces \( I_{Kr} \) amplitude [123] and when HERG and KCNE1 are coexpressed in HEK293 cells, the HERG current amplitude increases.

KCNH2 and KCNE2 have been identified as the loci of mutations associated with LQT2 and LQT6 of the Romano–Ward variant of cLQTS, respectively [4,124,125]. The cLQTS is a complex disease characterized by marked QT-interval prolongation and polymorphic ventricular tachycardias called torsades de pointes (TdP), causing syncope, seizures and sudden death [4,6,124,125]. More than 100 mutations in the KCNH2 gene have been described, including framershifts, insertions, deletions and missense and nonsense mutations [124–127]. Mutant channels cause a net reduction in outward \( K^+ \) current during repolarization that can result from different mechanisms, including generation of nonfunctional channels, altered channel gating and abnormal protein trafficking.

3.2.2.3. Pharmacology of \( I_{Kr} \). \( I_{Kr} \) is the target of class III antiarrhythmic drugs of the methanesulfonanilide group (almokalant, dofetilide, D-sotalol, E-4031, ibutilide, and MK-499) (Table 2). These drugs produce a voltage- and use-dependent block, shorten open times in a manner consistent with open-channel block and exhibit low affinity for closed and inactivated states [73,74,128,129]. Drugs that block both native \( I_{Kr} \) and heterologously expressed HERG channels are shown in Table 2 [6,12,71,122,123,129–158] and in Fig. 4A and B. \( I_{Kr} \) blockers prolong atrial and ventricular APD (QT prolongation) and refractoriness in the absence of significant changes in conduction velocity (AH, HV and PR intervals) [159]. In animal models, \( I_{Kr} \) blockers suppress ventricular tachycardia induced by programmed electrical stimulation or a new ischemic insult in dogs with prior infarct [159,160]. However, these drugs are probably not effective against triggered activity or increased automaticity [159].

Some LQT2-causing HERG mutations give rise to defects in channel trafficking resulting from improper protein folding and/or incorrect molecular assembly in the sarcoplasmic reticulum and/or Golgi apparatus, resulting in their retention and degradation by quality control machinery. Trafficking of some mutant channels (N470D, G601S) into the sarcolemma can be restored by HERG channel blockers (i.e. cisapride, terfenadine, astemizole, E-4031) [73,126,127], even when fexofenadine rescues mutant HERG channels at concentrations that do not cause channel block [127]. However, since \( I_{Kr} \) blockers failed to rescue other trafficking-defective mutants, it is evident that multiple mechanisms may exist for pharmacological rescue of LQT2 mutations [127]. Moreover, some drugs (almokalant [12], norpropoxyphene [161], azimilide [162], candesartan (Fig. 4C–E) [84] and E3174, the active metabolite of losartan [86]) can exhibit an “agonist” \( I_{Kr} \) action at low concentrations. Flufenamic acid and niflumic acid also increase HERG currents by accelerating channel opening [163]. These results open the possibility of developing new \( I_{Kr} \) openers for the treatment of patients with congenital (LQT2) or drug-induced LQTS. Lyosphosphati-dylcholine increases HERG currents, an effect that may contribute to \( K^+ \) loss in the ischemic heart [164].
An increasing number of drugs with diverse chemical structures block \( I_{Kr} \), delay ventricular repolarization, prolong the QT interval (acquired LQTS) and induce TdP (for review see Refs. [5,6,165,166]). Thus, it is important to understand the molecular determinants of drug binding to HERG channels as well as the consequences of drug action to develop safer and more effective drugs.

3.2.2.4. Structural basis of \( I_{Kr} \)/HERG blockade. Most \( I_{Kr} \) blockers gain access to a binding site from the intracellular side of the membrane and require channel opening before they can gain access to a high affinity binding site located inside the channel vestibule [73,74]. Once inside the pore, \( I_{Kr} \) blockers bind within the central cavity of the channel between the selectivity filter and the activation gate and can be trapped in the inner vestibule by closure of the activation gate when the channel deactivates during repolarization [167]. If a drug is charged and appropriately sized, then block is nearly irreversible as long as channels are not reopened even at negative potentials. However, the drug-trapping hypothesis predicts that unbinding and exit from the channel vestibule of a positively charged drug should be favored by membrane hyperpolarization if not impeded by the closed gate. Indeed, using a mutant HERG channel (D540K) that opens in response to hyperpolarization, it was found that channel reopening allows recovery from block by MK-499 [177]. The finding that drugs with such different chemical structures and sizes block \( I_{Kr} \) can be interpreted through two unique structural features of the HERG channel: (a) the lack of the PXP motif in the S6 segment (Fig. 2A) that makes larger volume of the inner vestibule of the channel pore thus increasing easy drug entry. Kv1–4 channels contain the PXP sequence that is believed to induce a sharp bend in the inner S6 helices of Kv channels [16] reducing the inner vestibule and precluding the trapping of large molecules like MK-499. (b) The presence of two aromatic residues (Y652, F656) located in the S6 domain facing the channel vestibule, that establishes specific interactions with aromatic moieties of several drugs by \( \pi \) electron stacking [129]. Alanine mutagenesis of both residues drastically reduces the potency of channel block by MK-499, dofetilide, quinidine, cisapride, terfenadine or chloroquine [129,136,168]. Mutations that result in loss of inactivation (S631A, G628C/S631C) reduce the affinity of methanesulfonanilides, while mutations that increase inactivation (T432S, A443S, A453S) enhance the blocking potency of dofetilide [74,169]. It has been hypothesized that the reduced affinity of noninactivating HERG mutant channels is not due to inactivation per se but to inactivation gating-associated reorientation of residues Y652 and F656 in the S6 domain that comprise a high-affinity binding site [168].
Very recently, the selective serotonin reuptake inhibitor fluvoxamine has been shown to exhibit HERG channel blocking properties that are different from those previously described. In fact, the S6 mutations, Y652A and F656A, and the pore helix mutant S631A only partially attenuate the fluvoxamine-induced blockade at concentrations causing profound inhibition of wild-type HERG channels [144]. Moreover, this blockade resembles that produced by canrenonic acid (CA), the main metabolite of spironolactone, on HERG channels expressed in CHO cells [135]. Fluvoxamine- and CA-induced HERG block is far more rapid (occurring within 10 msec) than that produced by the more potent methanesulfonanilides, suggesting that they exhibit either a closed-state block or extremely rapidly developing open channel blockade. Furthermore, channel inactivation was not a prerequisite for fluvoxamine- and CA-induced block.

3.2.2.5. Clinical consequences of IKr block. IKr blockers prolong atrial and ventricular APD and the QT interval and may cause TdP that can degenerate into ventricular fibrillation and sudden cardiac death. Proarrhythmia induced by IKr blockers is related to [4,5,165,166]: (a) excessive prolongation of APD near plateau voltages which favor the development of early after-depolarizations and (b) a more marked prolongation of the APD near plateau voltages which favor the development of TdP. The incidence of TdP is increased in the presence of hypokalemia, slow heart rates (recent conversion from AF), QT prolonging drugs, pre-existing cardiac diseases (hypertrophy, myocardial infarction), female gender or baseline QTc interval >0.46 s [4–6].

Unlike most other K+ currents, IKr amplitude increases upon elevation of [K+]o and decreases after removal of extracellular K+ [73,74]. Elevation of [K+]o reduces C-type inactivation and increases the single channel conductance of HERG channels [154]. This explains why the APD are shorter at higher [K+]o and longer at low [K+]o and the association between hypokalemia, APD prolongation and induction of TdP in patients treated with IKr blockers. On the contrary, modest elevations of [K+]o, using K+ supplements and spironolactone in patients given IKr blockers or with LQT2 significantly shortens the QT interval and may prevent TdP [170]. Moreover, the antiarrhythmic actions of IKr blockers can be reversed during ischemia, which is frequently accompanied by elevations of the [K+]o in the narrow intercellular spaces and by catecholamine surges that occur with exercise or other activities associated with fast heart rates [159,160].

Unfortunately, the prolongation of the APD produced by IKr blockers is more marked at normal resting potentials and slow heart rates, while at depolarized potentials or during tachycardia this prolongation is much less marked or even absent [5,159,160]. This reverse use-dependence limits antiarrhythmic efficacy, while maximizing the risk of TdP associated with bradycardia-dependent early afterdepolarizations. In guinea pigs, reverse use-dependence is attributed to a progressive IKr accumulation as the heart rate increased (due to the incomplete deactivation of this current), which shortens the APD and offsets the APD prolongation produced by the IKr blocker [73]. Another explanation is that IKr block itself is reverse use-dependent, this property being promoted by KCNE2 coexpression [120].

All these disadvantages, together with the finding that inherited mutations of KCNH2 and KCNE2 genes are linked to LQT2 and LQT6 forms of the Romano–Ward syndrome, have decreased the initial interest for developing selective IKr blockers.

3.2.2.6. Regulation of IKr. HERG channels presents four cAMP-dependent PKA phosphorylation sites and a cyclic nucleotide binding domain (CNBD) in the C-terminal and can be modulated via the cAMP-PKA phosphorylation pathway [121,171]. Activation of β-adrenergic receptors and elevation of intracellular cAMP levels regulate HERG channels both through PKA-mediated effects and by direct interaction with the protein [121]. PKA activation reduces HERG current amplitude and induces a depolarizing shift in the voltage-dependent activation curve [121,171]. This shift is inhibited by specific PKA inhibitors (H89, KT5720) and in channels missing all PKA phosphorylation sites (S283A, S890A, T895A, S1137A), while coexpression of HERG with KCNE1 or KCNE2 accentuates the cAMP-induced voltage shift [121]. The net result of these effects is a reduction in HERG current. However, isoproterenol increases IKr in guinea pig ventricular myocytes, an effect that was inhibited by bisindolylmaleimide [171]. In rabbit SA cells isoproterenol increases IKr and this effect is inhibited by H89, but not by bisindolylmaleimide [172]. These results suggest that regulation of IKr may be species- and tissue-specific and may also depend strongly on the experimental conditions.

HERG channels are modulated by PKC independently of direct phosphorylation of the channel [173]. PMA causes a positive shift of activation and reduces IKr, and its effects can still be observed when the PKC-dependent phosphorylation sites are deleted by mutagenesis. Changes in phosphatidyl 4,5-biphosphate (PIP2) levels result from activation of several adrenergic and muscarinic receptors. PIP2 increases HERG current and shifts the voltage-dependence of activation in a hyperpolarizing direction [174]. Moreover, in cells coexpressing the α1A-receptor and HERG, phenylephrine reduces HERG currents and this effect is prevented by PIP2 but not by PKC inhibition, suggesting that the mechanism is due to G-protein-coupled receptor stimulation of PLC resulting in the consumption of endogenous PIP2. Since there are several polycationic segments in the cytoplasmic C-terminus of HERG, it is possible that an electrostatic interaction between the negatively charged phosphate groups of PIP2 and positively charged amino acids in the
channel protein can maintain the channel in an open state [174]. In human ventricular myocytes, endothelin-1 inhibits \( I_{\text{Kr}} \), but fails to modify \( I_{\text{to}} \) and \( I_{\text{K1}} \) [175].

3.2.2.7. Pathological role. \( I_{\text{Kr}} \) and HERG currents are unchanged in patients with chronic AF [10] and homogeneously distributed in failing canine hearts [176]. ATP, derived from either glycolysis or oxidative phosphorylation, is critical for HERG function. Both hyper- and hypoglycemia inhibit HERG currents and can cause QT prolongation and ventricular arrhythmias [177]. \( I_{\text{Kr}} \) density and HERG mRNA levels are reduced in myocytes from infarcted canine ventricle [178]. However, \( I_{\text{Kr}} \) density increases in subendocardial Purkinje cells from the 48 h infarcted heart [8], an effect that may increase the proarrhythmic effects of \( I_{\text{Kr}} \) blockers in patients with myocardial infarction.

3.2.3. The slowly activating component of the delayed rectifier \( K^+ \) current, \( I_{KS} \)

\( I_{KS} \) is slowly activated at potentials positive to \(-30 \text{ mV}\) with a linear current–voltage relationship, reaching half-maximum activation at \(+20 \text{ mV}\) [71,74,179]. Thus, \( I_{KS} \) contributes to human atrial and ventricular repolarization, particularly during action potentials of long duration and is a dominant determinant of the physiological heart rate-dependent shortening of APD. As heart rate increases, \( I_{KS} \) channels have less time to deactivate, leading to an accumulation of open channels and a faster rate of repolarization [152,180]. In guinea pigs, the slow deactivation of \( I_{KS} \) results in a reduction of outward current that contributes to the slow diastolic depolarization of SA node cells [179].

In guinea pigs, \( I_{KS} \) density is greater in atrial than in ventricular myocytes and in subepicardial and M cells than in subendocardial cells [118], but is smaller in apical than in basal myocytes of the rabbit left ventricle [119]. There are no differences in \( I_{KS} \) density among cells from different regions of the canine right atria [31], while in the canine ventricle, \( I_{KS} \) density is higher in epicardial and endocardial cells than in the M cells [117] and in right than in left ventricular M cells [32]. The smaller \( I_{KS} \) density in M cells may explain their steeper APD-rate relations and their greater tendency to display pronounced action potential prolongation and to develop early afterdepolarization at slow heart rates or in response to QT prolonging drugs [117]. \( I_{KS} \) density is downregulated in all layers of the left ventricle of failing canine hearts [176] and a decrease in \( I_{KS} \) and KCNQ1/KCNE1 mRNA levels is found in myocytes from infarcted canine ventricle [178].

3.2.3.1. Molecular basis of \( I_{KS} \). \( I_{KS} \) is generated by the coassembly of four pore-forming KCNQ1 (676 amino acids) and two accessory KCNE1 \( \beta \)-subunits [181]. KCNQ1 (129 amino acids) exhibits a single transmembrane spanning domain; the N-terminus is extracellular and the C-terminus intracellular. Heterologous expression of KCNQ1 produces rapidly activating, slowly deactivating currents, while KCNE1, by itself, does not form functional channels. Coexpression of KCNQ1/KCNE1 slows activation and deactivation kinetics, shifts the voltage dependence of channel activation to more positive potentials and increases the macroscopic current amplitude, thus reproducing the biophysical properties of the native cardiac \( I_{KS} \) [182]. Additionally, KCNE1 confers regulation of \( I_{KS} \) by PKC and alters the pharmacology of KCNQ1 [74,183].

KCNQ1 is abundant in the SA node, but less abundant although homogeneously distributed throughout the ventricular wall and in the mouse heart expression is largely restricted to the conducting system. An alternatively spliced variant of KCNQ1 with a N-terminal deletion that produces a negative suppression of KCNQ1 is preferentially expressed in the M cells, which is consistent with the lower \( I_{KS} \) density in this region [184]. Transgenic mice overexpressing this spliced variant present abnormalities of SA and AV function, which suggests a role of KCNQ1 in normal automaticity [185]. KCNQ1 and KCNE1 expression and \( I_{KS} \) density increases in ventricular myocytes from hypothyroid rats, but decreases in hyperthyroidism [67] and in myocytes from infarcted canine ventricle [178]. Mutations in KCNQ1 and KCNE1 are associated with types 1 (LQT1) and 5 (LQT5) of the Romano–Ward variant of the cLQTS, respectively, and the Jervell–Lange–Nielsen syndrome associated with deafness arises in children who inherited abnormal KCNQ1 or KCNE1 alleles from both parents [124,186].

3.2.3.2. Pharmacology of \( I_{KS} \) and cellular consequences of \( I_{KS} \) channel blockade. \( I_{KS} \) is resistant to methanesulfonanilides, but selectively blocked by chromanols (293B, HMR-1556) [46,49,187], indapamide [188], thiopentone, propofol [189] and benzodiazepines (L-768,673, L-735,821 or L-7) [190,191]. The open channel block produced by chromanol is enantioselective, \((-)\)3R,4S-293B and \((-)\)3R,4S-HMR 1556 being potent \( I_{KS} \) blockers [192,193]. Table 2 summarizes the potency of various drugs to block \( I_{KS} \) and heterologously expressed KCNQ1/KCNE1 channels [44,130,133,139,190–195].

Initially, \( I_{KS} \) was considered an attractive target for class III antiarrhythmic drugs [159]. Because \( I_{KS} \) accumulates at fast driving rates due to its slow deactivation, \( I_{KS} \) blockers may be expected to be more effective in prolonging APD at fast rates [180]. Indeed, in human ventricular myocytes, 293B prolongs APD and refractoriness in a frequency-independent manner, so that it is proposed that \( I_{KS} \) blockade might have less proarrhythmic potency as compared to \( I_{Kr} \) blockers [46]. Furthermore, because \( I_{KS} \) activation occurs at around 0 mV and this voltage is more positive than the Purkinje fiber action potential plateau voltage, \( I_{KS} \) block should not be expected to prolong the APD at this level. Conversely, in ventricular muscle, the plateau voltage is more positive (\( \approx +20 \text{ mV} \)) allowing \( I_{KS} \) to be substantially more activated, so that \( I_{KS} \) block would be expected to increase APD markedly. The net result of both effects might
be less drug-induced dispersion in repolarization and reduced risk of arrhythmogenesis [196].

$I_{Ks}$ blockers prolong the cardiac APD and QT interval and suppress electrically induced ventricular tachyarrhythmias in animals with acute coronary ischemia and exercise superimposed on a healed myocardial infarction [49,159,187]. This QT prolongation can be accentuated by $\beta$-adrenergic stimulation. In arterially perfused canine left ventricle, 293B prolongs the ventricular APD and the QT interval, but does not induce TdP. However, in the presence of 293B, isoproterenol abbreviates the APD of epicardial and endocardial, but not in the M cells, accentuates transmural dispersion of repolarization and induces TdP [197]. These results explain the therapeutic actions of $\beta$-adrenergic blockers in patients with LQT1 and LQT5 and the increased risk of fatal cardiac arrhythmias under physical activity or stressful situations that increase sympathetic activity in these patients [124,125,198].

Furthermore, under normal conditions 293B and L-7 minimally prolong the APD regardless of pacing frequency in dog ventricular muscles and Purkinje fibers, probably because other $K^+$ currents may provide sufficient repolarizing reserve [190]. However, when the repolarizing reserve is decreased by QT prolonging drugs ($I_{Kr}$ or $I_{K1}$ blockers), remodeling (heart failure, cardiac hypertrophy) or inherited disorders, $I_{Ks}$ blockade can produce a marked prolongation of the ventricular APD, an enhanced dispersion of repolarization and TdP arrhythmias [196,199]. Moreover, $I_{Ks}$ or $I_{K1}$ inhibition results in an increased reverse use-dependence in the presence of an $I_{Ks}$ blocker [199]. These findings suggest that drugs that block several $K^+$ channels are probably more hazardous than specific channel blockers. Thus, as occurred with $I_{Kr}$ blockers, the characteristics of the $I_{Ks}$ blockade together with the finding of an association between mutations in KCNQ1 and KCNE1 genes and LQT1 and LQT5 [124,125] lowers the initial interest for the $I_{Ks}$ blockers as class III antiarrhythmic drugs.

3.2.3.3. KCNE1 modifies the pharmacology of $I_{Ks}$. KCNE1 modulates the effects of $I_{Ks}$ blockers and agonists. In fact, KCNQ1/KCNE1 channels have 6–100 fold higher affinity for $I_{Ks}$ blockers (293B, HMR-1556, XE991) than KCNQ1 channels [200,201] and the racemate and the enantiomers of 293B and HMR 1556 have different effects on $I_{Ks}$ and KCNQ1 channels expressed in Xenopus oocytes [49,193,200]. Mutations in two residues located in the pore loop and the S5 segment of the KCNQ1 channel (G272, V307) strongly decrease 293B sensitivity, which suggests that KCNE1 does not directly take part in chromanol binding but acts allosterically to facilitate drug binding to KCNQ1 subunit. Moreover, the $I_{Ks}$ activators mefenamic acid and DIDS have little effect on KCNQ1 channels, although they dramatically enhance KCNQ1/KCNE1 currents [200] and KCNE1 subunits are also responsible for the increase in $I_{Ks}$ produced by docosahexaenoic, oleic and lauric acids [202]. L-364373 (L3) increases $I_{Ks}$ and KCNQ1 currents and shortens the APD in guinea pig ventricular myocytes [203]. This effect is stereosepecific, as S-L3 blocks $I_{Ks}$. Moreover, R-L3 attenuates the prolongation of the APD and suppresses the early afterdepolarizations in dofetilide-treated and in hypertrophied rabbit ventricular myocytes [204]. However, R-L3 does not affect KCNQ1/KCNE1 channels, which indicates that KCNE1 interferes with the binding of R-L3 or prevents its action once bound to KCNQ1 subunits [203]. Thus, specific $I_{Ks}$ agonists may represent a new strategy to suppress arrhythmias resulting from excessive AP prolongation in patients with certain forms of LQTS or cardiac hypertrophy and failure.

3.2.3.4. Structural basis of $I_{Ks}$ blockade. In chimeric constructs of KCNQ1 and KCNQ2 (insensitive to $I_{Ks}$ blockers) expressed in Xenopus oocytes, the molecular determinants of channel block induced by 293B and L-7 are located in the pore loop (T312) and the S6 domain (I337, F339, F340 and A344) [191]. Mefenamic acid and DIDS bind to an extracellular N-terminal segment on KCNE1 [200] and restore $I_{Ks}$ channel gating in otherwise inactive KCNE1 C-terminal mutants, including the LQT5 mutant D76N [205]. It has been proposed that KCNE1 acts as an allosteric regulator of channel gating and permeation, so that in KCNE1 C-terminal mutants the conformational equilibrium is shifted towards an inactive closed state (i.e. a dominant negative phenotype) and DIDS and mefenamic acid bind to N-terminal residues and shift the equilibrium towards an activable closed state, leading to the functional recovery of wild-type $I_{Ks}$ phenotype [163]. These results provide interesting clues for the design of new antiarrhythmic therapies for restoring functional defects responsible for the LQT5.

3.2.3.5. Regulation of $I_{Ks}$. Lowering $[K^+]_o$ and $[Ca^{2+}]_o$ increase $I_{Ks}$ [74,179]. Stimulation of PKA by cAMP, phosphodiesterase inhibitors and $\beta$-adrenergic agonists increases $I_{Ks}$ density and produces a rate-dependent shortening of the APD [206,207]. The KCNQ1/KCNE1 channel forms a macromolecular signaling complex that is coordinated by the binding of the targeting protein yotiao to a leucine zipper motif in the C-terminus of KCNQ1. Yotiao is a scaffold protein which binds and recruits PKA and protein phosphatase 1 to the channel microdomain and regulates it via a phosphorylation of a residue (S27) located in the N-terminal [207]. Disruption of the leucine zipper domain of KCNQ1 and the mutation S27A prevents cAMP-dependent up-regulation of $I_{Ks}$, whereas in the absence of yotiao, KCNQ1/KCNE1 currents are not increased by intracellular cAMP. Phosphorylation of the KCNQ1 subunit by PKA blunts quinidine- and 293B-induced block, probably by inducing a change in the KCNQ1 conformation that modifies the drug access to the blocking site [208]. Thus, $\beta$-adrenergic stimulation, itself a potent proarrhythmic stimulus, may also decrease...
the antiarrhythmic effects of \( I_{Ks} \) blockers. PKC modulation of \( I_{Ks} \) current is complex, suggesting that there are two functionally distinct PKC sites on KCNQ1–KCNE1 channels [206]. Moreover, premodulation by PKC prevents \( I_{Ks} \) modulation by PKA, and PKC has no effect on \( I_{Ks} \) current after potentiation by PKA. These data indicate that \( I_{Ks} \) is modulated by PKC and PKA in a mutually exclusive manner and suggest that multiple interacting phosphorylation sites are involved. Endothelin-1 inhibits the \( I_{Ks} \) enhanced by isoproterenol and forskolin and prolongs APD duration via the ET\(_A\) receptor pertussis toxin (PTX)-sensitive G protein/PKA pathway [209].

4. Inward rectifying K\(^+\) channels

4.1. The inward rectifier K\(^+\) current, \( I_{K1} \)

\( I_{K1} \) is a strong rectifier that passes K\(^+\) currents over a limited range of membrane potentials [17]. At negative membrane potentials \( I_{K1} \) conductance is much larger than that of any other current, and so it clamps the resting membrane potential close to the \( E_K \). Upon depolarization, \( I_{K1} \) channels close almost immediately, remain closed throughout the plateau and open again at potentials negative to \(-20\) mV. Thus, \( I_{K1} \) contributes to terminal phase 3 of repolarization. \( I_{K1} \) density is higher in ventricular than in atrial myocytes, but is similar in epicardial, M and endocardial cells in canine and guinea pig hearts [14]. \( I_{K1} \) is downregulated in endocardial, epicardial and M cells of heart failure canine hearts [176]. \( I_{K1} \) density is very low in SA and AV pacemaker cells and, therefore, the maximum diastolic potential is more depolarized than in atrial and ventricular muscle cells [14].

4.1.1. Molecular basis of \( I_{K1} \)

Kir2.1 subunits (427 amino acids) encoded by the KCNJ2 gene, coassemble to form tetrameric channels [17]. Several \( I_{K1} \) channels with conductances of 9, 21, 35 and 41 pS are recorded in human atrial myocytes [210]. Likewise, different gene families (Kir2.1–2.3) have been found in human heart encoding \( I_{K1} \) [17]. Kir2.1 overexpression increases \( I_{K1} \) density, shortens the APD and hyperpolarizes the resting membrane potential in guinea pig myocytes, while genetic suppression of \( I_{K1} \) results in opposite changes and in a pacemaker phenotype accelerated by isoproterenol [211]. The proximal C-terminus and the M2 domain control the ability of Kir2.1 to interact with other Kir subunits [17].

<table>
<thead>
<tr>
<th>Drug</th>
<th>( I_{K1} )</th>
<th>( I_{K1,Ach} )</th>
<th>( I_{K1,ATP} )</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajmaline</td>
<td>&gt;20 ( \mu )M</td>
<td>1 ( \mu )M</td>
<td>145 ( \mu )M</td>
<td>TXO</td>
<td>[239]</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>100 ( \mu )M</td>
<td></td>
<td></td>
<td>HAM</td>
<td>[42]</td>
</tr>
<tr>
<td>Bepridil</td>
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<td>&gt;2 ( mM )</td>
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<td>TXO</td>
<td>[239]</td>
</tr>
<tr>
<td>Bretilium</td>
<td>6.6 ( \mu )M</td>
<td></td>
<td></td>
<td>TXO</td>
<td>[240]</td>
</tr>
<tr>
<td>Cibenzoline</td>
<td>6.6 ( \mu )M</td>
<td></td>
<td></td>
<td>TXO</td>
<td>[240]</td>
</tr>
<tr>
<td>Clofilium</td>
<td>3.3 ( \mu )M</td>
<td></td>
<td></td>
<td>TXO</td>
<td>[240]</td>
</tr>
<tr>
<td>Chloroquine</td>
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<td></td>
<td></td>
<td>CV</td>
<td>[45]</td>
</tr>
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<td></td>
<td>TXO</td>
<td>[239]</td>
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<tr>
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<td>17.8 ( \mu )M</td>
<td></td>
<td>GPV,TXO</td>
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<tr>
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<td></td>
<td>GPA</td>
<td>[223]</td>
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<td>Flecaïnine</td>
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<td>17.3 ( \mu )M</td>
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<tr>
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<td></td>
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<td>RAM, GPA, TXO</td>
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<td></td>
<td>RVM</td>
<td>[56]</td>
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<td>2 ( \mu )M</td>
<td></td>
<td>GPV,GPA</td>
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<td></td>
<td>TXO</td>
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Data are expressed as IC\(_{50}\) values.

4.1.2. Intrinsic gating of \( \text{I}_{\text{K1}} \)

The rectification of \( \text{I}_{\text{K1}} \) channels results from a voltage-dependent blockade of the inner channel pore by cytosolic \( \text{Mg}^{2+} \), \( \text{Ca}^{2+} \) and polyamines (spermine, spermidine, putrescine) [17]. \( \text{Mg}^{2+} \) blocks single \( \text{I}_{\text{K1}} \) channels with a low \( K_D \) (10.5 \( \mu \text{M} \) at +30 mV), so that at physiological concentrations of cytosplasmic \( \text{Mg}^{2+} \) (≈ 0.5–2 mM), the blocking rate is so fast that inward rectification appears as an instantaneous process. Polyamine-induced inward rectification depends on two negatively charged residues, D172 and E224, located in the M2 domain and in the C-terminal region, respectively [212].

4.1.3. Pharmacology of \( \text{I}_{\text{K1}} \)

Ba\(^{2+}\) is a potent \( \text{I}_{\text{K1}} \) blocker (IC\(_{50} \) = 20 \( \mu \text{M} \)) [210]. Other \( \text{I}_{\text{K1}} \) blockers are summarized in Table 3 [42,45,47,49,52,54,56,57,59,151,213]. \( \text{I}_{\text{K1}} \) blockers prolong atrial, AV node and ventricular APD and are effective against various types of experimental reentrant ventricular tachycardias [159]. Moreover, \( \text{I}_{\text{K1}} \) blockers produce membrane depolarization (an effect that slows conduction velocity due to a voltage-dependent inactivation of \( \text{Na}^+ \) channels) and prolongs the QT interval, both actions being proarrhythmic. Fast heart rates increase the \( [\text{K}^+] \) in the narrow intercellular space to several mM and the \( \text{I}_{\text{K1}} \) density, which results in a shortening of the APD that might offset the ability of \( \text{I}_{\text{Kr}} \) blockers to prolong the APD under these conditions.

4.1.4. Regulation of \( \text{I}_{\text{K1}} \)

Isoproterenol and forskolin inhibit \( \text{I}_{\text{K1}} \) in human ventricular myocytes [17,213], suggesting that \( \text{I}_{\text{K1}} \) can be suppressed by a PKA-mediated phosphorylation of the channel. Moreover, the effects of isoproterenol are inhibited by propranolol and acetylcholine [214]. PMA inhibits \( \text{I}_{\text{K1}} \) in human atrial myocytes and in Kir2.1b channels but this effect is inhibited by chelerytrine [215] and mutations of PKC phosphorylation sites (S64A, T353A). In human atrial myocytes, methoxamine inhibits \( \text{I}_{\text{K1}} \) and this effect is prevented by H-9, a specific PKC inhibitor, suggesting that \( \alpha_1 \)-adrenergic stimulation reduces \( \text{I}_{\text{K1}} \) via PKC-dependent pathways [216]. Kir2.1 channels are negatively controlled by a tyrosine kinase-dependent phosphorylation process [217]. Acidosis modulates \( \text{I}_{\text{K1}} \) but its effect is species-dependent [17]. PIP\(_2\) directly activates \( \text{I}_{\text{K1}} \), perhaps by an electrostatic interaction of the negatively charged head groups of PIP\(_2\) with charged residues in the C-terminal region of the Kir channel [17].

4.1.5. Pathology

\( \text{I}_{\text{K1}} \) is downregulated in spontaneously hypertensive rats, in models of cardiac hypertrophy and in patients with severe heart failure and cardiomyopathies [7,9,35]. The reduction in ventricular \( \text{I}_{\text{K1}} \) density is transmurally homogeneous in failing canine hearts [176]. Reduced \( \text{I}_{\text{K1}} \) density is observed in canine Purkinje cells after myocardial infarction [8] and in a 3-day-old infarcted rat heart, but this reduction is greater in epicardial than in endocardial myocytes [66]. The ventricular myocytes from patients with idiopathic dilated cardiomyopathy exhibit decreased channel activity, longer APD and lower resting membrane potentials than those from patients with ischemic cardiomyopathy [218]. The downregulation of \( \text{I}_{\text{K1}} \) produces membrane depolarization, prolongation of the APD and both early and delayed afterdepolarizations [7,9]. However, \( \text{I}_{\text{K1}} \) density increases in patients with chronic AF [219]. Mutations of KCNJ2 encoding Kir2.1 result in dominant negative effects on the current and have been associated with Andersen’s syndrome, an inherited disease characterized by periodic paralysis, dysmorphic features, QT prolongation and ventricular arrhythmias [220].

4.2. The acetylcholine-activated \( K^+ \) current, \( \text{I}_{\text{KAch}} \)

Acetylcholine activates \( K^+ \) channels (\( \text{K}_{\text{Ach}} \)) in pacemaker SA and atrial cells, thus regulating heart rate [19]. \( \text{K}_{\text{Ach}} \) density is ≈ 6 times greater in the atrium than in the ventricle [14] and results from a tetrameric complex of two Kir3.1 (501 amino acids) and two Kir3.4 (419 amino acids) subunits [221]. Following the binding of acetylcholine to muscarinic M2 receptors, the G\( \beta \gamma \) subunits of the PTX-sensitive G protein (\( G_\beta G_\gamma \) family) activate the \( \text{K}_{\text{Ach}} \) channel by interacting directly to its cytoplasmatic N- and C-termini. Activation of \( \text{I}_{\text{KAch}} \) hyperpolarizes the membrane potential, slows the spontaneous firing rate of the pacemaker cells of the SA and AV nodes and delays AV conduction [1,12]. These effects explain why vagal maneuvers or intravenous adenosine can terminate atrioventricular reentrant tachyarrhythmias. \( \text{I}_{\text{KAch}} \) activity can be stimulated by intracellular ATP, PIP\(_2\) and ET\(_A\) endothelin, \( \mu \) opioid, \( \alpha_2 \)-adrenergic and A1 adenosine receptor agonists [11,12,222] and inhibited by intracellular acidification and several antiarrhythmic drugs (Table 3) [223–225]. Disopyramide, procanamide and pilsicainide mainly block the muscarinic receptors, while flecaïnide and propafenone act as open channel blockers [224]. Atrial \( \text{K}_{\text{Ach}} \) channels are inhibited by membrane stretch, possibly serving as a mechanoelectrical feedback pathway, a property conferred by the Kir3.4 subunit [226]. Vagal stimulation produces a nonuniform shortening of the atrial APD and refractoriness mediated by activation of \( \text{I}_{\text{KAch}} \), an effect that may contribute to the perpetuation of AF [227]. Chronic AF reduces \( \text{I}_{\text{KAch}} \) density possibly to counteract the AF-induced nonuniform shortening of the atrial refractoriness [219].

4.3. ATP-sensitive \( K^+ \) channels, \( \text{K}_{\text{ATP}} \)

Cardiac \( \text{K}_{\text{ATP}} \) (~ 70–90 pS) are inhibited by physiological intracellular ATP levels (ATP\(_i\)), coupling cell metabolism to membrane potential [18,228]. The mechanism of inward rectification involves an open channel blockade by intracellular Mg\(^{2+}\) and Na\(^+\) ions [229].
4.3.1. Molecular basis of $I_{KATP}$

Cardiac $K_{ATP}$ results from the coassembly of four inwardly rectifying channel $\alpha$-subunits (Kir6.2) and four regulatory SUR2A subunits (Fig. 2B) [18,228]. Kir6.2 subunits confer inhibition by ATP [230]. The SUR2A subunit has three transmembrane domains (TMD0, TMD1, and TMD2), each of which consists of five, five, and six membrane spanning regions and two nucleotide binding folds (NBF-1 and NBF-2) located in the loop between TMD1 and TMD2 and in the C-terminus, respectively [18,231]. The SUR2A subunit confers sensitivity to MgADP, sulfonylureas and K$^+$ channel openers (KCOs) and ATP hydrolysis at each NBF gates the K$^+$ permeation through the Kir6.2 $\alpha$-subunit [18,228]. There is an endoplasmic reticulum retention motif (RKR) in the C-terminal region in Kir6.2 and in an intracellular loop between TMD1 and NBF-1 in SUR2A that prevents their surface expression in the absence of the other subunit [232].

4.3.2. Role of $K_{ATP}$ channels

Opening of $K_{ATP}$ during myocardial ischemia shortens the APD and decreases Ca$^{2+}$ influx through L-type channels. Both effects prevent cardiac Ca$^{2+}$ overload, preserve ATP levels, and increase cell survival [233,234]. Activation of $K_{ATP}$ plays an important role in ischemic preconditioning (IPC), wherein single or multiple brief periods of myocardial ischemia confer protection against a subsequent prolonged ischemia, reducing myocardial infarct size, severity of stunning and incidence of cardiac arrhythmias. However, activation of $K_{ATP}$ also results in shortening of the APD, accumulation of extracellular K$^+$, membrane depolarization and slow conduction velocity, effects that render the ischemic heart vulnerable to reentrant arrhythmias [159]. Kir6.2 $\alpha$-subunits (Kir6.2/C0) mice lack K$_{ATP}$ and present an aberrant regulation of cardiac excitability, inadequate Ca$^{2+}$ handling, ventricular arrhythmias and sudden death following sympathetic stimulation [235]. These results suggest that Kir6.2 is required for adaptation to stress.

4.3.3. Pharmacology of $I_{KATP}$

$K_{ATP}$ are inhibited by ATP and activated by MgADP, so that the channel activity is regulated by the ATP/ADP ratio [16]. Several factors desensitize $K_{ATP}$ to inhibition by ATP, including nucleotide diphosphates, lactate, oxygen-derived free radicals and adenosine A1 receptor stimulation [18,228].

KCOs (pinacidil, cromakalim, rimakalim and nicorandil) bind to two distinct regions of TMD2, the intracellular loop joining TM13 and TM14 and TM16 and TM17 (residues K1249 and T1253) [18,228,236]. They exert cardioprotective effects in experimental models of myocardial ischemia/reperfusion and in patients with acute myocardial infarction [159,233]. However, KCOs also activate vascular $K_{ATP}$ (Kir6.1/SUR2B) and produce hypotensive effects that limit their use in the treatment of myocardial ischemia. Moreover, since $I_{KATP}$ density is larger in the epicardium, KCOs produce a more marked shortening of APD in epicardial cells, leading to a marked dispersion of repolarization and to the development of extrasystolic activity via a mechanism of phase 2 reentry [237]. However, KCOs shorten the APD (QT interval), reduce transmural dispersion of repolarization and suppress early and late afterdepolarizations induced in patients with LQT1 [238]. Thus, KCOs may prevent spontaneous TdP when congenital or acquired LQTs is secondary to reduced $I_{Kr}$ or $I_{Ks}$.

$K_{ATP}$ are blocked by sulfonylureas (i.e. glibenclamide, gliclazide, glipizide, glimepiride, tolbutamide), gliburide (repaglinide, nateglinide) and various antiarrhythmic drugs (Table 3) [12,239–243]. The sulfonylurea binding sites include the region between TM15 and TM16 in TMD2 and S1237, located in the loop between TM15 and TM16 [18,244]. Cardiac $I_{KATP}$ blockers prevent the shortening of the APD and the incidence of ventricular fibrillation during the myocardial ischemia, even when in models of ischemia–reperfusion they are mainly arrhythmogenic [159,233,234]. The clinical effects of glibenclamide are also contradictory in type 2 diabetic patients with coronary artery disease [245]. Moreover, since $K_{ATP}$ are present in pancreatic $\beta$-cells and smooth muscle [18], $I_{KATP}$ blockers can produce hypoglycemia and coronary vasoconstriction, effects that may preclude their interest as antiarrhythmic agents. Cardioselective $I_{KATP}$ blockers (clamikalant, HMR 1098) inhibit hypoxia-induced APD shortening and prevent ventricular fibrillation induced by coronary artery occlusion in post-infarcted conscious dogs at doses that have no effect on insulin release, blood pressure or coronary blood flow [233,234]. Thus, they may represent a new therapeutic approach to the treatment of ventricular arrhythmias in patients with coronary heart disease.

Recently, it has been postulated that mitochondrial $K_{ATP}$ (mito$K_{ATP}$) rather than sarcolemmal $K_{ATP}$ (sarc$K_{ATP}$) are responsible for IPC [233]. In fact, diazoxide, a selective mito$K_{ATP}$ agonist mimicks IPC, whereas 5-hydroxydecanoate (5-HD), a selective mito$K_{ATP}$ blocker, suppresses the cardioprotection induced by IPC and diazoxide. Since cardiac mito$K_{ATP}$ appear to be pharmacologically distinct from sarc$K_{ATP}$, it seems possible to develop selective mito$K_{ATP}$ openers devoid of hemodynamic and cardiac electrophysiological side effects of first generation $K_{ATP}$ openers. The selective mito$K_{ATP}$ opener BMS-191095 exerts cardioprotective effects without shortening APD, which may explain the reduced propensity for reentrant arrhythmias [246]. However, recent evidence has demonstrated that diazoxide also activates sarc$K_{ATP}$ channels [247], an effect that is inhibited by HMR1098 but not by 5-HD and that it does not improve contractile function in Kir2.2 $\alpha$-subunit mice [248]. These data suggest that activation of sarc$K_{ATP}$ rather than of mito$K_{ATP}$ is responsible for its cardioprotective effects. Therefore, further research is needed to determine the molecular basis of mito$K_{ATP}$ and the cardioprotection induced by specific mito$K_{ATP}$ openers [233].
4.3.4. Regulation of $K_{\text{ATP}}$

Both PKC and adenosine A1 receptor activation enhance $K_{\text{ATP}}$ and induce IPC and these effects are suppressed by chelerythrine or bisindolylmaleimide [249]. The effects of PKC on $K_{\text{ATP}}$ activity are associated with phosphorylation at T180 in the Kir6.2 subunit. $K_{\text{ATP}}$ activated by glucose-free anoxia close immediately upon reoxygenation, but activation of both novel and conventional PKC isoforms contributes to the persistent opening of the channels under these circumstances [250]. $K_{\text{ATP}}$ can be activated indirectly by PKA-coupled and other protein kinase-coupled receptors via phosphorylation of channel proteins or by depleting cellular ATP levels [244]. The PKA site responsible for channel stimulation includes the residues S372 and T224.

$\text{PIP}_2$ directly interacts with positively charged residues (R176, R177) at the C-terminus of Kir6.2 subunit stabilizing the open state of the channel and antagonizes ATP inhibition of $K_{\text{ATP}}$ [17,251], whereas breakdown of $\text{PIP}_2$ by PLC the open state of the channel and antagonizes ATP inhibition of $K_{\text{ATP}}$ [252]. Moreover, $\text{PIP}_2$ mimics ATP in preventing current rundown and in rescuing activity after rundown [251]. Protein tyrosine kinases (PTKs) are also mediators of ischemic preconditioning. In guinea pig ventricular myocytes the PTK inhibitor genistein elicited $I_{\text{KATP}}$. Stimulation of receptor PTKs with epidermal growth factor, nerve growth factor or insulin attenuates, while the protein tyrosine phosphatase inhibitor orthovanadate prevents the effects of genistein on $I_{\text{KATP}}$ [249]. The effects of PKA-coupled and other protein kinase-coupled receptors are activated by NO donors in rabbit hearts may underlie the background K$^+$ current active during the plateau of the action potential ($I_{\text{Kp}}$) [20].

The release of nitric oxide (NO) and bradykinin during ischemia can also play a role in IPC. $K_{\text{ATP}}$ are activated via the NO-cGMP-PKG signaling pathway, which phosphorylates some serine–threonine residues and this activation is reversed by protein phosphatase 2A [254]. PKC-$\epsilon$ and PKC-$\eta$ are activated by NO donors in rabbit hearts [255] and their infarct-size limiting effects are abolished by chelerythrine. Interferon-$\alpha$ inhibits $I_{\text{KATP}}$ in rabbit ventricular cells and this effect is blocked by genistein, but is not affected by H-7, an inhibitor of PKC and PKA [256]. These findings suggest that tyrosine kinase-mediated inhibition of $I_{\text{KATP}}$ by cytokines may aggravate cell damage during myocardial ischemia.

5. Two pore or background K$^+$ channels, $K_{2P}$

Cardiac cells have $K_{2P}$ currents with little time- or voltage-dependency, termed background currents, that regulate resting membrane potential and cell excitability (Fig. 2C) [20–22]. $K_{2P}$ are insensitive to conventional K$^+$ channel blockers (4-AP, TEA, Ba$^{2+}$, Cs$^+$, glibenclamide), but show differential sensitivity to membrane stretch, changes in pH, fatty acids and inhalation (e.g. halothane, isoflurane, chloroform) and local (e.g. lidocaine, bupivacaine) anesthetic agents and are regulated by second messenger phos-}

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1: currents inhibited, ↑: currents increased, 0: no effects, a: activates an inactive channel.

PKA: protein kinase A, PKC: protein kinase C.
(1) PKA was activated with cAMP, forskolin and 1-methyl-3-isobutylxanthine (IBMX).
(2) PKC was activated with phorbol-12-myristate 13-acetate (PMA).

Cardiac expression of TWIK-1 may underlie the background K$^+$ current active during the plateau of the action potential ($I_{\text{Kp}}$) [20].

TASK currents are highly sensitive to variations in the extracellular pH in the physiological range. Mutations of the residue H98 (H98D) which lies immediately distal to the first pore domain reduces pH sensitivity [22]. TASK currents are insensitive to arachidonic acid, stretch and PKC activators, but are inhibited by local anesthetics and submicromolar concentrations of the endocannabinoid anandamide and increased by volatile anesthetics [22]. TREK and TRAAK channels are activated by membrane stretch, cell swelling, negative pressure and lysophosphatidylcholine. TREK and THIK-1 are stimulated by arachidonic acid and polyunsaturated fatty acids and THIK-1 channels are inhibited by intracellular acidification. TWIK currents are increased by arachydonic acid and PKC activation and inhibited by intracellular acidosis, but are insensitive to PKA activation. In contrast, TREK channels are modulated by $G_{q}$, $G_{i}$ and $G_{s}$-coupled receptors, inhibited by activators of PKA and PKC and increased by intracellular acidosis. Thus, far from being passive leaks, as initially thought, $K_{2P}$ channels are sensitive to changes in pH, membrane stretch and phosphorylation state, possibly acting as cellular sensors and transducers, translating these stimuli into a change in the membrane potential and excitability [22]. If so, some $K_{2P}$ channels will represent important therapeutic targets in the near future.

6. Conclusions and perspectives

Cardiac K$^+$ channels have been recognized as potential therapeutic targets. However, the rational design of safer and more effective K$^+$ channel blockers/openers and the
the recent identification of the structures of several K+ messengers and ligands have been characterized. Moreover, structural biophysical properties, subunit stoichiometry, channel assembly and modulation by intracellular second messengers and ligands have been characterized. Moreover, the precise role that each K+ channel gene product plays subunits and splice variants that underlie the various K+ disorders (Romano–Ward and Jervell–Lange–Nielsen long QT syndromes). Moreover, given the diversity of α- and β-subunits and splice variants that underlie the various K+ channels and the potential for heteromeric channel assembly, the precise role that each K+ channel gene product plays in the regional heterogeneity of native currents or in the cellular pathophysiology in the human heart is uncertain. Similarly, very little is known on the possible transcriptional, translational and post-translational mechanisms regulating the function and expression of K+ channel α and β subunits as well as on the gene products required to maintain its normal function. All this information is the basis to understand the pathophysiology and arrhythmogenesis of the diseased hearts and to identify new targets for the development of K+ channel modulators safer and more effective than those actually prescribed.

Ion channel blockers tested in clinical trials have shown no decrease mortality outcome, but an increase in proarhythmia. Thus, and despite the success of electrical devices and the promising alternative of somatic gene transfer, it is clear that we need safer and more effective antiarrhythmic drugs. In this review we indicated new promising targets, including atrioselective \( I_{Kr} \) blockers, \( I_{Ks} \) agonists and the possibility of drug-induced restoring trafficking defects of HERG channels. However, there are several strategies for the development of new K+ channel modulators. Because of the multiple mechanisms of rhythm disturbances drugs with multiple modes of action (i.e. amiodarone-like drugs) are needed, even when we do not know how to optimize chemical structures for the rational targeting of multiple ion channels. Another possibility is to combine \( I_{Kr}/I_{Ks} \) with β-adrenergic blockade or \( I_{CaL} \) and blockade in order to reduce the risk of proarhythmia. An unexplored perspective is to develop drugs that affect Kv channel gating by interacting at regions away from the ion conducting pore to produce the desired changes in cardiac refractoriness.

Other possible targets include accessory β-subunits and intracellular signalling molecules (PKA, PKC, cyclic nucleotides). Finally, to optimize drug–channel interaction thousands of chemical entities must be screened to identify a few candidate drugs. The new technological developments (planar-array chip-based approaches) address the high throughput restrictions of classical patch clamp electrophysiology and allow recordings from hundreds of cells per day and provide new perspectives for drug discovery. All this information is the basis to understand the pathophysiology and arrhythmogenesis of the diseased hearts and to identify new targets for the development of K+ channel modulators safer and more effective than those actually prescribed.

Acknowledgements

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References


Volders PG, Sigpo KD, Carmeliet E, et al. Repolarizing K+ currents i(to) and i(kb) are larger in right than left canine ventricular midmyocardium. Circulation 1999;99:206–10.


Bodi I, Muth JN, Hahn HS, et al. Electrical remodeling in hearts

Antzelevitch C, Sicouri S, Litovsky SH, et al. Heterogeneity within

Li GR, Feng J, Yue L, et al. Evidence for two components of delayed

Tristani-Firouzi M, Sanguinetti MC. Structural determinants and bio-

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Grissmer S, Nguyen AN, Aiyar J, et al. Pharmacological character-


Sanguinetti M, Jurkiewicz N. Two components of cardiac delayed

Nattel S, Yue L, Wang Z. Cardiac ultrarapid delayed rectifiers. A


Tseng G-N, Iqbal M. The hERG channel. J Mol Cell Cardiol 2001;33:

Tristani-Firouzi M, Sanguinetti MC. Structural determinants and bio-

Li GR, Feng J, Yue L, et al. Evidence for two components of delayed

Snyders D, Knoth K, Roberds S, et al. Time-, voltage-, and state-

Franqueza L, Longobardo M, Vicente J, et al. Molecular determinants

Wang Z, Fermini B, Nattel S. Sustained depolarization-induced out-

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Grisser M, Nguyen AN, Aiyar J, et al. Pharmacological character-

Nattel S, Matthews C, De Blasio E, et al. Dose-dependence of


Kobayashi S, Reien Y, Ogura T, et al. Inhibitory effect of bepridil on


and inhibits the K’ current through a human cardiac cloned channel


González T, Longobordo M, Caballero R, et al. Effects of bupiva-

caine and a novel local anesthetic, IQB-9302, on human cardiac K’


Caballero R, Delpón E, Valenzuela C, et al. Direct effects of cande-

sartan and eprosartan on human cloned potassium channels involved


Rampe D, Roy ML, Dennis A, et al. A mechanism for the proar-

rhythmic effects of cisapride (Propulsid): high affinity blockade of

the human cardiac potassium channel HERG. FEBS Lett 1997;

417:28–32.

Caballero R, Delpón E, Valenzuela C, et al. Losartan and its metab-

olite E3174 modify cardiac delayed rectifier K* currents. Circulation


Valenzuela C, Delpón E, Franqueza L, et al. Comparative effects of

nonsedating histamine H1 receptor antagonists, ebastine and terfe-

nadine, on human Kv1.5 channels. Eur J Pharmacol 1997;326:

257–63.

Moreno I, Caballero R, González T, et al. Effects of ibersartan on

cloned potassium channels involved in human cardiac repolarization.

J Pharmacol Exp Ther 2003;304:862–73.


channels by loratadine: voltage-, time- and use-dependent block at

concentrations above therapeutic levels. Cardiovasc Res 1997;35:

341–50.

Matuda T, Masumiya H, Tanaka N, et al. Inhibition by a novel anti-

arrhythmic agent, NIP-142, of cloned human cardiac K’ channel


Choe H, Lee YK, Lee YT, et al. Papaverine blocks hKv1.5 channel

current and human atrial ultrarapid delayed rectifier K’ currents.


cardiac K’ channel by the neuroleptic pimozide. Eur J Pharmacol


Franqueza L, Valenzuela C, Delpón E, et al. Effects of propafenone

and 5-hydroxy-propafenone on hKv1.5 channels. Br J Pharmacol

1998;124:969–78.

Snyders D, Knoth K, Roberds S, et al. Time-, voltage-, and state-

dependent block by quinidine of a cloned human cardiac channel.


Valenzuela C, Delpón E, Franqueza L, et al. Effects of ropivacaine

on a potassium channel (hKv1.5) cloned from human ventricle.


Yeola SW, Rich TC, Ubele BN, et al. Molecular analysis of a

binding site for quinidine in a human cardiac delayed rectifier K’

channel. Role of S6 in antiarrhythmic drug binding. Circ Res


Franqueza L, Longobardo M, Vicente J, et al. Molecular determin-

ants of stereoselective bupivacaine block of hKv1.5 channels. Circ


Longobardo M, Delpón E, Caballero R, et al. Structural determinants

of potency and stereoselective block of hKv1.5 channels induced by


Caballero R, Moreno I, González T, et al. Putative binding sites for

benzocaine on a human cardiac cloned channel (Kv1.5). Cardiovasc


Zhang X, Anderson JW, Fedida D. Characterization of nifedipine

block of the human heart delayed rectifier, hKv1.5. J Pharmacol Exp

Ther 1997;281:1247–56.


channel block: conservation of drug binding sites among voltage-


cardiac delayed-rectifier K’ channel (Kv1.5) by polyunsaturated fatty


Varro, A, Balati, B, Iost, N, et al. The role of the delayed rectifier $I_{Ks}$ in guinea pig cardiac myocytes and guinea pig $I_{Ks}$ channels by the chromanol 293B. Pflügers Arch 1996;432:1094–6.


Tong, Y, Brandt, GS, Li, M, et al. Tyroside decaging leads to substan-


Dobrev, D, Graf, E, Wettwer, E, et al. Molecular basis of downregu-


Cho, H, Hwang, JY, Kim, D, et al. Acetycholine-induced phosphati-

Guillemare, E, Marion, A, Nisato, D, et al. Inhibitory effects of dro-


