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Cardiac and renal inward rectifier potassium channel pharmacology: emerging tools for integrative physiology and therapeutics

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Inward rectifier potassium (Kir) channels play fundamental roles in cardiac and renal function and may represent unexploited drug targets for cardiovascular diseases. However, the limited pharmacology of Kir channels has slowed progress toward exploring their integrative physiology and therapeutic potential. Here, we review recent progress toward developing the small-molecule pharmacology for Kir2.x, Kir4.1, and Kir7.1 and discuss common mechanistic themes that may help guide future Kir channel-directed drug discovery efforts.

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Introduction

Inward rectifier potassium (Kir) channels play key roles in cardiac excitation-contraction coupling, renal water and solute transport, and other vital physiological and pathophysiological processes [1]. In mammals, the channel superfamily is comprised of at least 16 genes (*KCNJx*) and 7 subfamilies (Kir1.x-7.x) that share a common molecular structure [2]. Kir channels are tetramers of identical (homomeric) or similar (heteromeric) subunits assembled around an aqueous membrane-spanning pore. They lack regulatory voltage-sensing domains, but are gated by polyvalent cations (e.g. polyamines and Mg^{2+} [3–5]) that occlude the pore at cell potentials more positive than the K^+ equilibrium potential (E_K). Kir channels thus function as biological diodes by limiting the extent of outward, but not inward, K^+ current, a property is termed inward rectification. *Strong rectifiers* exhibit a sharp cutoff of outward current due to the presence of negatively charged pore-lining residues that stabilize electrostatic interactions with pore-blocking cations (Figure 1), whereas *weak rectifiers*, have uncharged amino acids at some of the equivalent positions that weaken block and enable the channels to remain open well above E_K [1].

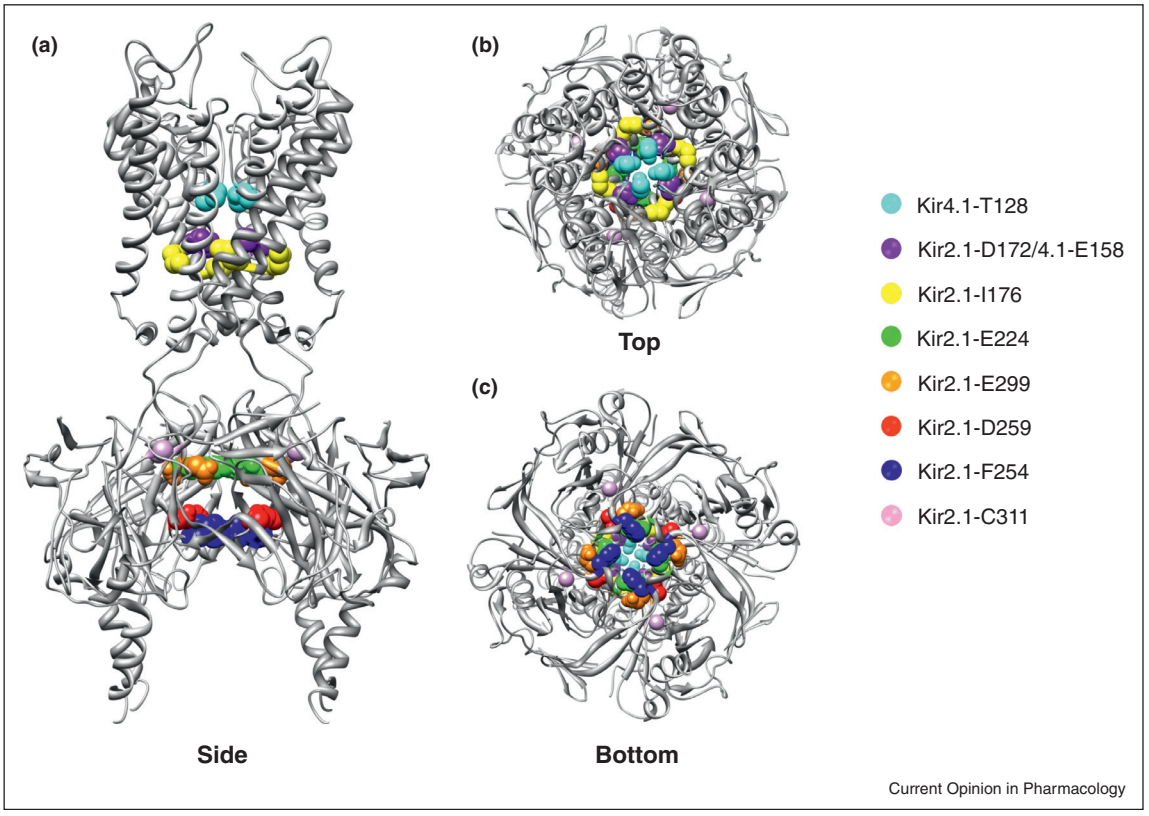
Kir channels are also gated through interactions with the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2). X-ray structures of Kir2.2 revealed that binding of a PIP_2-derivative induces conformational changes in the cytoplasmic domain (Figure 1) that opens the pore [6]. Growing evidence suggests that some cationic amphiphilic drugs (e.g. carvedilol, mefloquone, thiopental) inhibit Kir channels by interfering with channel-PIP_2 interactions [7–9]. These have been discussed recently elsewhere [10] and will not be considered further.

As discussed below, emerging physiological, genetic, and pharmacological data point to specific Kir channels as novel drug targets for cardiac and renal diseases. With few exceptions, the rudimentary pharmacology has mired efforts to explore their integrative physiology and therapeutic potential of Kir channels. This review provides a snapshot of the current state of the field, highlighting recent progress and opportunities for developing the pharmacology of cardiac and renal Kir channels.

Cardiac Kir2.x channels

The ability of the heart to function as a pump requires that atrial and ventricular chambers contract in a highly stereotyped and synchronized fashion. Action potentials (AP) originating in the sinoatrial node (SAN) spread through the atria and then ventricles to initiate contractions. Three Kir channel subfamilies contribute to cardiac excitability. Heteromeric Kir3.1/3.4 channels comprising the muscarinic M2 receptor-activated I_{KACh} current slows SAN pacemaker discharge and heart rate in response to parasympathetic nerve stimulation. Activation I_{KATP} carried by heteromeric Kir6.2/SUR channels during metabolic stress contributes to ischemic preconditioning that protects heart function during prolonged ischemia. The molecular physiology, pathophysiology, and pharmacology of I_{KACh} and I_{KATP} have been reviewed extensively [1,11–14] and will not be discussed here. The major focus of this review is on the molecular pharmacology of the third group of cardiac Kir channels: the strong

Figure 1



Putative small-molecule binding sites in a model Kir channel. **(a)** Side, **(b)** top and **(c)** bottom view of chicken Kir2.2 crystal structure with equivalent residues implicated in small-molecule binding highlighted with sphere. Channel-specific residues are indicated in the legend. See text and Table 1 for details.

rectifiers Kir2.1, Kir2.2, and Kir2.3. Homomeric and heteromeric assemblies of Kir2.x subunits underlie the I_{K1} current that dominates the resting K^+ conductance and shapes late-phase action potential (AP) repolarization in cardiac myocytes [12]. Genetic loss-of-function and gain-of-function mutations in Kir2.1 (*KCNJ2*) prolong and shorten, respectively, the AP duration and increase the susceptibility to lethal ventricular arrhythmias [15,16]. No disease-causing mutations in Kir2.2 (*KCNJ12*) and Kir2.3 (*KCNJ4*) have been reported.

It is clear that Kir2.x channels play important roles in heart pump function, but their species and regional heterogeneity and lack of specific pharmacological probes has slowed efforts to develop a comprehensive understanding of their integrative physiology and druggability in cardiac diseases. Below we discuss recent progress toward developing these critically needed tools for overcoming this barrier.

Chloroquine

The 4-aminoquinoline derivative chloroquine (Table 1) is used widely as an anti-malarial drug in developing countries. However, prolonged treatment or overdose can induce lethal ventricular arrhythmias through inhibition of various cardiac ion channels [17]. Sanchez-Chapula and colleagues [18••] found that chloroquine blocks Kir2.1 at clinically relevant doses (half-maximal inhibitor concentration [IC_{50}] = 8.7 μM) and in a voltage-dependent manner consistent with 'knock-off' of the drug from the intracellular pore [19]. Direct application of chloroquine to the cytoplasmic face of Kir2.1 results in channel inhibition that is much faster (~15 seconds) than that observed when applied extracellularly (~8 min), suggesting chloroquine must cross the plasma membrane to reach an intracellular binding site. Indeed, alanine-scanning mutagenesis revealed that mutation of four residues in the cytoplasmic domain of Kir2.1 led to progressive loss of chloroquine sensitivity with the following rank-order: E224 > D259 > E299 > F254 (Figure 1). Molecular modeling identified an energetically favorable docking pose between chloroquine and the channel involving electrostatic interactions between E224, D259, and E299 as well as aromatic pi-stacking with P254. Identifying the putative chloroquine binding site creates opportunities for designing safer analogs exhibiting reduced Kir2.1 activity and cardiotoxicity.