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Review

Towards the Development of AgoKirs: New Pharmacological Activators to Study $K_{ir}2.x$ Channel and Target Cardiac Disease

Laura van der Schoor [1,†], Emma J. van Hattum [1,†], Sophie M. de Wilde [1,†], Netanja I. Harlianto [1,†], Aart-Jan van Weert [1,†], Meye Bloothooft [2] and Marcel A. G. van der Heyden [2,*]

1 Honours Program CRU+ Bachelor, University Medical Center Utrecht, Heidelberglaan 100, 3584 CM Utrecht, The Netherlands; l.vanderschoor@students.uu.nl (L.v.d.S.); e.j.vanhattum@students.uu.nl (E.J.v.H.); s.m.dewilde@students.uu.nl (S.M.d.W.); n.i.harlianto@students.uu.nl (N.I.H.); a.vanweert@students.uu.nl (A.-J.v.W.)

2 Department of Medical Physiology, Division of Heart & Lungs, University Medical Center Utrecht, Yalelaan 50, 3584 CM Utrecht, The Netherlands; M.Bloothooft-3@umcutrecht.nl

* Correspondence: m.a.g.vanderheyden@umcutrecht.nl; Tel.: +31-88-755-8901

† These authors contributed equally to this work.

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Abstract: Inward rectifier potassium ion channels (I_{K1}-channels) of the $K_{ir}2.x$ family are responsible for maintaining a stable negative resting membrane potential in excitable cells, but also play a role in processes of non-excitable tissues, such as bone development. I_{K1}-channel loss-of-function, either congenital or acquired, has been associated with cardiac disease. Currently, basic research and specific treatment are hindered by the absence of specific and efficient $K_{ir}2.x$ channel activators. However, twelve different compounds, including approved drugs, show off-target I_{K1} activation. Therefore, these compounds contain valuable information towards the development of agonists of K_{ir} channels, AgoKirs. We reviewed the mechanism of I_{K1} channel activation of these compounds, which can be classified as direct or indirect activators. Subsequently, we examined the most viable starting points for rationalized drug development and possible safety concerns with emphasis on cardiac and skeletal muscle adverse effects of AgoKirs. Finally, the potential value of AgoKirs is discussed in view of the current clinical applications of potentiators and activators in cystic fibrosis therapy.

Keywords: inward rectifier channel; $K_{ir}2$; agonist; I_{K1}; Andersen syndrome; heart failure

1. $K_{ir}2.x$ Expression, Structure and Rectification

$K_{ir}2.x$ potassium channels are part of the inward rectifier K^+ (I_{K1}) channels family, which are of major importance for stabilizing the resting membrane potential of excitable cells and contribute to final action potential repolarization in cardiomyocytes [1,2]. Additionally, I_{K1} has an important role in non-excitable cells, for example, in osteoblasts, in which I_{K1} channels are involved in chondrogenesis and osteoblastogenesis [3].

$K_{ir}2.x$ channels are expressed in several excitable tissues, such as skeletal muscle ($K_{ir}2.1$, $K_{ir}2.2$, and $K_{ir}2.6$), brain ($K_{ir}2.1$, $K_{ir}2.2$, and $K_{ir}2.3$), and heart ($K_{ir}2.1$, $K_{ir}2.2$, and $K_{ir}2.3$) [4]. Within tissues, isoform expression can vary. For example, $K_{ir}2.3$ is dominantly expressed in the atria, whereas $K_{ir}2.1$ is mainly present in the ventricles and has a higher channel density [5]. An intermediate level of expression of $K_{ir}2.x$ channels is present in smooth muscle tissue and the retina [4].

I_{K1} channels are homo- or heterotetrameric assemblies of $K_{ir}2.x$ monomeric subunits [6,7]. An individual subunit consists of a transmembrane and a cytoplasmic region. The transmembrane

region of the channel controls ion selectivity and channel gating, and the cytoplasmic domain functions as a gating regulator [1].

Depending on the membrane potential (V_m) relative to the potassium equilibrium potential (E_k), I_{K1} channels conduct either inward or outward current. The outward currents are smaller due to rectification properties. Inward rectification is based on binding of polyamines and Mg^{2+} in the conducting pore region, at V_m more positive than E_k and inhibiting outward current [8]. Polyamine and Mg^{2+} dependent blocks are absent at more negative V_m than E_k, which results in inward current [9]. Each $K_{ir}2.x$ isoform displays its own characteristic rectification profile [5].

Whereas much knowledge on $K_{ir}2.x$ function has been derived from experimental in vitro studies and transgenic mice, insights on the physiological contributions of I_{K1} in larger organisms can only be deduced from a few experimental large animal studies using specific inhibitors [10,11] and patients with gain- or loss-of-function mutations [5]. For example, specific I_{K1} inhibition in awake dogs induced adverse effects like premature ventricular contractions, respiratory distress, and mild generalized muscle weakness [11]. On the contrary, the effects of specific I_{K1} activation have not been investigated in large animals. Therefore, as argued before [12], specific I_{K1} modifying compounds, i.e., inhibitors and activators, would benefit experimental studies on the role of I_{K1} in muscle contraction, cardiac function, neuronal excitation, and bone development.

2. $K_{ir}2.x$ Disease Relationships

I_{K1} function and disease have a bidirectional relationship. On the one hand, mutations can affect $K_{ir}2.x$ function, while, on the other hand, diseased states influence $K_{ir}2.x$ function. Both congenital and acquired loss-of-function may result from impaired functional $K_{ir}2.x$ expression at the plasma membrane, for example, due to aberrant transcription, trafficking, or gating kinetics.

Gain-of-function mutations in the *KCNJ2* gene encoding $K_{ir}2.1$ are associated with ventricular arrhythmia like short QT syndrome type 3 [13] or atrial arrhythmia such as congenital atrial fibrillation (AF) [14,15]. Loss-of-function mutations in *KCNJ2* are associated with autosomal dominant Andersen syndrome (AS) [16]. This disease is characterized by symptoms like ventricular arrhythmias, periodic muscle paralysis, and dysmorphologies such as a broad forehead, a cleft palate, and small hands and feet [17].

AF results in increased I_{K1} densities in atrial tissue [18–20], which thereafter causes a shortening of the atrial effective refractory period that ultimately promotes and stabilizes atrial re-entry. Chronic heart failure (HF) induces loss-of-function of I_{K1}. In experimental models of HF, I_{K1} is reduced in canine and rabbit ventricles [21–23]. More recently, heart failure following myocardial infarction (MI) in a porcine model shows significantly decreased I_{K1} density [24]. Also, declined density of whole-cell I_{K1} was also found in terminal HF patients [25].

Inward rectifier current inhibition has been suggested as a potential avenue in AF therapy [26]. Indeed, in preclinical and clinical settings, pharmacological I_{K1} inhibition is an effective method of AF reduction [10,27]. Moreover, pharmacological activators have been developed for several ion channels and successfully implemented in clinical therapy to treat cystic fibrosis, hypertension, and alopecia, to name a few applications [28–30]. Currently, no drugs are available that specifically target impaired I_{K1}, whereas such a treatment may be effective in AS and HF. However, twelve drugs and compounds exert I_{K1} activating capacity (Figure 1, Table 1). We will review these drugs for indirect and direct properties to activate the different $K_{ir}2.x$ channels, focusing on $K_{ir}2.1$, $K_{ir}2.2$, and $K_{ir}2.3$.

Table 1. Compounds with I_{K1} activating properties.

Compound	Readout	Test System	Dose-Effect Relation	Mechanism of Action	Ref.
Direct activators					
Flecainide	C	CHO cells	$I_{Kir2.1}$ EC_{50}/Emax (−50 mV) = 0.4 ± 0.01 μM/53.9 ± 3.6% $I_{Kir2.1}$ EC_{50}/Emax (−120 mV) = 0.8 ± 0.01 μM/22.0 ± 1.96%	Interaction with Cys311	[31]
	C	guinea pig ventricular cmc	I_{K1} 1 μM: 19.5 ± 3.2% (−120 mV); 38.0 ± 9.5% (−40 mV)	Interaction with Cys311	[31]
PREGS	C	*Xenopus* oocytes	$I_{Kir2.3}$ EC_{50} (−70 mV) = 15.6 ± 0.9 μM	Binding extracellular site	[32]
Propafenone	C	CHO cells	$I_{Kir2.1}$ EC_{50}/Emax (−50 mV) = 12.0 ± 3.0 nM/42.0 ± 2.6%	Interaction with Cys311	[33]
	C	guinea pig ventricular cmc	I_{K1} 0.5 μM: approx 45 ± 5% (−40 mV)	Interaction with Cys311	[33]
Timolol	C	CHO cells	$I_{Kir2.1}$ EC_{50} (−50 mV) = 3.2 ± 0.3 nM	Interaction with Cys311	[33]
Indirect activators					
Aldosterone	C	rabbit ventricular cmc	I_{K1} 10 nM: 1.6-fold increase NPo of 30 pS current	MR-independent activation	[34]
		rat ventricle	K_{ir}2.1 2.24 mg/h/kg 4 wks: approx. 1.57 ± 0.14 fold K_{ir}2.3 2.24 mg/h/kg 4 wks: approx. 1.26 ± 0.15 fold (ns)	Unknown Unknown	[35]
Isoproterenol	C	*Xenopus* oocytes	$I_{Kir2.1}$ EC_{50} = 27.4 nM * $I_{Kir2.2}$ EC_{50} = 17.8 nM *	PKC dependent via β3-AR PKA dependent via β3-AR	[36]
Valsartan	R,P	rat ventricle	10 mg/kg/day for 7 days prevented K_{ir}2.1 downregulation	Casein Kinase 2 inhibition and/or Th1 immune response inhibition and/or NF-κB-miR-16 pathway	[37–39]
	R,P	ventricular cmc, H9c2 cells	20 μM (48 h) prevented K_{ir}2.1 downregulation	Casein Kinase 2 inhibition and/or Th1 immune response inhibition and/or NF-κB-miR-16 pathway	[37–39]
	C	rat ventricular cmc	20 μM (48 h) prevented I_{K1} inward current downregulation	Th1 immune response inhibition	[38]
Zacopride	C	rat atrial cmc, HEK-293 cells	$I_{Kir2.1}$ EC_{50} (−50 mV) = 30.7 nM $I_{Kir2.1}$ (−50 mV) = 40.7 ± 9.7%	PKA dependent	[40]
LPS	P,C	mouse pulmonary micro-vascular endothelial cells	10 ng/mL (up to 24 h): 1.5-fold increase K_{ir}2.1 10 ng/mL $I_{Kir2.1}$ (inward/outward) = approx. 1.7-fold	Unknown	[41]
Morphine	C	rabbit ventricular cmc	I_{K1} (−60 mV) = 25 ± 9% (0.1 μM); 32 ± 11% (1 μM)	Opioid-receptor pathway independent	[42]
Polydatin	C	rat ventricular cmc	I_{K1} 10 μM: approx. 40% (−100 mV)	Unknown	[43]
Tenidap	C	CHO cells	$I_{Kir2.3}$ EC_{50} = 1.3 μM *	Extracellularly, unaffected by pA2, PKC, and AA secondary pathways	[44]

C, current; cmc, cardiomyocyte; P, protein; R, mRNA. * Voltages unknown.

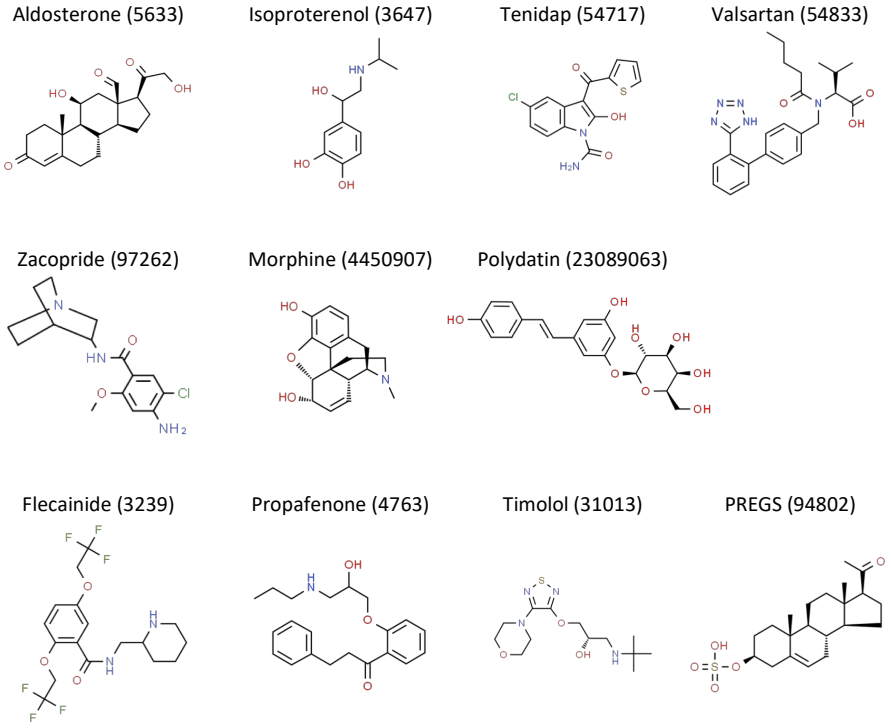

Figure 1. Chemical structures of I_{K1} activating compounds, obtained from the Royal Society of Chemistry-owned ChemSpider website (accessed on 12 June 2020). Structure name and ChemSpider ID is given. Full records can be retrieved at http://www.chemspider.com/Chemical-Structure.ID.html, in which ID should be substituted by the ID number provided above the structure.

3. AgoKirs, Agonists of $K_{ir}2.x$ Function

3.1. Indirect Activators

3.1.1. Aldosterone

Aldosterone is part of the renin-angiotensin-aldosterone-system (RAAS) and is essential in Na^+ homeostasis. Activation of aldosterone and the mineralocorticoïdreceptor (MR) play an important role in the pathophysiology of cardiovascular disease.

In vitro studies using isolated rabbit ventricular cardiomyocytes demonstrated that 10 nmol/L of aldosterone caused a rapid activation (within minutes to days) of a 30 pS K^+-selective current.

This current had pharmacological and biophysical properties that are consistent with those of the I_{K1}-current. By use of RU28318, a specific MR-antagonist, and potassium canrenoate, a non-specific MR-antagonist, the underlying mechanism was determined as independent of the MR pathway [34].

Furthermore, cardiac tissue of rats implanted with osmotic minipumps showed a dose-dependent aldosterone effect on $K_{ir}2.1$ expression after four weeks. At a dose of 0.5 µg/h, a moderate increase of $K_{ir}2.1$ and $K_{ir}2.3$ was observed [35], whereas, in another study, decreased expression levels of $K_{ir}2.1$ surprisingly was shown at 1 µg/h aldosterone [45].

3.1.2. Isoproterenol

Isoproterenol, or isoprenaline (ISO), is a synthetic β-adrenoceptor (β-AR) agonist that has a cardiac muscle stimulating and bronchodilating effect. β3-AR stimulation leads to intracellular signal transduction pathways, including protein kinase A and C (PKA and PKC) [46]. In *Xenopus* oocytes, 10 µM ISO activated $K_{ir}2.2$ currents via a PKA-dependent pathway [36], consistent with findings on the role of PKA in $K_{ir}2.2$ activation [47]. Furthermore, ISO also activated $K_{ir}2.1$ currents via a PKC-dependent pathway [36].

3.1.3. Tenidap

Tenidap is a non-steroidal cyclooxygenase and lipoxygenase inhibitor [48] but also a potent $K_{ir}2.3$ opener. By use of $^{86}Rb^+$ cell efflux and patch clamp electrophysiology, it was found that tenidap increased $K_{ir}2.3$ carried inward and outward current in Chinese hamster ovary (CHO) cells [44]. Tenidap showed some channel specificity as it did not enhance $K_{ir}2.1$ and $K_v1.5$ channel activity [44]; however, it did activate I_{KATP} channels [49].

3.1.4. Valsartan

Valsartan is a highly selective angiotensin type 1 receptor antagonist and is widely used in the treatment of mild to moderate essential hypertension [50]. In rats with MI, induced by coronary artery ligation, $K_{ir}2.1$ mRNA, protein, and the resulting I_{K1} became downregulated, which was associated with ventricular arrhythmias [51]. Such downregulation of $K_{ir}2.1/I_{K1}$ was prevented by Valsartan [37–39], possibly involving multiple mechanisms.

Firstly, casein kinase 2 dependent $K_{ir}2.1$ downregulation after MI was prevented by valsartan. Secondly, valsartan treatment after MI decreased T-helper cell levels and thereby ameliorated $I_{K1}/K_{ir}2.1$ downregulation. Thirdly, valsartan reduced miRNA-16 levels by the prevention of NF-κB upregulation and thereby prevented downregulation of $K_{ir}2.1$/*KCNJ2*/I_{K1} in infarcted hearts. Currently, it is unknown to what extent the working mechanism of valsartan on each individual signaling pathway contributed to restoring normal $K_{ir}2.1$ expression in MI rat hearts. Nevertheless, the authors stated that a direct interaction of valsartan with the $K_{ir}2.1$ ion channel, resulting in its activation, appeared improbable.

3.1.5. Zacopride

Zacopride (ZAC) is an antiemetic, gastroprokinetic, and anxiolytic drug. It is a selective antagonist of the 5-hydroxytryptamine (5-HT)3 receptor and agonist of 5-HT4 receptor. In the adrenal glands, ZAC is known to stimulate the secretion of aldosterone [52].

Rats treated with ZAC showed elevated levels of $K_{ir}2.1$ protein in left ventricular tissue [53]. Furthermore, ZAC treatment prevented ischemia-mediated downregulation of left ventricular $K_{ir}2.1$ protein [54] (note [55]). Additionally, ZAC enhanced both the inward and outward I_{K1} current in rat ventricular myocytes [56,57] but not in atrial cardiac myocytes [40]. ZAC-induced $K_{ir}2.1$ channel activation appeared to be mediated by PKA-dependent phosphorylation of Ser425 in the $K_{ir}2.1$ C-terminus [40]. In human embryonic kidney 293 cells and CHO cells, ZAC increased I_{K1} carried by ectopic homotetrameric $K_{ir}2.1$ channels but not current carried by homotetrameric $K_{ir}2.2$ or $K_{ir}2.3$ channels or heterotetrameric channels containing $K_{ir}2.1$, $K_{ir}2.2$, or $K_{ir}2.3$ [40,57].

3.2. Direct Activators

Within the class of direct activators, interactions with the extracellular domain and cytoplasmic domain have been indicated. The direct activators currently described display isoform specificity and may open the way towards tissue-specific activation of I_{K1}.

3.2.1. Flecainide

Flecainide, a class Ic antiarrhythmic drug, is known to block Na^+ channels and voltage-dependent K^+ (K_v) channels. Thereby, flecainide effectively prolongs action potential duration (APD) in the atria but not in the ventricles [58]. On the other hand, flecainide increases I_{K1} selectively in the ventricles, offering a possible explanation for a difference in effect on atrial and ventricular APD [31].

Flecainide specifically activated $K_{ir}2.1$ channels by a mechanism involving Cysteine311 (Cys311) and had no effect on $K_{ir}2.2$ or $K_{ir}2.3$ channels that contain an alanine instead of a cysteine residue on their equivalent positions (312 and 303, respectively) [31].

Flecainide's mode of action likely involved antagonizing spermine-mediated rectification, resulting in increased outward current. Spermine was shown to inhibit I_{K1} in a concentration-dependent

manner. The presence of flecainide decreased spermine's inhibiting effect, as a rightward shift in the concentration-effect curve was observed [31]. The fact that the E_{max} of spermine was saturated at 82.1 ± 5.5% in the presence of flecainide suggests that spermine block was decreased in a noncompetitive manner by flecainide through allosteric changes to the binding site for polyamines [31].

3.2.2. Propafenone

Propafenone, just like flecainide, is a class Ic antiarrhythmic drug, mainly used in the treatment of ventricular tachycardias. Supra-therapeutic concentrations (>1 μM) of propafenone inhibited $K_{ir}2.x$ current [59]. Propafenone interacted with the cytoplasmic domain of the channel, which decreased the negative charge of the pore and the channel affinity for PIP_2, a lipid critical for channel activation [59].

Given its structural similarities with flecainide, the effect of propafenone on $K_{ir}2.1$ carried current was tested at lower concentrations. At 0.5 μM, propafenone enlarged inward and outward $K_{ir}2.1$ current [33]. Propafenone significantly decreased spermine-induced block and thus relieved inward rectification, similarly as observed for flecainide [33]. However, propafenone failed to enhance both inward and outward $K_{ir}2.2$ and $K_{ir}2.3$ carried current [33]. In cells expressing $K_{ir}2.1/2.2$, $K_{ir}2.1/2.3$, and $K_{ir}2.2/2.3$ heterotetrameric channels, propafenone also failed to increase inward and outward current [33]. Propafenone also did not modify I_{K1} recorded in human right atrial myocytes [33].

The molecular structure of propafenone can be described as an L-like shape, in which a long and short arm are joined by an aromatic ring [33]. Molecular dynamics simulations predicted interaction with $K_{ir}2.1$, in which the hydrophobic long arm of propafenone embeds in a hydrophobic pocket formed by subunit A of the channel. The short arm, on the other hand, contains a group that formed a hydrogen bond with cysteine residue Cys311, which is part of the G-loop [33,60]. This critical hydrogen bond between Cys311 and propafenone was promoted due to the binding orientation of the alkylamino tail, which enabled propafenone's hydroxyl group to be in close proximity to Cys311 [33].

To determine a potential role of Cys311 and propafenone interaction, mutations creating a cysteine were made in $K_{ir}2.2$ (A312C) and $K_{ir}2.3$ (A303C). These mutations made the channels responsive to activation with 0.5 μM propafenone. With respect to channel kinetics, it was found that opening frequency and mean open probability were increased at all tested voltages [33].

3.2.3. Timolol

Structural similarities and the common Cys311 binding site of flecainide and propafenone have led to the development of a pharmacophore model for binding to this particular site. The model predicted that a drug must have the following molecular properties that enable interaction with Cys311: (1) an "L-like" configuration with a short and long arm, joined by an aromatic ring at an angle of approximately 100 degrees; (2) a hydrogen bond acceptor/donor group in the short arm that interacts with Cys311; (3) a hydrophobic group in the long arm that interacts with a hydrophobic pocket in subunit A of $K_{ir}2.1$; (4) a hydrogen bond between Arg67 or Glu63 and the drug for stabilization. By screening drugs for these properties, timolol was found as a potential activator of $K_{ir}2.1$ [33]. Timolol, a non-selective β-receptor antagonist, selectively activated $K_{ir}2.1$ by directly binding to Cys311 and thereby increased I_{K1} in the ventricles [33].

3.2.4. Pregnenolone Sulfate

Pregnenolone sulfate (PREGS) is an endogenous neurosteroid. PREGS modulates the function of multiple neurotransmitter receptors and channels, among which were voltage-gated K^+ channels [61]. PREGS-enhanced $K_{ir}2.3$ carried current in *Xenopus* oocytes when applied extracellularly only, whereas no current response in $K_{ir}1.1$, $K_{ir}2.1$, $K_{ir}2.2$, or $K_{ir}3.1/K_{ir}3.2$ channels was observed [32].

3.3. Unknown Mechanism of Activation

3.3.1. LPS (lipopolysaccharides)

Lipopolysaccharides (LPS) are an important constituent of the cell wall of gram-negative bacteria. LPS can injure pulmonary microvascular walls. In those vessels, $K_{ir}2.1$ plays a role in vasodilation by modulating the membrane potential and intracellular Ca^{2+} concentration. Treatment of mouse pulmonary microvascular endothelial cells with LPS enhanced $K_{ir}2.1$ channel expression and Ba^{2+} sensitive I_{K1} current [41].

3.3.2. Morphine

Morphine is the main element of opium and is used as an anesthetic or sedative as agonist of the μ, δ, and κ opioid receptors [62]. In rabbit ventricular myocytes, morphine significantly increased I_{K1}, independent of the opioid-receptor pathway [42]. In human atrial myocytes, morphine was unable to enlarge I_{K1} [63].

3.3.3. Polydatin

Polydatin (PD), also known as piceid (3,4′,5-trihydroxystilbene-3-β-D-glucoside), is a monocrystalline compound found, for example, in *Polygonum cuspidatum* Sieb. et Zucc. (Polygonaceae), peanuts, and grapes. PD has a therapeutic effect on hypertension, arrhythmia, hypertrophy, cardiac ischemia, and heart failure by means of manipulation of Ca^{2+} mobilization [64]. In rat ventricular myocytes, PD increased I_{K1} in a concentration-independent manner [43].

4. Lead Compounds and Clinical Perspective

Due to their similar chemical structure, the drugs flecainide, propafenone, and timolol are able to directly activate $K_{ir}2.1$ channels by means of their interaction with cysteine residue Cys311. This off-target effect can be exploited for rationalized drug development towards specific I_{K1} activators. This provides a promising perspective in the search for a suitable AgoKir.

As mentioned above, timolol has been found to be an activator of $K_{ir}2.1$ after being selected based on the criteria proposed by a pharmacophore model developed by [33]. This opens the possibility to develop a new drug with a higher specificity for $K_{ir}2.1$ channels that meets the requirements of the model. Medicinal chemistry approaches involving modifications to the long and short arm of one of these three existing drugs might be an important step to explore properties that increase specificity for the $K_{ir}2.1$ channel. For example, flecainide's principal mechanism of action is the inhibition of cardiac $Na_v1.5$ sodium channels [65]. It might be possible to modify parts of the flecainide molecule that, as per the pharmacophore model, are not essential for binding to $K_{ir}2.1$, but are important for interaction with $Na_v1.5$ to steer specificity.

Out of all the drugs reviewed in this article, the ones directly activating $K_{ir}2.1$ by binding to Cys311 provide the most viable basis for the development of AgoKirs. As discussed earlier, I_{K1} channels are homo- or heterotetrameric assemblies of $K_{ir}2.x$ monomeric subunits. Only the $K_{ir}2.1$ subunit has the cysteine residue Cys311 as part of its G-loop. $K_{ir}2.2$ and $K_{ir}2.3$ subunits contain an alanine instead of a cysteine residue on their equivalent positions (312 and 303, respectively) [31]. Propafenone and flecainide both cannot activate homo- or heterotetramers of $K_{ir}2.2$ and $K_{ir}2.3$ channels. These observations indicate the potential for isoform-specific $K_{ir}2.x$ activating compound development by targeting this channel domain but also indicate that other regions of the channel protein should be targeted for the development of multi-isoform activators. Furthermore, high throughput screening methods, such as automated patch-clamp, optical membrane potential detection, and ion-flux measurements, are being developed that will further aid the generation of AgoKirs [66].

The reports on aldosterone and valsartan on $K_{ir}2.x/I_{K1}$ regulation/activation are contradictory. Aldosterone production is part of RAAS, whereas valsartan is an antagonist of that system. Therefore, one would expect that those compounds have opposite effects on the activation and expression of the

$K_{ir}2.1$ subunits. The findings, however, showed that valsartan was able to ameliorate MI-induced $K_{ir}2.1$ downregulation. Unfortunately, the results for aldosterone were inconclusive. Two studies demonstrated upregulation, and only one study proved downregulation and therefore matched our expectations. This inconsistency between the outcomes of aldosterone and valsartan might indicate that both compounds use additional, RAAS independent pathways in order to activate I_{K1}.

Specific AgoKirs may come with potential adverse effects. Gain-of-function mutations indicate the potential of developing reentry-based arrhythmias, either atrial, ventricular, or both. The underlying mechanism is likely AP shortening and reduction in the effective refractory period. Furthermore, due to the strong stabilization of the resting membrane potential, muscle and neuronal cells harboring I_{K1} may become less or even unexcitable. Therefore, it would be beneficial to develop (1) $K_{ir}2$ isoform-specific AgoKirs and (2) AgoKirs with a wide therapeutic range.

Ion channel activators have been pursued in several other research fields, and, for some, they entered clinical practice. The research field advanced furthest with respect to channel activation is undoubtedly that of cystic fibrosis. This disease results mainly from insufficient cystic fibrosis transmembrane conductance regulator (CFTR) channel activity at the plasma membrane of glandular epithelial cells of, for example, the lungs, sweat glands, and gastrointestinal tract [29]. The CFTR channel research field developed both potentiators and correctors. Potentiators act on plasma membrane-localized channels and increase their open probability, whereas correctors address the impaired trafficking deficiencies and enhance forward trafficking of the channels by approaching several different steps of the trafficking machinery [29]. Clinically, a combination of potentiators and correctors appeared most effective (e.g., [67,68]). With respect to cardiac potassium channel activators, progress has been made for $K_v11.1/I_{Kr}$. Delayed rectifier I_{Kr} loss-of-function is associated with long QT syndrome type 2 and cardiac arrhythmia [69]. A number of compounds have been developed and tested in animal models for antiarrhythmic properties [70–73]. Currently, these activators mainly function to enhance channel kinetics resulting in increased potassium current. However, many of the forms of I_{Kr} loss-of-function are due to defective forward trafficking of the $K_v11.1$ proteins [74–76], and the development of compounds directly addressing this issue may be favorable. Unfortunately, existing enhancers of forward trafficking are also strong $K_v11.1$ inhibitors [77]. However, molecular insights in the rescue mechanism, including drug-channel interactions, may result in new rescuers of trafficking that do not display inhibition effects. In addition, activators alone might be sufficient in several conditions to increase current to sufficient levels to counteract arrhythmia [78]. With respect to inward rectifier currents, advances have been made in the application of drugs targeting I_{KATP} channels. For example, minoxidil, an I_{KATP} channel opener, is used as a vasodilator in the treatment of resistant hypertension [28] and to promote hair growth in androgenetic alopecia patients [30]. To our knowledge, specific AgoKirs have not been developed, but giving the new insights from the direct channel activators (flecainide, propafenone, timolol, PREGS) and the successes in other research fields, this development will be a valid way to counteract disease-associated loss-of-function of I_{K1} channels.

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Abbreviations

AF	Atrial Fibrillation
AgoKir	Agonist of Kir channel
AS	Andersen Syndrome
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CHO	Chinese Hamster Ovary
E_K	Potassium equilibrium potential

HEK	Human Embryonal Kidney
HF	Heart Failure
I_{K1}	Inward rectifier current
I_{KATP}	ATP regulated inward rectifier current
ISO	Isoproterenol/isoprenaline
$K_{ir}2.x$	Isoform x of the inward rectifier protein $K_{ir}2$ family
LPS	Lipopolysacharides
MI	Myocardial Infarction
MR	Mineral corticoid Receptor
PD	Polydatin
PKA	Protein Kinase A
PKC	Protein Kinase C
PREGS	Pregnenolone Sulfate
RAAS	Renin-Angiotensin-Aldosterone-System
V_m	Membrane potential
ZAC	Zacopride

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