The mammalian KIR2.x inward rectifier ion channel family: expression pattern and pathophysiology.

<table>
<thead>
<tr>
<th><strong>Journal:</strong></th>
<th><em>Acta Physiologica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Manuscript ID:</strong></td>
<td>APH-2009-10-0262.R1</td>
</tr>
<tr>
<td><strong>Manuscript Type:</strong></td>
<td>Review Article</td>
</tr>
<tr>
<td><strong>Date Submitted by the Author:</strong></td>
<td>21-Jan-2010</td>
</tr>
</tbody>
</table>
| **Complete List of Authors:** | de Boer, Teun; UMC Utrecht, Medical Physiology  
Houtman, Marien; UMC Utrecht, Medical Physiology  
Compier, Marieke; UMC Utrecht, Medical Physiology  
vander Heyden, Marcel; UMC Utrecht, Medical Physiology |
| **Key Words:** | Andersen-Tawil syndrome, Kir2.x, inward rectifier current, expression pattern, barium, Thyrotoxic Periodic Paralysis |
The mammalian $K_{IR}2.x$ inward rectifier ion channel family: expression pattern and pathophysiology.

Teun P de Boer, Marien JC Houtman, Marieke Compier, Marcel AG van der Heyden*

Department of Medical Physiology, Division Heart & Lungs, UMCU, Utrecht, The Netherlands

Running title: $K_{IR}2.x$ expression patterns and function

Word count: 8920

*Address for correspondence
Dr. Marcel AG van der Heyden
Department of Medical Physiology
Division Heart & Lungs, UMC
Yalelaan 50
3584 CM Utrecht.
Phone: +31 30 2538900
Fax: +31 30 2539036
Email: m.a.g.vanderheyden@umcutrecht.nl
Abstract

Inward rectifier currents based on $K_{IR}2.x$ subunits are regarded as essential components for establishing a stable and negative resting membrane potential in many excitable cell types. Pharmacological inhibition, null mutation in mice and dominant positive and negative mutations in patients reveal some of the important functions of these channels in their native tissues. Here we review the complex mammalian expression pattern of $K_{IR}2.x$ subunits and relate these to the outcomes of functional inhibition of the resultant channels. Correlations between expression and function in muscle and bone tissue are observed, while we recognize a discrepancy between neuronal expression and function.

Keywords Andersen-Tawil syndrome, barium, expression pattern, inward rectifier current, $K_{IR}2.x$, Thyrotoxic Periodic Paralysis.
Introduction

The membrane potential of cells is determined by a combination of unequal distribution of ions between intra- and extracellular compartments and ion specific channels. In excitable cells membrane potential can quickly change in response to voltage dependent opening and closing of ion channels producing an action potential (AP). Between subsequent action potentials, resting membrane potential (RMP) is kept constant by inward rectifier currents ($I_{K1}$). This prevents premature action potential formation that otherwise may lead to inappropriate muscle contraction, chaotic neuronal activation or cardiac arrhythmia to name some. At membrane potentials ($V_M$) negative from the potassium equilibrium potential ($E_K$), the $I_{K1}$ channel provides inward potassium current, at potentials positive of $E_K$ outward potassium current is conducted. By this mechanism, $I_{K1}$ exerts a strong stabilising effect on RMP. Inward rectification means that these channels conduct more potassium ions into the cell at a given $V_M$ negative from $E_K$ than at an equivalent positioned $V_M$ positive from $E_K$, thereby permitting AP formation by temporary relief of the stabilising effect on $V_M$. Inward rectification is accomplished by binding of Mg$^{2+}$ or polyamines at specific sites in the pore region at $V_m$ positive of $E_K$, thereby blocking outward current (Bichet et al. 2003). Membrane potentials negative of $E_K$ lead to the fast removal of blocking molecules from their binding sites. Therefore, affinity of the channel for blocking molecules influences its rectification capacity. $I_{K1}$ rectification profiles for different cell types are variable and depend on the relative amount of the underlying $I_{K1}$ channel subunit expressed. Variable rectification profiles
lead, in combination with the presence of sodium and calcium channels, to differences in excitability of various tissues (Lopatin & Nichols 2001).

A muscle type $I_{K1}$ channel was first described by Katz in 1949, then called anomalous rectifier channel due to its contra-intuitive behaviour (Katz 1949). In the late sixties of the 20th century, the current became known as $I_{K1}$. The first molecular counterparts were cloned in 1993 (Ho et al. 1993; Kubo et al. 1993). Soon a complete family of inward rectifier genes was identified, cloned and analysed in various expression systems.

The $K_{IR}$ channel family is encoded by the $KCNJ$-genes (Dhamoon & Jalife 2005) and currently includes 7 subfamilies ($K_{IR}$1-7) (Fig. 1a). $K_{IR}$ channel proteins consist of two transmembrane domains, a short pore loop that harbours the potassium selectivity filter (GYG), and intracellularly located N- and C-termini (Fig. 1b). Between subfamilies, sequence homology is 40%. Within subfamilies, sequence identity rises to approximately 60%. The subfamilies functionally differ in their rectifying strength and display differential expression patterns (Bichet et al. 2003) (Fig. 2). The $K_{IR}$ protein family is involved in many physiological processes like cardiac ($K_{IR}$2) and neural ($K_{IR}$2; $K_{IR}$3) excitability, insulin release ($K_{IR}$6), vascular tone ($K_{IR}$2) and potassium homeostasis ($K_{IR}$1 and $K_{IR}$4). In this review we will focus on $K_{IR}$2.x expression patterns to explain phenotypes upon pharmacological intervention or mutation of $K_{IR}$2.x channel proteins.

$K_{IR}$2.x channel subunits

The $K_{IR}$2.x proteins form the main $I_{K1}$ channels in cardiac tissue exemplified by prominent and severe effects on cardiac electrophysiology when gain- or loss-of-function or pharmacological intervention interfere in $K_{IR}$2.x action. The mammalian $K_{IR}$2.x
subfamily consists of five members: $K_{\text{IR}}2.1$, 2.2, 2.3, 2.4 and 2.6 that can form functional tetramers. The $K_{\text{IR}}2.x$ subfamily is closest related to the $K_{\text{IR}}5.x$ subfamily, of which $K_{\text{IR}}5.1$ is the only known member today. The four different subunit proteins are between 427-445 amino acids in length. $K^+$-selectivity is determined by a GYG sequence within the P-loop. Each subunit is stabilized by electrostatic interactions and salt bridges. Disulfide bonds between subunits are important in correct folding and assembly of the active channel. Genes underlying individual subunits have been identified (Table 1). $K_{\text{IR}}2.1$ is encoded by the $KCNJ2$-gene, $K_{\text{IR}}2.2$ by $KCNJ12$, $K_{\text{IR}}2.3$ by $KCNJ4$, $K_{\text{IR}}2.4$ by $KCNJ14$ and $K_{\text{IR}}2.6$ by $KCNJ18$.

Functionally, $K_{\text{IR}}2.x$ subunits form homotetramers and in some cases heterotetramers (Preisig-Müller et al. 2002; Schram et al. 2002; Zobel et al. 2003; Dhamoon et al. 2004). $K_{\text{IR}}2.1$, $K_{\text{IR}}2.2$ and $K_{\text{IR}}2.6$ homotetramers display highest levels of conductance while $K_{\text{IR}}2.3$ and $K_{\text{IR}}2.4$ homotetramers display two- to threefold lower conductance levels (Table 1) (reviewed in Lopatin & Nichols 2001; Ryan et al. 2010). With respect to rectification strength, the subfamily can be ordered as $K_{\text{IR}}2.4>K_{\text{IR}}2.2>K_{\text{IR}}2.3>\text{K}_{\text{IR}}2.1$ (Table 1). In heterotetramers, channel characteristics may be, but not necessarily, intermediate of the individual subunit homotetramers, *i.e.* activation kinetics or $\text{Ba}^{2+}$ sensitivity (Schram et al. 2003; Panama et al. 2007), or one subunit type may confer means of regulation to the channel, *i.e.* pH sensitivity, drug mediated potentiation or adrenergic regulation (Muñoz et al. 2007; Scherer et al. 2007; Kobayashi et al. 2009).
Clinical manifestations of \textit{KCNJ2} mutations or altered expression

The physiological relevance of a given protein can often be inferred from its mutant phenotype. In 2001, a landmark paper of Plaster and co-workers established a causal relationship between mutant human \(K_{\text{IR}2.1}\) and Andersen-Tawil syndrome (ATS1) (Plaster \textit{et al.} 2001). Many \(K_{\text{IR}2.1}\) mutations associated with ATS have been described since (Donaldson \textit{et al.} 2004). ATS1 is characterized by periodic muscle paralysis, often in combination with hypo- or hyperkalemia (Tawil \textit{et al.} 1994). Upon aging the paralytic periods become less frequent. Secondly, cardiac arrhythmias, often biventricular tachycardias are observed. ECG recordings in asymptomatic patients regularly, but not always, show long repolarisation times (LQT). Thirdly, skeletal abnormalities classified as broad forehead, hypoplastic mandible, hypotelorism, low-set ears, clinodactyly and syndactyly, mild microcephaly. Less frequently observed are the absence of lateral incisors and a high-arched palate, a cleft palate, and small hands and feet. Also, scoliosis, cardiovascular malformations and kidney dysplasia might be associated with the disease (Andelfinger \textit{et al.} 2002). Finally, neurocognitive abnormalities might be present in the syndrome (Yoon \textit{et al.} 2006), however, this issue certainly needs further studies on large patient populations (reviewed in Sansone & Tawil 2007).

The ATS1 phenotype indicates important roles for \(K_{\text{IR}2.1}\) in striated muscle, both skeletal and cardiac, and during embryonic bone development, but probably also in neural crest development, migration and/or differentiation.

In contrast to ATS1, which can lead to prolongation of repolarisation (LQT), a mutation in \textit{KCNJ2} has also been identified that has the opposite effect. As described by Priori \textit{et al.} (2005), short QT syndrome (SQT) can be caused by gain of function mutations in
KCNH2 (SQT1), KCNQ1 (SQT2) or KCNJ2 (SQT3). The D172N mutation in the KCNJ2 gene resulted in enhanced outward $I_{K1}$, shorter action potentials in a human ventricular action potential model and susceptibility to ventricular arrhythmia in the patient.

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is characterised by induction of bidirectional or polymorphic ventricular arrhythmia by adrenergic stimulation. In approximately 50 to 60% of patients, this disease has been linked to mutations in RyR2 or calsequestrin-2 (CASQ2), but for the remaining patients there was no genetic explanation available (Lehnart et al. 2007). However, a recent study described the case of a patient with CPVT-like symptoms that did not have mutations in RyR2 or CASQ2, but in KCNJ2 instead (Vega et al. 2009) This mutation, V227F, was demonstrated to result in increased sensitivity of $K_{IR2.1}$ channels to phosphorylation by PKA, which may explain how in this patient, adrenergic stimulation resulted in ventricular tachycardia through inhibition of $I_{K1}$.

Atrial Fibrillation (AF) is the most common arrhythmia among hospitalized patients diagnosed with cardiac arrhythmia (Chugh et al. 2001). AF often develops into a chronic, sustained arrhythmia, probably because of electrical and structural remodelling of the atrial myocardium. Electrical remodelling involves shortening and triangularisation of the AP which facilitates the appearance of sustained re-entry circuits (for a review, see Ravens & Cerbai 2008). Both $I_{K,ACCh}$ and $I_{K1}$ channels have been implicated in AF. $I_{K,ACCh}$ channels in human AF patients can become constitutively active and $I_{K1}$ density was increased, thereby enhancing repolarisation (Dobrev et al. 2005). Enhanced expression of $I_{K1}$ channels has been linked to reduced expression of the mIR-1 micro-RNA (Yang et al.
2007, Girmatsion et al. 2009). Upregulation of $I_{K1}$ is an important factor in AF, which is confirmed by computer modelling (Zhang et al. 2005) and the observation that transgenic overexpression of $KCNJ2$ in mice causes atrial fibrillation (Li et al. 2004). In addition, a $KCNJ2$ gain-of-function mutation (V93I) was linked to hereditary AF (Xia et al. 2005).

The clinical phenotypes of mutations in the $KCNJ2$ gene illustrate the importance of $KIR2.1$ based $I_{K1}$ for normal repolarisation in the human heart.

**Clinical manifestation of $KCNJ18$ mutations**

Thyrotoxic hypokalemic periodic paralysis (TPP) occurs as a serious complication of hyperthyroidism and is characterized by skeletal muscle paralysis and hypokalemia predominantly affecting young adult male patients of Asian decent (reviewed in Kung 2006). Recently, mutations in the $KCNJ18$ gene, a paralog of $KCNJ12$, have been described that cause susceptibility to TPP (Ryan et al. 2010). The gene is expressed in skeletal muscle and is transcriptionally regulated by thyroid hormone via a thyroid responsive element in the $KCNJ18$ promoter region. Mutations are mainly localized in the intracellular C-terminus. Upon ectopic expression, mutations are found to result in decreased $I_{K1}$ densities while rectification properties are not affected. The altered electrophysiological characteristics most likely undermine setting of the resting membrane potential and its stability.

**$KIR2.1$ and $KIR2.2$ knockout mice**

Apart from natural occurring mutations as described above, directed removal of a gene of interest in animal models proves very efficient in establishing its function. Null mutations
of KCNJ2 and -12 were generated by complete removal of the genes open reading frames (Zaritsky et al. 2000). KIR2.1-/- mice died between 8 and 12 hours after birth. Pups became cyanotic, gasped for breath and displayed gradual swelling of their stomach and small bowel with air. Maxilla were slightly smaller but no other first pharyngeal arch derived bone or cartilage defects were observed. All knockout pups presented complete and wide cleft of the secondary palate, however other facial midline structures were not affected. No defects in cardiac anatomy were observed. IK1 was absent from neonatal cerebral arterial myocytes and cerebral arteries failed to dilate in response to increased extracellular potassium concentrations. In contrast, Ca2+-induced constriction and forskolin mediated dilatation were not affected. In KIR2.1-/- neonatal ventricular cardiomyocytes no IK1 could be detected at physiological extracellular potassium levels (Zaritsky et al. 2001). KIR2.1-/- myocytes displayed lengthening of the AP, at 90% as well as at 50% of complete repolarization (APD90 and APD50). The majority of the KIR2.1-/- cells displayed spontaneous beating activity, in contrast to wildtype cells (Zaritsky et al. 2001). Finally, ECG analysis revealed no ectopic or reentry based activity, but lengthening of the RR, PR and QT intervals was evident, as well as broadening of the QRS complex (Zaritsky et al. 2001).

KIR2.2-/- mice were viable and fertile, appeared normal and no brain or cardiac abnormalities were detected (Zaritsky et al. 2000 and 2001). Neonatal ventricular cardiomyocytes from KIR2.2-/- displayed a 50% reduction in IK1 compared to wildtype cells (Zaritsky et al. 2001). No changes in ECG parameters were observed compared to wildtype of heterozygote littermates (Zaritsky et al. 2001). KIR2.2-/- animals displayed a
transient depressed hypercapnic ventilatory response on postnatal days 14-15 compared to wildtype animals (Oyamada et al. 2005).

Currently, we are not aware of any studies reporting $\text{K}_{\text{IR}2.3}$, $\text{K}_{\text{IR}2.4}$ or $\text{K}_{\text{IR}2.6}$ null mutation.

**Pharmacological investigations**

$\text{K}_{\text{IR}2.x}$ mediated $I_{\text{K1}}$ is sensitive to $\text{Ba}^{2+}$ mediated current block. Therefore, but only to a certain extent, acute and chronic $\text{Ba}^{2+}$ induced phenotypes can be correlated to the expression of $\text{K}_{\text{IR}2.x}$ based $I_{\text{K1}}$ currents. $\text{Ba}^{2+}$ containing minerals have been first described by William Withering in the 18th century (Withering 1784). Soon after, the physiological effects of applying $\text{Ba}^{2+}$ containing compounds to dogs were described by James Watt (Watt 1790). Since then many detailed accounts have been made on its devastating effects on frog, rabbit, hare, cat and dog physiology (e.g. Brodie 1812; Boehm 1875; Smith et al. 1940; Roza & Berman 1971). In general, slow $\text{BaCl}_2$ infusion led to, in chronological order, arterial hypertension, ectopic cardiac activity, spontaneous muscle contractions, salivation, diarrhoea and spontaneous urine loss, muscle twitching and increased respiratory rate, worsening ectopic cardiac activity, strong hypotension, flaccid paralysis of skeletal muscle, asphyxia. Similar symptoms were observed in human cases of severe $\text{Ba}^{2+}$ poisoning (e.g. Tenenbein 1985; Johnson & VanTassell 1991; Jacobs et al. 2002). By and large, the overall picture of skeletal, cardiac and smooth muscle dysfunction arises. However, no seizures were reported which is remarkable, since neural tissue strongly express $\text{K}_{\text{IR}2.x}$ based $I_{\text{K1}}$ channels too.
These acute experiments and sad poisoning cases correlate in part with symptoms of null mutation and ATS1, apart from the dysmorphic features in the latter case. However, BaCl₂ application to chicken eggs resulted in development of abnormal and small feet (Ridgway & Karnofsky 1952), which partly phenocopies ATS1.

An increasing number of drugs is found to possess $I_{K1}$ inhibitory activity (Table 2), often by interfering in the channel pore (e.g. chloroquine, pentamidine) or its regulation (e.g. tamoxifen) (Rodríguez-Menchaca et al. 2008; Ponce-Balbuena et al. 2009; De Boer et al. 2010). Most of the compounds display inhibitory effect on other ion channels too, either directly by occupying the channel pore region, indirectly by interfering in protein trafficking, regulation by lipid interaction and/or phosphorylation, or by a combination of direct and indirect mechanisms. These pleiotropic effects make it difficult to ascribe a drug-induced physiological effect to the function of a particular ion channel, in this case $I_{K1}$. To further illustrate this, Fig. 3a depicts the effects of modulating $I_{K1}$ levels with respect to the cardiac AP using a computer model. Increasing the amount of $I_{K1}$ resulted in AP shortening, mainly due to increasing late repolarisation leaving upstroke and plateau phase unaffected. Lowering the amount of $I_{K1}$ yielded mild AP prolongation by extending the late repolarisation phase. AP recordings from isolated canine cardiomyocytes showed that Ba²⁺ application resulted in mild AP potential prolongation as seen in the model (Fig. 3b). In contrast, application of the multiple ion channel affecting drug chlorpromazine resulted in distinct changes in action potential morphology (marked lowering of the plateau phase, action potential shortening) resulting from a combination of its inhibitory effects on different ion channels, including $I_{K1}$ (Fig. 3c).
Variability as seen in ATS1 penetrance and the pleiothropic effects of Ba\textsuperscript{2+}-mediated inhibition and null mutations suggest a complex expression pattern of the underlying genes, understanding of which may help to explain the compound phenotype. Starting with the cloning of the molecular counterparts of the $I_{K1}$ channels, large parts of this intriguing expression pattern have been described in many individual studies that used a plethora of experimental setups. We will review the scattered information in the remaining part of this paper.

**$K_{IR2.x}$ expression profiles**

Studies on $K_{IR2.x}$ subunit expression have been performed in a number of different mammalian species, each having their own advantages as a model system. In general, $K_{IR2.x}$ expression patterns display very little variance between species investigated thus far. Therefore, summarized and detailed expression data from adult rat, mouse, guinea pig, rabbit, dog, hamster and human are presented in Table 3. One exception worthwhile to mention is prominent $K_{IR2.3}$ expression in sheep atria (Dhamoon et al. 2004), where other species express mainly $K_{IR2.1}$ and $K_{IR2.2}$ in their atria.

In general $K_{IR2.x}$ is expressed in brain, cardiac, (arterial) smooth and skeletal muscle cells. In brain, $K_{IR2.1-2.3}$ subunits display a broad expression pattern. Marked differences are the virtual absence of $K_{IR2.1}$ and $K_{IR2.3}$ from the cerebellum, where $K_{IR2.2}$ is highly expressed instead. $K_{IR2.4}$ has a very restricted expression pattern and is mainly confined to cranial nerve motor nuclei. In heart, $K_{IR2.1}$ and to a lesser extent $K_{IR2.2}$ are the main subunits expressed in the working myocardium of atria and in particular the ventricles, while the existence of a functional role of $K_{IR2.4}$ in the heart is still under debate.
Skeletal muscle mainly expresses $K_{\text{IR}2.1}$, $K_{\text{IR}2.2}$ and $K_{\text{IR}2.6}$. In contrast, arterial smooth muscle displays mainly $K_{\text{IR}2.1}$ as a functional channel.

**Developmental changes in $K_{\text{IR}2.x}$ expression**

In the embryonic rat, $K_{\text{IR}2.1}$ subunit transcript expression is found as early as day E12. From E12 to E17, $K_{\text{IR}2.1}$ is primarily present in bone forming structures in the head, limbs and body. In addition, at E17, transcripts are clearly present in the metanephros and heart (Karschin & Karschin 1997). During embryonic brain development, $K_{\text{IR}2.1}$ transcript expression is uniformly present throughout the brain in embryonic neuroepithelium at early stages and becomes more restricted in late embryonic and postnatal phases, like in the cortex from E19 and in hippocampus from P10 onwards. In addition, after birth $K_{\text{IR}2.1}$ expression disappears from the thalamus and appears in hippocampus (Karschin & Karschin, 1997) and Purkinje cells (Miyashita & Kubo, 1997). During murine postnatal inner ear development, $K_{\text{IR}2.1}$ is temporarily expressed in hair cells of the cochlea, with increasing amounts of transcripts from P0 to P12, while expression was absent at P20 (Ruan et al. 2008). Expression of $K_{\text{IR}2.1}$ in the heart during embryogenesis is relatively high and increases after birth to adulthood (Grandy et al. 2007; Harrell et al. 2007).

$K_{\text{IR}2.2}$ expression is first found on day 12 during rat embryonic development. It is expressed in epithelia, at low levels in the heart, metanephros and mainly in the peripheral nervous system (Karschin & Karschin, 1997). In rat brain, $K_{\text{IR}2.2}$ is expressed in cortex and thalamus from day 19 of embryonic development and 10 days after birth in the cerebellum and hippocampus (Karschin & Karschin, 1997). Compared to 9-10 days
after birth, expression of K\textsubscript{IR2.2} in mouse brain seems to be upregulated 14-15 days after birth. As suggested, this may correlate to involvement of K\textsubscript{IR2.2} in the hypercapnic ventilary response (Oyamada et al. 2005).

During rat embryonic development, K\textsubscript{IR2.3} expression is mainly restricted to the brain (Karchin & Karschin 1997) and only present at low levels in the heart. After birth, the expression of K\textsubscript{IR2.3} in the human heart does not increase further (Harrell et al. 2007). Expression of K\textsubscript{IR2.3} in the rat brain starts at day 21 of embryonic development in the cortex. After birth, K\textsubscript{IR2.3} also becomes expressed in the thalamus and hippocampus (Karschin & Karschin 1997).

The expression of K\textsubscript{IR2.4} in the heart is already present during mouse embryonic development, although levels are low. After birth, the expression levels do not increase and thus remain very low compared to the other K\textsubscript{IR2} isoforms (Harrell et al. 2007).

Functional expression of K\textsubscript{IR2.x}

Functional expression of K\textsubscript{IR2.x} subunits have been inferred from close examination of current properties, like single channel conductance and rectification factor and from pharmacological block, RNA-interference strategies and knockout studies. Many of these investigations are hampered however by the presence of functional heterotetramers that often present intermediate electrical characteristic compared to homotetramers.

Despite detailed K\textsubscript{IR2.x} expression studies at the molecular level in neural tissues, functional distinction between expression of the individual subunits has gained less attention for neural than for cardiac cells. Studies on hypercapnic ventilatory response in K\textsubscript{IR2.2}\textsuperscript{-/-} mice suggest a functional, but transient, involvement of the K\textsubscript{IR2.2} subunit in
central ventilatory chemosensitivity in the postnatal brainstem (Oyamada et al. 2005). K\textsubscript{IR}2.1 expression in dentate gyrus cells of the hippocampus regulates excitability of the neurons, and during maturation of the brain expression increases (Mongiat et al. 2009). Ba\textsuperscript{2+} concentration dependent \(I_{K1}\) block in hypoglossal motoneurons in rat brain stem slices identified a prominent role for K\textsubscript{IR}2.4 in the cranial nerve nuclei (Töpert et al. 1998).

\(I_{K1}\) current density was found to increase during cardiomyocyte development. Interestingly, in fetal rat cardiomyocytes (day 12) an 11 pS \(I_{K1}\) conductance was observed that disappeared later in development. At later stages (fetal day 18, neonatal day 5) a 31 pS conducting \(I_{K1}\) channel was found (Masuda & Sperelakis 1993), and a 42 pS channel was observed in adult rat cardiomyocytes (Wahler 1992). In adult rabbit cardiomyocytes, a 31 pS channel was observed (Chen et al. 1991). In guinea pig, Xin Liu et al. compared cardiomyocyte \(I_{K1}\) with cloned guinea pig K\textsubscript{IR}2.x subunits and concluded that K\textsubscript{IR}2.2 is the largest contributor to cardiac \(I_{K1}\) in that species (Xin Liu et al. 2001). In adult rabbit ventricular cardiomyocytes it is concluded, based on specific dominant negative K\textsubscript{IR}2.x subunit overexpression, that K\textsubscript{IR}2.1 and K\textsubscript{IR}2.2 heterotetramers contributed to \(I_{K1}\) mainly (Zobel et al. 2003). Studying outward characteristics, Dhamoon et al. concluded that sheep atrial cardiomyocyte \(I_{K1}\) is based on K\textsubscript{IR}2.3 mainly, while sheep ventricular and guinea pig atrial and ventricular cardiomyocytes rely on K\textsubscript{IR}2.1 (Dhamoon et al. 2004). Using antisense K\textsubscript{IR}2.1 oligonucleotides \(I_{K1}\) was substantially inhibited in adult rat ventricular myocytes (Nakamura et al. 1998). More efficient RNAi approaches directed against K\textsubscript{IR}2.1 resulted in more that 90% reduction of rat adult ventricular myocyte \(I_{K1}\) (Rinne et al. 2006). Targeted deletion of K\textsubscript{IR}2.1 resulted in an almost complete absence
of \( I_{K1} \) in neonatal mouse ventricular myocytes, whereas \( \text{Kir}2.2 \) deletion decreased \( I_{K1} \) in these cells to 50% (Zaritsky et al. 2001). Finally, analyzing activation kinetics, Panama et al. conclude that \( \text{Kir}2.3 \) has no significant contribution to \( I_{K1} \) in the mouse heart (Panama et al. 2007). In general, these data are consistent with an upregulation of \( \text{Kir}2.1 \) and \( \text{Kir}2.2 \) levels during heart development, while initial \( \text{Kir}2.3 \) and \( \text{Kir}2.4 \) expression levels do not increase during subsequent maturation. In adult ventricular cardiomyocytes, \( \text{Kir}2.1 \) and to a lesser extent \( \text{Kir}2.2 \) are the main molecular determinants of \( I_{K1} \).

Based on current characteristics and pharmacological properties it has been concluded that \( I_{K1} \) in vascular smooth muscle cells is carried by \( \text{Kir}2.1 \) (Bradley et al. 1999). This was subsequently confirmed in human bronchial smooth muscle cells using \( \text{Kir}2.1 \) antisense oligonucleotides (Oonuma et al. 2002). A similar conclusion was reached for murine cerebral artery smooth muscle by studying of the \( \text{Kir}2.1 \) knock out model. Moreover, \( \text{Kir}2.1 \) appeared to be involved in the \( K^+ \) mediated vasodilation in contrast to \( \text{Kir}2.2 \) (Zaritsky et al. 2000). However, apart from \( \text{Kir}2.1 \), Tennant et al. report a predominant role for \( \text{Kir}2.4 \) in cultured human pulmonary artery smooth muscle cells (Tennant et al. 2006).

In human aortic endothelial cells, \( I_{K1} \) is mainly carried by \( \text{Kir}2.1 \) and \( \text{Kir}2.2 \) with a dominant role of the latter as determined by pharmacological and channel characteristics and the use of dominant negative \( \text{Kir}2. \) mutants (Fang et al. 2005). In bovine corneal endothelial cells, \( I_{K1} \) is carried by \( \text{Kir}2.1 \) as was deduced from \( I_{K1} \) pharmacology (Yang et al. 2003).

Rat juxtaglomerular cells display an \( I_{K1} \) with a single channel conductance of 31.5 pS, excluding \( \text{Kir}2.3 \) and \( \text{Kir}2.4 \) from being the underlying component. Based on other
electrophysiological properties such as Cs\(^+\) blockade and inactivation at hyperpolarisation, the authors conclude that K\(_{\text{IR}}\)2.1 is the most likely candidate (Leichtle \textit{et al.} 2004), however a functional contribution of K\(_{\text{IR}}\)2.2 cannot be ruled out at present. Finally, the K\(_{\text{IR}}\)2.1\(^{-/-}\) animals present a complete penetrance of cleft palate, indicating an essential functional role of this subunit in palate development. Furthermore, homozygous animals present slight narrowing of the maxilla. These results are the only experimental evidence for K\(_{\text{IR}}\)2.1 involvement in bony structure development so far (Zaritsky \textit{et al.} 2000).

**Expression pattern - phenotype considerations**

\textit{Genetic phenotypes}

A number of ATS1 related disorders can directly be correlated to the K\(_{\text{IR}}\)2.1 expression pattern, like paralysis (expression in skeletal muscle), cardiac arrhythmias (cardiomyocyte expression) and skeletal defects (transient expression in developing bone structures). The same can be stated for the even rarer cases of KCNJ2 mutation associated forms of CPVT, AF and SQT3. Partly overlapping conclusions can be drawn from the K\(_{\text{IR}}\)2.1 knockout studies. Remarkably, given the wide expression in neural tissues, no obvious neurologic anomalies like seizures have been described thus far in ATS1. However, cognitive impairment may be present. An explanation may be found in strong redundancy in neural tissues which express besides K\(_{\text{IR}}\)2.1, several other inward rectifier subunits or even subfamilies. Another explanation may be found in a potential fundamentally different function of K\(_{\text{IR}}\)2.1 between brain and muscle cells, where in muscle K\(_{\text{IR}}\)2.1 may be mainly involved in establishing a stable resting membrane
potential, in brain $K_{ir}2.1$ could have a more prominent role in determining neuron
excitability instead, either directly by influencing input resistance, or indirectly through
its role in buffering of extracellular $K^+$ by astrocytes.

Also of note is the difference of phenotypes between ATS1 and $K_{ir}2.1^{+/}$ heterozygous
mice. According to Zaritsky et al., (2000 and 2001), $K_{ir}2.1^{+/}$ animals can be considered
as control animals ($K_{ir}2.1^{+/+}$) implicating the absence of an at least obvious phenotype.
Most likely, the ATS1 phenotype is more severe due to a dominant negative effect of the
mutant protein (e.g. Andelfinger et al. 2002; Lange et al. 2003). Therefore, the generation
of a mouse model bearing a dominant negative $K_{ir}2.1$ mutation would be a valuable tool
in a better understanding of ATS1 characteristics, and in general on the role of $K_{ir}2.x$
based $I_{K1}$ in development and adult physiology.

The recently identified $KCNJ18$ gene and its mutations associated to TTP correlate well
to its expression in skeletal muscle. In the years ahead the $K_{ir}2.6$ expression pattern will
be refined that might be related to more subtle characteristics of TTP.

Currently, no disease processes have been linked to (dominant) negative mutation of
$KCNJ4$, 12 or 14 genes. In our opinion, these will be discovered although penetrance is
probably lower than that of the $KCNJ2$ gene.

**Pharmacologic phenotype**

Currently, there is no selective pharmacologic inhibitor of $K_{ir}2.x$ based $I_{K1}$. A relatively
low concentration of $Ba^{2+}$ (10-100 µM) is thus far the most specific inhibitor, resulting in
a number of pathophysiologival reactions correlating well with the $K_{ir}2.x$ expression
patterns. In contrast to symptoms seen with impaired $I_{K1}$ function in ATS1, strong effects
on blood pressure are noted that may be related to KIR2.x expression in arterial smooth muscle and endothelium. Again, no neurological defects have been reported, emphasizing again the potential redundancy of KIR2.x in neural tissue.

Further progress of this field will be helped by availability of specific pharmacological inhibitors. Recent studies on the molecular interactions of compounds inhibiting KIR2.x based IK1 (Rodriguez-Menchaca et al. 2008; De Boer et al. 2010) will help to define the molecular profile of an effective and efficient IK1 blocker that will certainly reveal some of the remaining enigmas of KIR2.x function.

Acknowledgements

We thank Dr. M. Peschar (NOTOX, Den Bosch) for providing canine sinus rhythm cardiomyocytes.

Conflict of interest

There is no conflict of interest.
References


Dhamoon AS & Jalife J. 2005. The inward rectifier current ($I_{K1}$) controls cardiac excitability and is involved in arrhythmogenesis. *Heart Rhythm* 2, 316-324.


rectifier \( K^+ \) 2.x family of inward rectifier channels by interfering with phosphatidylinositol 4,5-bisphosphate-channel interactions. *J Pharmacol Exp Ther* **331**, 563-573.


Smith, P.K., Winkler, A.W. & Hoff, H.E. 1940. Cardiovascular changes following the intravenous administration of barium chloride. *J Pharmacol Exp Therap* 68, 113-122.


Legends

Figure 1
Organisation of the $K_{IR}$ gene family and basic structural characteristics. (a) Phylogram of the human inward rectifier gene family. Amino acid sequences were aligned by Clustal-W algorithm, phylogeny analysis was performed using the Neighbor-Joining method using MEGA4 software. *Burkholderia thailandensis* KirBac1.4 was used as outgroup. The scale beneath the tree measures the distance between family members and units indicate the number of substitution events. Support for each node (numbers) was determined by interior-branch test. (b) Snake plot representation of human $K_{IR}2.1$ indicates its overall topology. Plasma membrane is represented as gray bar. Potassium selectivity filter (GYG) is highlighted. See text for additional details.

Figure 2
Schematic representation of overall inward rectifier family member expression pattern in indicated organs and tissues. Blank indicates no expression, black indicates prominent expression, and striations indicate intermediate levels of expression.

Figure 3
Pharmacological modulation of $I_{K1}$ and action potential morphology. (A) Computer simulation of the effect of $I_{K1}$ density on action potential duration. The human ventricular epicardial cardiomyocyte model by Ten Tusscher *et al.* (2004) was used with reduced or increased $I_{K1}$ densities as indicated. Simulations were performed using Cellular Open Resource (COR), see Garny *et al.*, 2009. Dashed lines indicate 0 mV, scale provided
applies to all panels. (b) Prolongation of ventricular action potential after $I_{K1}$ block using 10 µM BaCl$_2$ in isolated left ventricular cardiomyocytes from normal sinus rhythm dogs. (c) Severe shortening of canine left ventricular action potential after application of chlorpromazine (CPZ), a non-specific blocker of $I_{K1}$. 
Table 1  Basic characteristics of the human K_{IR}2.x protein family

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>Chromosome</th>
<th>Aminoacids</th>
<th>Predicted M_{w}</th>
<th>Conductance</th>
<th>Rectification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_{IR}2.1</td>
<td>KCNJ2</td>
<td>17q23.1-q24.2</td>
<td>427</td>
<td>48.2 kD</td>
<td>~30 pS</td>
<td>~0.61</td>
</tr>
<tr>
<td>K_{IR}2.2</td>
<td>KCNJ12</td>
<td>17p11.2</td>
<td>433</td>
<td>48.9 kD</td>
<td>~34-41 pS</td>
<td>~0.37</td>
</tr>
<tr>
<td>K_{IR}2.3</td>
<td>KCNJ14</td>
<td>22q13.1</td>
<td>445</td>
<td>49.4 kD</td>
<td>~10-13 pS</td>
<td>~0.50</td>
</tr>
<tr>
<td>K_{IR}2.4</td>
<td>KCNJ4</td>
<td>19q13</td>
<td>436</td>
<td>47.7 kD</td>
<td>~15 pS</td>
<td>~0.25</td>
</tr>
<tr>
<td>K_{IR}2.6</td>
<td>KCNJ18</td>
<td>17p11.1-2</td>
<td>433</td>
<td>48.7 kD</td>
<td>~34 pS</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Rectification factor is determined by maximal outward current divided by maximal inward current within the voltage range -100 to + 10 mV. Rectification factors for K_{IR}2.1 – 3 were taken from Anumonwo (2004) or calculated using data published by Schram et al. (2002) (K_{IR}2.4). n.d. not determined.
Table 2  Direct $K_{IR2.x}/I_{K1}$ ion channel blockers

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$</th>
<th>Inhibitory activity on additional ion channels$^1$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba$^{2+}$</td>
<td>0.5-10.3 µM</td>
<td>specific</td>
<td>Liu et al. (2001)</td>
</tr>
<tr>
<td>Celastrol</td>
<td>&gt;20 µM</td>
<td>$I_{Kr}$</td>
<td>Sun et al. (2006)</td>
</tr>
<tr>
<td>Chloroethylclonicine</td>
<td>37 µM</td>
<td>unknown; $\alpha$-adrenergic agonist</td>
<td>Barrett-Jolley et al. (1999)</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>1.1 µM</td>
<td>$I_{Kr}$, $I_{Na}$, $I_{Ca-l}$</td>
<td>Rodriguez-Menchaca et al. (2008)</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>6.1 µM</td>
<td>$I_{Kr}$, $I_{Na}$, $I_{Ca-l}$, $I_{K(ATP)}$</td>
<td>Kon et al. (1994)</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>0.17 µM</td>
<td>$I_{Kr}$</td>
<td>De Boer et al. (2010)</td>
</tr>
</tbody>
</table>

$^1$Inhibitory action of compounds on ion channels other than $K_{IR2.x}$ may be either direct by interacting in the pore region, by affecting ion channel trafficking towards the plasmamembrane or by both actions. $^2$IC$_{50}$ = 235 µM (Liu et al. 2001).
<table>
<thead>
<tr>
<th>Isoform</th>
<th>Organ</th>
<th>Expression pattern</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIR2.1</td>
<td>Brain</td>
<td>cerebral cortex, piriform cortex, hippocampus, thalamus, purkinje cells, striatal</td>
<td>Bredt et al. (1995), Horio et al. (1996), Howe et al. (2008), Kang et al.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>neurons, pyramidal cells, postmitotic granule cells, olfactory bulb, olfactory</td>
<td>(2008), Karschin &amp; Karschin (1997), Karschin et al. (1996), Miyashita et</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bulb astrocytes, superior and inferior colliculi, macroglia, ependyma, caudate</td>
<td>al 1997, Morishige et al. (1994), Pruss et al. (2005), Stonehouse et al.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>putamen, trigeminal nucleus, pontine nucleus, corpus callosum, capillary endothelium</td>
<td>(1999)</td>
</tr>
<tr>
<td>Eye</td>
<td>retina</td>
<td>retina inner nuclear and ganglion cell layers, corneal endothelium, Müller cells,</td>
<td>Kofuji et al. (2002), Raap et al. (2002), Tian et al. (2003), Yang et al.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>retinal pigment epithelium</td>
<td>(2003, 2008)</td>
</tr>
<tr>
<td>Heart</td>
<td>ventricle, atrium, ventricular and atrial</td>
<td>ventricle, atrium, ventricular and atrial cardiomyocytes, purkinje fibers, SA-node, pulmonary vein</td>
<td>Chilton et al. (2005), Domenighetti et al. (2007), Fang et al. (2005), Fülöp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cardiomyocytes, myofibroblast, aortic endothelium</td>
<td>et al. (2006), Gaborit et al. (2007), Harrel et al. (2007), Melnyk et al.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2005), Xin Liu et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Smooth muscle</td>
<td>mesenteric, coronary, cerebral, pulmonary, renal afferent and efferent artery,</td>
<td>Bradley et al. (1999), Jantzzi et al. (2006), Karkanis et al. (2003), Kim et</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hamster retractor muscle feed artery smooth muscle cells, bronchial smooth muscle</td>
<td>al. (2005), Oonuma et al. (2002), Smith et al. (2008), Tennant et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Skeletal muscle</td>
<td>myoblast, myotube, skeletal muscle cells</td>
<td>Fischer-Lougheed et al. (2001)</td>
</tr>
</tbody>
</table>
Placenta  first, second, third trimester placenta, cytотrophoblast cells  Mylona et al. (1998)
Kidney  juxtaglomerular cells  Leichtle et al. (2004)
KIR2.2 Brain  cerebral cortex, thalamus, hippocampus, cerebellum, hypothalamus, spinal cord, medulla, ependyma, brainstem, olfactory bulb and tubercle, caudate putamen, piriform cortex, macroglia, telencephalic neurons, corpus striatum, pituitary, corpus callosum, capillary endothelium  Bredt et al. (1995), Horio et al. (1996), Karschin & Karschin (1997), Karschin et al. (1996), Pruss et al. (2005), Stonehouse et al. (1999), Wulfsen et al. (2000)
Eye  neural epithelium, retinal pigment epithelium, Müller cells  Raap et al. (2002), Yang et al. (2008)
Heart  ventricle, atrium, Purkinje fibers, ventricular and atrial cardiomyocyte, aortic endothelium  Fang et al. (2005), Gaborit et al. (2007), Xin Liu et al. (2001)
Smooth muscle mesenteric, coronary, cerebral and basilar smooth muscle, hamster retractor muscle feed artery smooth muscle cells  Jantzi et al. (2006), Karkanis et al. (2003)
KIR2.3 Brain  piriform cortex, neocortex, stria terminalis of thalamus, caudate putamen, indisium griseum, supraoptic nucleus, facial nucleus, cerebellar Purkinje cells, pituitary, olfactory bulb, hippocampus, macroglia, corpus callosum, ependyma  Falk et al. (1995), Horio et al. (1996), Karschin & Karschin (1997), Karschin et al. (1996), Pruss et al. (2005), Stonehouse et al. (1999)
Eye  neural retina, retina inner nuclear and ganglion cell layers  Tian et al. (2003), Yang et al. (2008)
Heart  atrium, ventricle, Purkinje fibers, cardiomyocytes, capillary and aortic endothelium, pulmonary vein cardiomyocytes  Fang et al. (2005), Gaborit et al. (2007), Harrell et al. (2007), Melnyk et al. (2005)
<table>
<thead>
<tr>
<th>Smooth muscle</th>
<th>cerebral smooth muscle</th>
<th>Xin Liu et al. (2001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K\textsubscript{IR}2.4</td>
<td>Brain</td>
<td>cranial nerve motor nuclei in midbrain, pons, medulla</td>
</tr>
<tr>
<td>Eye</td>
<td>neural retina, Müller cells</td>
<td>Hughes et al. (2000), Raap et al. (2002), Yang et al. (2008)</td>
</tr>
<tr>
<td>Heart</td>
<td>aortic endothelial cells</td>
<td>Fang et al. (2005)</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>pulmonary arterial smooth muscle</td>
<td>Tennant et al. (2006)</td>
</tr>
<tr>
<td>K\textsubscript{IR}2.6</td>
<td>Skeletal muscle</td>
<td>no cell types specified</td>
</tr>
</tbody>
</table>
Figure 1
Figure 3
De Boer et al., 2010