REVIEW ARTICLE

What have we learned from two-pore potassium channels? Their molecular configuration and function in the human heart

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ABSTRACT

Two-pore domain potassium channels (K_{2P}) control excitability, stabilize the resting membrane potential below firing threshold, and accelerate repolarisation in different cells. Until now, fifteen different genes for the six K_{2P} channel subfamily were cloned. The pore-forming part is translated from two genes and they are built up from a dimer of two two-unit transmembrane domains functioning with a wide spectrum of physiological profiles. K_{2P} ion channels were discovered in the last two decades and gave novel opportunity to recognize the complex molecular mechanism of the potassium ion flux, and may lead to the design of individual drug targeting in the future. In this review, we summarise the structure, function, channelopathies and pharmacological silhouette of the two-pore potassium channels in the human tissues. In addition, we present the computer model of the partially reconstructed wild type K_{2P}1/TWIK1 lacking the intracellular C and N terminal loops.

KEY WORDS

inward rectifier channels, K_{2P} two-pore domain potassium ion channels, TWIK-1 prediction model

The structure of the two-pore domain channels

The inward rectifier potassium channels (I_{KR}) are responsible for the time course of the action potential (AP) (Dhamoon et al. 2005). I_{Kr} and the background currents (I_{Kb}), both called leak currents, contribute to the resting membrane potential (Lesage et al. 2000a; Bayliss et al. 2008), and strongly influence the final repolarisation in cardiomyocytes, renal and neuronal cells (Périr et al. 1994; Nichols et al. 1997; Karle et al. 2002; Nerbonne et al. 2005; Millar et al. 2006; Bayliss et al. 2008). Furthermore, they also regulate diastolic membrane conductance (Dhamoon et al. 2004; Miale et al. 2003; Zobel et al. 2003) in the heart. During the time course of the AP, both I_{Kr} and I_{Kb} currents are active but their molecular basis is poorly understood in the heart and kidney, contrary to the brain. The strong inward rectifier current is determined by Kir2.x ion channels, while the I_{Kb} current is conducted by the two-pore ion channels (K_{2P}) (Lesage et al. 1996a, 2000a; Goldstein et al. 2001; Gierten et al. 2008). Although, they are structurally very different (Nerbonne et al. 2001, 2005), both the Kir2.x and K_{2P} channel subunits are thought to establish the structural and molecular basis of the I_{Kr} ion channels in cardiomyocytes. The expression and properties of the above mentioned K+ channels are altered in cardiac diseases (arrhythmias, Long QT syndromes, hypertrophic cardiomyopathy, heart failure) or other, i.e. renal and neuronal diseases (Bayliss et al. 2008; Hedley et al. 2009; Gaborit et al. 2009; Greiser et al. 2009; Es-Salah-Lamoureux et al. 2010).

K_{2P} structure and their nomenclature

In the literature, the conventional name is frequently used instead of the systematic nomenclature accepted by the Human Genome Organization (HUGO; Goldstein et al. 2005) for genes (KCNK1-18) and proteins (K_{2P}1-18) of K_{2P} channels. In this review, we mark both the latest and the old nomenclature.

Lesage et al. (1996a, b) have reported the structure of the first member of the two-pore channel family, although it was

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postulated much earlier. Their sequences are diverse; however, the $K_{\alpha}$ channels have two common structural features. First, the $K_{\alpha}$ channels possess two concatenated pore domains on a single protein chain protomer (Fig. 1). Each pore domain of this tandem formation contains an outward and an inward membrane-spanning helix flanking the potassium ion-selectivity filter segment and the concatenated membrane-re-entrant pore helix (Lesage et al. 2000a; Nerbonne et al. 2001, 2005). The other similarity is that the $K_{\alpha}$ channels hold a segment of about 60 amino acids on the extracellular surface inserted between the first outward transmembrane helix and the first pore domain (Fig. 1a). The role of this “extracellular loop” in the channel function has not been revealed yet. The crystal structure of the human $K_{\alpha}$ channels has remained less well characterized compared to other potassium channels until now. Recently the structures of the TWIK1/K$\alpha$1 and TRAAK1/K2P4 channels were determined by Miller et al. (2012) and by Brohawn et al. (2012), respectively.

The proteins with two P-loop domains, named as K$\alpha$1.1/TWIK1, were cloned from human kidney (Lesage et al. 1996a) and further analysis gave evidence for the existence of the four transmembrane domains (TM1-4). Following this recognition, 15 different K$\alpha$ channels were identified with two P-loop domains and four TM domains. They have been classified into six subfamilies on the basis of sequence and functional similarity (TWIK, TREK, TASK, TALK, THIK and TRESK; see Table 1) (Périer et al. 1994; Lesage et al. 2000a, b; Nerbonne et al. 2001, 2005; Bayliss et al. 2008; Enyedi et al. 2010). Based on the protein sequence a structural model showed that all subunits for the K$\alpha$ channels are assembled as a dimer to form principally homodimeric or heterodimeric ion channels as it was presented by Berg et al. (2004) with one exception between K$\alpha$3.1/TASK1 and K$\alpha$9.1/TASK3 (Berg et al. 2004; Czirjak et al. 2002). Three of the 15 K$\alpha$ channels have yet unknown physiological function yet because their current cannot be measured (K$\alpha$7.1/KCNK7; K$\alpha$12.1/THIK2; K$\alpha$15.1/TASK5). Different animal species express diverse types of the physiologically active K$\alpha$ channels with the 4TM/2P structure, such as ORK1 in Drosophila sp. (Goldstein et al. 1996), and more than 40 types in Caenorhabditis sp. and in plants.

**Binding partners of K$\alpha$ channels**

The membrane potential is essential to the cardiac and neuronal activity; therefore, the “leak” current regulation is an essential dynamical mechanism to control the cellular excitability (Bayliss et al. 2003, Patel et al. 2001, Goldstein et al. 2001). The trafficking by the different two-pore channels, their post-translational modification and localization in the membrane are different (Table 2). The auxiliary subunits bound to the pore-forming $\alpha$-subunits of K$\alpha$ channels were summarised by Plant et al. (2005) and Mathie et al. (2010) as shown in Table 2. Among those binding partners, SUMO (small ubiquitin-related modifier protein), 14-3-3 (HIV encoded partner) and Vpu1 (Viral protein U 1) have direct interactions with K$\alpha$ channels and the investigation of K$\alpha$ have revealed new regulatory pathways (Czirjak et al. 2008). The 14-3-3 proteins are members of the adaptor family proteins and they play a role in many cellular processes including apoptosis, metabolism, and membrane protein trafficking (Mrowiec et al. 2006). Furthermore, the 14-3-3 proteins were shown to be important in the intracellular trafficking and the promotion of forward trafficking between the ER and the plasma membrane.

The associated partners are modulators together with strong controlling external chemical and physical stimuli.
Structure and function of two-pore potassium channels in the heart

Table 1. mRNA expression and special function of two-pore channels in the human tissues. (reviews by: Lesage et al. 1996a,b, 2000a,b; Lazdunsky et al. 2000; Bayliss et al. 2008; Enyedi et al. 2010).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Acc.No.</th>
<th>Protein</th>
<th>Expression</th>
<th>Function</th>
<th>References</th>
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<tr>
<td>KCNK1</td>
<td>NM_002245</td>
<td>TWIK-1/</td>
<td>brain, heart, kidney, liver, lung, ovary, pancreas, placenta, prostate, intestine, testis, colon</td>
<td>week inward rectifier; cell volume regulation; in K⁺ secretion; may inhibit growth of tumor cells; deafness-associated sensitization</td>
<td>Lazdunsky et al. 2000; Millar et al. 2006; Gaborit et al. 2007</td>
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<td></td>
<td>Kᵥ₆.1</td>
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<td>KCNK6</td>
<td>NM_004823</td>
<td>TWIK-2/</td>
<td>heart, kidney, liver, lung, ovary, pancreas, placenta, prostate, intestine, spleen, thymus</td>
<td>week inward rectifier; in K⁺ secretion;</td>
<td>Millar et al. 2006</td>
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<td>Kᵥ₆.6</td>
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<td></td>
<td>NM_033348 v. B</td>
<td>Kᵥ₇.1</td>
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<td>BC004367</td>
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<td>KCNK2</td>
<td>AF129399 v1</td>
<td>TREK-1/</td>
<td>brain, heart, kidney, ovary, prostate, skeletal muscle, intestine, testis</td>
<td>temperature and mechanical pain sensation; neuroprotection by PUFA; nociception; cardioprotection.</td>
<td>Lazdunsky et al. 2000; Cohen et al. 2008, 2009b</td>
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<td>KCNK10</td>
<td>NM_021161 v. 1</td>
<td>TREK-2/</td>
<td>brain, kidney, heart</td>
<td>mechanical stretching</td>
<td>Kim et al. 2000a; Lesage et al. 2000a,b; Kazanski et al. 2010</td>
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<td>KCNK4</td>
<td>AF247042</td>
<td>TRAAK/</td>
<td>brain, kidney, placenta, prostate, intestine, testis</td>
<td>modulated by PUFA and temperature</td>
<td>Kim et al. 2000b; Kang et al. 2005</td>
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<td></td>
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<td>Kᵥ₄.1</td>
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<td>KCNK3</td>
<td>NM_001017424v.1</td>
<td>TASK-1/</td>
<td>brain, heart, kidney, liver, lung, pancreas, placenta, prostate, intestine</td>
<td>sensitive to changes in extracellular acidic pH</td>
<td>Cohen et al. 2008</td>
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<td>NM_014217 v.2</td>
<td>Kᵥ₃.1</td>
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<td>NM_002246 v.3</td>
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<td>KCNK5</td>
<td>NM_003740</td>
<td>TASK-2/</td>
<td>heart, kidney, liver, lung, pancreas, placenta, ovary</td>
<td>renal acidosis; regulation of respiration; osmotic volume regulation; sensitive to changes in extracellular alkaline pH</td>
<td>Gestreau et al. 2010; Olszewski et al. 2008; Hosten et al. 2007</td>
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<td>Kᵥ₅.1</td>
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<tr>
<td>KCNK9</td>
<td>NM_016601</td>
<td>TASK-3/</td>
<td>brain, heart, kidney, liver, lung</td>
<td>sensitive to changes in extracellular alkaline pH; characteristic dismorphism</td>
<td>Cohen et al. 2008, 2009b</td>
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<td></td>
<td>NG_012842</td>
<td>Kᵥ₉.1</td>
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<td>KCNK16</td>
<td>NM_001153105v.1</td>
<td>TALK-1/</td>
<td>brain, heart, kidney, liver</td>
<td>contribute to bicarbonate reabsorption; control of apoptotic volume of kidney proximal cells</td>
<td>Cho et al. 2012; Duprat et al. 2005</td>
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<tr>
<td></td>
<td>NM_032115 v2</td>
<td>Kᵥ₁₆.1</td>
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<td>NM_001153106v.3</td>
<td>TALK-2/</td>
<td>exocrine pancreas, heart</td>
<td>role in the ischemic stroke</td>
<td>Domingues-Montanaria et al. 2010; Girard et al. 2001</td>
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<td>NM_001153107v.4</td>
<td>Kᵥ₁₇.1</td>
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<tr>
<td>KCNK17</td>
<td>NM_031460 v.1</td>
<td>TASK-5/</td>
<td>olfactory bulb mitral cells, cerebellar Purkinje cells</td>
<td>sensitive to changes in extracellular alkaline pH; functional currents not known</td>
<td>Marsh et al. 2012</td>
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<td>NM_001153111v.2</td>
<td>Kᵥ₁₅.1</td>
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<tr>
<td>KCNK15</td>
<td>NM_022358</td>
<td>THIK-1/</td>
<td>in the proximal and distal nephron of kidney</td>
<td>stimulated by arachidonic acid</td>
<td>Rajan et al. 2001; Theilig et al. 2008; Morrison et al. 2010</td>
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<td>Kᵥ₁₃.1</td>
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<td>KCNK13</td>
<td>NM_022054</td>
<td>THIK-2/</td>
<td>kidney, brain, heart</td>
<td>functional currents not known</td>
<td>Nyholt et al. 2008; Theilig et al. 2008</td>
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<td>Kᵥ₁₂.1</td>
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<tr>
<td>KCNK12</td>
<td>NM_022055</td>
<td>TREK-2/</td>
<td>brain temperature detection; nociception; migraine and related disorders; immunomodulators</td>
<td>King et al. 2004; Lafrenière et al. 2011</td>
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<td>Kᵥ₁₈.1</td>
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including oxygen tension, pH, lipids, mechanical stretch, neurotransmitters and G-protein coupled receptors (Goldstein et al. 2002; Lesage et al. 2000b). SUMO silences Kᵥ₃.1 plasma membrane channels, phosphorylation of Kᵥ₃.3 enables 14-3-3 binding to affect forward trafficking, whereas it decreases the open probability of Kᵥ₂.2; and, Vpu1, an HIV encoded partner, mediates an assembly-dependent degradation of Kᵥ₃. NOX4 confers O₂ sensitivity to channel Kᵥ₃.1/TASK1.

The TWIK1 associated protein partners are SUMO and ADP-ribosylation factor 6/exchange factor for ADP-ribosy-
Table 2. Associated proteins involved in the regulation and modulation of two-pore channels. Acronyms: ADP-ribosylation Factor 6, ARF6; A-kinase anchoring protein 150, AKAP150; protein kinase A, PKA; Coatomer protein complex 1 COPI; Endoplasmic reticulum, ER; Exchange Factor for ADP-ribosylation factor 6, Ef6; microtubule-associated protein, Mtap2; protein kinase C, PKC; HIV encoded partner, 14-3-3; NADPH oxidase 4, NOX4; Viral protein U 1, Pvu1; Polyunsaturated fatty acid, PUFA; Small Ubiquitin-related Modifier Protein, SUMO; Tyrosine kinase, TK.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Binding partners</th>
<th>Presumed role</th>
<th>References</th>
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<tbody>
<tr>
<td>KCNK1</td>
<td>TWIK1/1, K&lt;sub&gt;3.1&lt;/sub&gt;</td>
<td>SUMO, ARF6/EF6</td>
<td>‘Silences’ the channel: controls open probability by reversible linkage to K&lt;sub&gt;3.1&lt;/sub&gt;</td>
<td>Rajan et al.2005 Mathie et al. 2010</td>
</tr>
<tr>
<td>KCNK6</td>
<td>TWIK2/1, K&lt;sub&gt;6.1&lt;/sub&gt;</td>
<td>TK</td>
<td>TK inhibition reduces beneficial effects on cardiac ischaemic preconditioning and cardioprotection</td>
<td>Gierten et al. 2008 Cho et al. 2005 Gierten et al. 2008</td>
</tr>
<tr>
<td>KCNK3</td>
<td>TASK1/1, K&lt;sub&gt;3.3&lt;/sub&gt;</td>
<td>14-3-3, COPI, PKA</td>
<td>Increases the surface expression of the channel</td>
<td>Rajan et al.2002 Zuzarte et al. 2009 Mathie et al. 2010 O’Kelly et al. 2002 Gierten et al. 2008</td>
</tr>
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<td>KCNK5</td>
<td>TASK2/1, K&lt;sub&gt;5.5&lt;/sub&gt;</td>
<td>14-3-3</td>
<td>Increases the surface expression of the channel</td>
<td>Rajan et al.2002</td>
</tr>
<tr>
<td>KCNK9</td>
<td>TASK3/1, K&lt;sub&gt;9.3&lt;/sub&gt;</td>
<td>14-3-3, COPI, TK</td>
<td>Regulates channel number by controlling the forward transport to the plasma membrane</td>
<td>Zuzarte et al. 2009 Mathie et al. 2010 O’Kelly et al. 2002</td>
</tr>
<tr>
<td>KCNK2</td>
<td>TREK1/1, K&lt;sub&gt;2.1&lt;/sub&gt;</td>
<td>Mtap2, AKAP150, PKC</td>
<td>Enhances surface expression and current density</td>
<td>Rajan et al.2002 Sandoz et al. 2006 Sandoz et al. 2008</td>
</tr>
<tr>
<td>KCNK13</td>
<td>THIK-1/1, K&lt;sub&gt;13.1&lt;/sub&gt;</td>
<td>TK</td>
<td>Effects on cardiac ischaemic preconditioning and cardioprotection</td>
<td>Gierten et al. 2008</td>
</tr>
<tr>
<td>KCNK18</td>
<td>TRESK/K&lt;sub&gt;18.1&lt;/sub&gt;</td>
<td>14-3-3, calcineurin</td>
<td>Regulates calcineurin-mediated activation of the channel</td>
<td>Czirjak et al. 2008</td>
</tr>
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The mechanosensitive TREK and TRAAK channels are able to form an amphipathic α-helix that is different from other 2P channels. Recently, Milac et al. (2011) have built a model of TREK channels forming a parallel dimeric coiled-coil in closed and open conformations. Brohawn et al. (2012) also presented the crystal structure of the human K2P4/TRAAK channel at a resolution of 3.8 Angstrom.

Our 3D structure of TWIK-1 was built by homology modelling based on the experimental structure (Miller et al. 2012). It was necessary to reconstruct the wild type sequence because the experimental structure is lacking of two fragments (residues 94-99 and 169-174) and contains mutated residues replaced for Cys22 and Asn95 (Fig. 2). A relaxed structure was obtained by energy minimization and the arrangement of the side chains in the critical pore domain was found to be identical with that of the experimental structure.

Discussion

The two pore loops and helices are different within one chain. One important difference may be the presence of Leu in pore 2 (Fig. 3B pore 2) in place of Tyr located in the same position in pore 1 (Fig. 3A pore 1). It is stated by the experiments with engineered 2P channels that this Tyr is essential.
The bulkier side chain of Tyr with hydrogen bonding capacity may rigidify the pore, compared to Leu. Another major difference is the presence of His in pore 1 (Fig. 3A) in place of an Asp in the same position of pore 2 (Fig. 3B). This Asp can be found in many homotetrameric potassium channels too, and it is stated to be responsible for the stability of the open state of the channel (Chapman et al. 2001). Altogether, the GYGD motif in the pore loop of a potassium channel is essential resulting in a selectivity ratio of ca. 1000 between K⁺ and Na⁺. In TWIK1, none of the pore loops possesses this motif, which may be the reason that in the case of lower physiological concentration of extracellular potassium the channel starts to transport sodium leading to fibrillation and arrhythmia (Ma et al. 2011). Further differences can be found between P1 and P2. In identical position of the pore helices there are a Phe and a Tyr in P1 and P2, respectively, with obviously different H-bonding capacities. There is a Glu at the N-terminal end of P2 not being present in P1. A Glu in a similar position can also be found in many potassium channels, facing to the above-mentioned Asp.

Expression and function of two-pore domains channels

Genomic and functional studies pointed to an important role of various K₂P channels in brain, kidney and heart diseases, but so far, only poor and contradictory molecular data have been described. At the past decades, numerous channels were investigated in different ways, including DNA sequencing, microarray analysis, immunocytochemistry and breeding transgenic animals differently expressing various types of K₂P channel proteins compared to wild type undiseased animals. These channels are essential in numerous physiological pro-

Figure 2. The dimer of the wild type channel. The reconstituted residues of the chains are shown in yellow and the cistin bridge by atom type (C in green, O in red, N in blue).

Figure 3. The specific residues in selectivity filter 1 and in pore helix 1 (A) and in selectivity filter 2 and in pore helix 2 (B). Residues in the selectivity filters are coloured by atom type (C in green, O in red, N in blue), the differing Tyr and Phe in the pore helices shown in yellow.
cesses, and recently have been shown to participate in human pathologies as well. These channels were also overexpressed in various human kidney, melanoma, brain and heart cell lines and tissues.

$K_{\text{2P}}$ channels conduct potassium-selective “leak” currents that are time- and voltage-independent, playing pivotal role to set and control the resting membrane potential, the potassium homeostasis and the volume of the cells. Furthermore, they modulate various physiological functions related to altered membrane potential, i.e. neurotransmitter and hormone secretion, as well as neuronal and muscular excitability (Lesage et al. 1996a). Regional expression of $K_{\text{2P}1.1}/\text{TWIK1}$ in the heart was highly expressed at both mRNA and protein level in the right and left atria as well as in Purkinje fibers and in about a 30% lesser extent in the right and left ventricle (Gaborit et al. 2007). We detected $K_{\text{2P}1.1}/\text{TWIK1}$ by immunofluorescence in the intercalated discs of the left ventricle (Fig. 4a). Confocal microscopy studies showed that both TWIK1 and TASK1 were expressed in the intercalated discs of the ventricle (Fig. 4A, 4B). $K_{\text{2P}3.1}/\text{TASK1}$ mRNA expression is one magnitude

![Figure 4. The protein expression of two-pore domain ion channels in the human left ventricle of the heart. (A) K2P1.1/TWIK-1 immunolocalized in the intercalated discs of the left ventricle. (B) K2P3.1/TASK1 proteins expressed in the intercalated discs and the lateral side of sarcolemma.](image-url)
less expressed in the ventricle.

Recently we showed gender differences of several Kv ion channels from donor hearts (Gaborit et al. 2010). Its importance is raised because Kv channels are known to be important determinants of the human arrhythmic risk involving Kv channels. However, our study revealed that there are no gender differences in the expression of either the most abundant Kv1.1/TWIK1 or the Kv5.1/TASK2 channels. In contrast, the transcripts for Kv3.1/TASK1 were 3-fold more abundant in female left ventricle (LV) endocardium and epicardium (Gaborit et al. 2010) which might be considerably different in normal physiology conditions in contrast to diseased states.

Kv1.1/TWIK1 and Kv6.1/TWIK2 ion channels are known as weak inward rectifiers. Kv2.1/TREK1 and Kv4.1/TRAALK are polyunsaturated fatty acid (PUFA) and stretch-activated Kv channels whereas Kv3.1/TASK1 and Kv5.1/TASK2 are acid-sensitive channels. Kv2.1/TREK-1 is the only known outward rectifier channel. However, KCNK6 and KCNK7 are possibly silent genes.

Kv1.1/TWIK1 channels are expressed in the heart (Wang et al. 1998), cerebellum, thalamus (Talley et al. 2001), and kidney (Levy et al. 2004). Under in vitro conditions, Kv1.1/TWIK1 was detected mainly in recycling endosomes (RE) in native and transfected cells. Kv1.1/TWIK1 gene inactivation leads to the loss of nonselective cationic conductance, an unexpected effect that was attributed to the adaptive regulation of other channels (Millar et al. 2006) in renal principal cells.

It was reported that TWIK1 is responsible for the paradoxical depolarization of the human heart during pathological hypokalemia (Ma et al. 2011). The ion selectivity of TWIK1 is highly modulated by the extracellular pH. Although TWIK1 is K+ selective at neutral pH, it becomes permeable to Na+ at the acidic pH found in endosomes (Chatelain et al. 2012). At acidic pH, typical in the secretory and endocytic/recycling pathways, TWIK1 conducts Na+ leaving a nonselective cationic channel in RE. The transitions from the Na+-conducting state to the K+-selective state are very slow, suggesting that TWIK1 delivered to the cell surface will remain nonselective for several minutes at the plasma membrane. This phenomenon could explain the loss of cationic conductance and the hyperpolarisation of the resting membrane potential recorded in TWIK1−/−knock out kidney cells. It was also shown that TWIK1−/−pancreatic β cells are hyperpolarized compared with the control wild type cells, particularly when the ATP-sensitive K+ channels are turned off by the presence of glucose in the medium endosomes (Chatelain et al. 2012).

TASK (TWIK-related Acid-Sensitive K channels channel family) was one of the first Kv channels discovered, and it was intensively studied being it was essential in anaesthesia and in the therapy of brain diseases. Regional expression of TASK1, TASK3, and TASK5 was reported in the brain (Karschin et al. 2001). They were also detected in the heart (Fig. 4A, 4B), kidney, liver, lung, pancreas, placenta, and ovary. In the right and left atria, the mRNA expression for TASK1 was abundant, but one order of magnitude less nucleic acid was detected in the Purkinje fibre and the ventricle of the heart (Gaborit et al. 2007). TASK2 was also expressed with the same ratio as TASK1 in different regions of the heart but much lower amount was detected compared with TASK1 or to TWIK1. We detected TASK1 in the intercalated discs similarly to TWIK1 in the ventricle (Fig. 4A, B).

All types of Kv α-subunits are expressed in the human kidney, TWIK1 and TASK2 are the most abundant, similarly to the heart (Table 1). TWIK1 was immunolocalized in the rat kidney, where it is highly expressed in the brush-border membrane of the proximal convoluted tubules, in the thick ascending limb of the loop of Henle, and in the collecting duct intercalated cells, both by intracellular and apical localization (Cluzeaud et al. 1998).

Similarly to the kidney, all Kv subunits are expressed in the brain and mostly different Kv channels are mapped. TWIK1 is abundant in the human brain and plays essential roles in diverse physiological functions in neuronal cells (Bayliss et al. 2008; Enyedi et al. 2010).

Kv2.1/TREK1 is expressed in rat left and right atria, ventricles and septum (Terrenoire et al. 2001; Tan et al. 2004) but not reported in the human myocardium. TREK1 is located in the longitudinal stripes at the surface of cardiomyocytes that conforms to the sensing of longitudinal stretch in myocytes (Li et al. 2006). However, there is no evidence for colocalization with membrane-associated cytoskeletal proteins in the “costamers” (Kostin et al. 1998).

Kv2.1/TREK1 channel exhibits two distinct types of current, the large and small conductance states, at positive potential in rat cardiomyocytes (Li et al. 2006). It exists in two distinct splice variants, TREK1a and TREK1b in the myocytes. It highlights the importance of the post-translational regulation of TREK1 that might affect the conductance of this channel and it is supposed that TREK1 may be involved in balancing the membrane potential and the duration of the action potential as an outward rectifying current (Terrenoire et al. 2001; Li et al. 2006).

TREK1, TREK2, Kv1/2/Kv10 (TWIK-Related K channels) and TRAAK (TWIK-Related Arachidonic Acid (AA)-stimulated K channel) channels have diverse electrophysiological properties (Kim et al. 2001a, b; Lesage et al. 2000a, b). Kv2.1/TREK1 is a mechanogating stretch-activated channel, which is also activated by PUFAs, phospholipids, volatile anaesthetics, intracellular acidification (decreased pH) and increased temperature. They are inhibited by Gzα- and Gαq-linked GPCRs through protein kinase A (PKA) and PKC pathways, respectively, and activated by a PKG-mediated mechanism. TREK-1 is reciprocally sensitive to extracel-
lular Mg²⁺ changes than are the TREK-2 and TRAAK channels (Kim et al. 2001a, b). Regulation of Kᵥ channels by G protein-coupled receptors was also investigated in neuronal tissues (Mathie A 2007).

TREK1 is abundantly expressed in smooth muscle and the endothelial layer of the basilar artery, and is essential in the modulation of the cerebrovascular tone.

The studies of the mechanically gated channels, TREK1/ Kᵥ2.1), TREK2/Kᵥ10.1), and TRPC6 (Transient Receptor Potential Cation channel 6) channels study showed (Kazanski et al. 2010), that NO plays a role in the regulation of the activity of these channels. Furthermore, the endothelial NO-synthase predominates as NO source in cardiomyocyte response to stretching.

Kᵥ13.1/THIK1 and Kᵥ12.1/THIK2 are arachidonic acid sensitive K2P channels, and they are expressed in the kidney, brain and in the vasculature. The mRNA for Kᵥ13.1/THIK1 but not that of Kᵥ12.1/THIK2 was detected in the heart (Kim et al. 1998). In the vasculature the arachidonic acid sensitive Kᵥ channels are localized in the arteries and play an important role (Gurney et al. 2009) in the endothelial cells in the regulation of the vascular tone (Blondeau et al. 2007; Garry et al. 2007). The rat mesenteric and femoral arteries express TREK1, TREK2 and THIK. TRAAK is detected in mesenteric artery but not in femoral artery (Gardener et al. 2004; Goonetilleke et al. 2007).

Kᵥ4.1/TRAAK (TWIK-related arachidonic acid-stimulated potassium channel protein) is a lipid- and mechanosensitive K⁺ channel in Kᵥ family. This protein forms homodimers and functions as an outwardly rectifying channel. It is expressed principally in neural tissues and stimulated by membrane stretch and polyunsaturated fatty acids. Proteins in TREK family (TREK1, TREK2 and TRAAK) are inactivated by hypo-osmolarity and phosphorylation by protein kinase A and C (Lesage et al. 2003).

Kᵥ16.1/TALK1 and Kᵥ17.1/TALK2 are expressed in the exocrine pancreas, and also in the heart (Girard et al. 2001) and sensitive to external alkalic pH such as TASK1 and TASK2 (Duprat et al. 2005). However, TASKs are expressed in the islets of Langerhans. Nitric oxide (NO) specifically activates TALK1 and TALK 2, and they are might be under NO dependent stimulation (NOS). Interestingly, the current, generated by TALK2 channel, is low at physiological pH and NOS is mostly active on TALK2 suggesting that the NOS plays a role in the preconditioning as well (Duprat et al. 2005).

Physiological effects on the Kᵥ domain channels and their pharmacological targeting

Physiology and pharmacology of Kᵥ channels are intensively studied in the past decades mainly in the central nervous system (Kim 2005; Duprat et al. 2007; Cohen et al. 2009a, b; Enyedi et al. 2010), however, the role of these channels in the cardiovascular system is still to be explored. Here we also present evidence focusing on the latest years’ results from pharmacological and regulatory aspects in the cardiovascular system.

Physiological roles and pharmacological properties

The non voltage-gated Kᵥ potassium channels, TASK1, TASK3, and TREK1 contribute to anaesthetic-induced hypnosis and immobilization. Buckler et al. investigated TASK-like channels in rat arterial chemoreceptor cells studied oxygen-, acid-, and anaesthetic-sensitivity. Duprat et al. (2007) have shown that TASK channels function as chemo-, and nutrient sensors. However, TASK channels are not required for brainstem control of breathing by CO₂ or pH despite wide expression in these areas. TASK channels are necessary for adrenal aldosterone secretion. TREK-1 channels contribute to temperature and mechanical pain sensation, neuroprotection by PUFAs, and mood regulation. TASK2 channel is necessary for HCO₃⁻ reabsorption and osmotic regulation in the kidney proximal tubules (Bayliss et al. 2008).

Experiments with transgenic mice presented evidence that TREK channels mediate the effect of PUFAs during an ischemic attack, not only by hyperpolarizing neurons (and thus reducing the susceptibility to ischemia), but also enhancing the circulation in/or nearby the affected region (Blondeau et al. 2007; Enyedi et al. 2010).

The expression and the pharmacological properties of TASK-1 were investigated in human pulmonary artery smooth muscle cells (Olschewski et al. 2006) resulting in an evidence that moderate hypoxia is a blocker of TASK1. However, treprostinil activates this channel mediated by PKA, which may indicate the pivotal mechanism underlying the vasorelaxing properties of prostanooids.

Localization of pain is important in all organs/tissues but understanding the molecular mechanism of nociception has just started only a few years ago, and it is still far from elucidation. Cohen’s group highlighted the importance of Kᵥ2.1/TREK1 channels in the process of sensitization of pain signalling and its modulation (Cohen et al. 2008, 2009b).

Kᵥ18/TRESK (TWIK-related spinal cord K⁺ channel, KCNK18) channels are mainly expressed in the dorsal root ganglion (DRG) neurons and they have been suggested to play a key role in pain disorders (Kang et al. 2004; Dobler et al. 2007; Huang et al. 2008; Mathie A 2010; Tulleuda et al. 2011). Examining the TRESK channel Liu et al. (2004) highlighted the possibility of activation of the human Kᵥ2 channels by clinical concentrations of volatile anaesthetics.

Furthermore, the dominant-negative mutation of TRESK1 channel was reported to be linked to familial migraine with aura (Lafreniere et al. 2010; Braun et al. 2011). Studying TRESK channels in the heart requires concerted efforts of multiple research laboratories.
$K_{\text{p}}$ channels are relatively insensitive to classical $K^+$ channel blockers such as TEA, Ba$^{2+}$, and Cs$^+$. TASK1 channels are selectively inhibited by the endocannabinoid anandamide. TREK1 is a stretch-activated and heat sensitive channel. TREK1, TREK2, TRAAK and THIK1 are stimulated by arachidonic acid and PUFAs. $K_{\text{p}}$ channels are generally sensitive to inhalation and local anaesthetics, but TWIK2 and TRAAK channels show no sensitivities to anaesthetics (O’Connell et al. 2002). Several laboratories reported that inhalational anaesthetics activate $K_{\text{p}}$ channels, hyperpolarizing neurons in the mammalian central nervous system (Nicoll et al. 1982; Patel et al. 1999; Buckler et al. 2000). Different effects of volatile anaesthetics on human TASK-1 channels expressed in Xenopus oocytes are found. They are insensitive to propofol but inhibited by etomidate and stimulated by sevoflurane and isoflurane (Putzke et al. 2007a, b).

**Pharmacological effects on cardiac $K_{\text{p}}$ channels**

Slowing of the intrinsic heart rate may be explained by the increased expression of TASK1, TWIK1, TWIK2, K(ir)2.4 channels, calsequestrin 2, and the A1 adenosine receptor in the sinoatrial node (SAN) in the heart (Yanni et al. 2011). Alterations in the gene expression of the ion channels have also occurred in the sinoatrial node dysfunction induced by heart failure.

The endocardial and epicardial differences and the regional differences in the stretch activation of ventricle (Tan et al. 2004) are possibly caused by TREK1. This leads to the differential mechano-electrical feedback and reduces the action potential repolarization in areas of the myocardium where the conduction velocity is lower (Lab 1999; Ravens 2003; Gurney et al. 2009).

The antidepressant fluoxetine inhibits cardiac and cerebral TREK1 channels expressed in HEK-293 cells and Xenopus oocytes. Sensitivity was decreased by 70% when first 52 amino acids were removed (Eckert et al. 2011).

The class III antiarrhythmic drug amiodarone prolongs the action potential and suppresses arrhythmia in human TASK1 channels expressed in Xenopus oocytes (Gierten et al. 2010). TASK1 channels in rat cardiac myocytes substantially contribute to outward potassium current during plateau phase and this current can be inhibited by the $\alpha_1$A adrenergic agonist methoxamine. A293 was a selective blocker of TASK1 channels expressed in Xenopus oocytes (Putzke et al. 2007a, b).

$K_{\text{p}}$ channel related regulation, trafficking and diseases with treatments

**Molecular mechanism of the regulations of two-pore domain channels**

$K_{\text{p}}$ channels exhibit different pharmacological effects and there are diverse regulatory and signalling pathways behind this. In the myocytes, contraction is stimulated by the rise in the Ca$^{2+}$ concentration of the cytosol in myocytes where an increased Ca$^{2+}$ concentration is the endpoint of many different pathways. Small proteins or carbohydrates are frequently attached to $K_{\text{p}}$ channels to modulate their physiological effects. The mechanism of the trafficking, post-translational modification, and localization in the membrane are different for the two-pore channels. The auxiliary subunits binding to the pore-forming $\alpha$-subunits of the $K_{\text{p}}$ channels were summarised (see in Table 2; Plant et al. 2005; Mathie et al. 2010). $K_{\text{p}}$ channels can be activated by different stimuli such as cell membrane stretch, lysophospholipids and arachidonic acids, and inactivated or modulated by physicochemical factors, endogenous neurochemicals and clinically relevant drugs (Bayliss et al. 2008; Gurney et al. 2009).

TWIK1 regulation is strongly effected by the ADP-ribosylation factor 6 (ARF6). The small protein, ARF6 which is a part of the RAS superfamily, was recently cloned (acc.no. NM_001663.3, the translated protein id. is NP_001654.1. It is abundantly expressed in the heart, widely present in other tissues (Tsuchiya et al. 1991) and functionally and physially associated with the Golgi complex (Stearns et al. 1990; Deutsch C. 2003). A sequence homology of 100% was identified in vertebrates and high homology was found even with *Saccharomyces pombe*. This factor is a member of the human ARF gene family, and the ARF gene encodes the small guanine nucleotide-binding protein (175aa) that stimulates the ADP-ribosyltransferase activity of cholera toxin (Welsh et al. 1994). ARF6 plays a role in the vesicular trafficking and acts as an activator of phospholipase D (D’Souza-Schorey et al. 1995). It is localized in the plasma membrane, and regulates the vesicular trafficking via remodelling the membrane lipids and signalling pathways that most probably lead to actin remodelling. A pseudogene of this gene is located on chromosome 7 under the $\alpha$-adrenerg regulation.

In the heart, Doroudgar et al. (2009) investigated the stress response of the endoplasmic reticulum under ischemic conditions where the AT6 branch has been activated. Possibly the Toll-like receptor 9-mediated signalling plays a role in this mechanism (Wu et al. 2012).

$K_{\text{p}3}$/TASK1, TASK3, and the non-functional TASK5 associate to 14-3-3 proteins in recombinant and native form (Girard et al. 2002). When COPI interacts with TASK channels, the surface expression of these channels as well as the accumulation of the channels are decreased in the ER. Thus 14-3-3 and COPI act in opposite ways either enhancing the TASK channels’ forward trafficking towards the membrane or retaining the TASK channels in the ER, respectively. Modulation of TASK channels by protein kinase C has also been investigated (Gierten et al. 2010).

Recently it was shown that the TASK1 channel protein interacts directly with NADPH oxidase 4 (NOX4) (Park et al. 2009) to confer oxygen sensitivity to TASK1 and mediate the oxygen-sensitive K$^+$ current response in carotid and
neuro-epithelial bodies (Lee et al. 2006; Duprat et al. 2007). Recently, it has been evidenced that TASK1 is neuroprotective under ischemic conditions (Meuth et al. 2009).

TREK-1 channels are activated by PUFAs, phospholipids, stretch, volatile anesthetics, intracellular acidification (decreased pH) and by elevated temperature. They are inhibited by Gtọs- and Gtq-linked GPCRs through protein kinase A (PKA) and PKC, but activated by a PKG-mediated mechanism (Patel et al. 1995; Honore et al. 2007).

Mathie et al. (2010) examined the TASK channel trafficking from the endoplasmic reticulum (ER) to the plasma membrane via the Golgi apparatus and then focused on the established regulatory mechanisms for K_{2P} channel trafficking. Localisation of TREK channels to particular regions of the neuronal membrane and the involvement of binding partners such as A-kinase anchoring protein 150 (AKAP150) and microtubule-associated protein (Mtap2) in this localisation was reviewed. The binding partners regulating the localisation of TWIK1 are ARF6 together with EFA6, and Vpu for TASK1 Mathie et al. (2010).

Post-translational regulation of K_{2P} channel trafficking controls the number of functional channels and has an effect on the functional properties in different cells (neurons, cardiomyocytes, endothelial cells) (Mathie et al. 2010; Enyedi et al. 2010). It was reported that P11 might promote forward trafficking together with 14-3-3, which binds to the last three amino acids of the carboxy terminal for transferring the associated K_{2P} complex to the plasma membrane. On the other hand, P11 is able to associate with TASK1 too in the presence of 14-3-3.

Different agents via calcineurin-mediated pathway (Czirják et al. 2004) can activate calcineurin. The pivotal role of endogenous calcineurin in TREK activation has been verified in mammalian cells (Czirják et al. 2006). The regulation of TREK by calcineurin is in good accordance with the previous result that TREK and calcineurin are associated via direct protein-protein interaction (Kang et al. 2006, 2008). Mathie et al. (2007) showed that MARK directly phosphorylates TREK in vitro and specifically inhibits the TREK1 channels. Mutagenesis studies proved that the phosphorylation of Serine 264 and the direct interaction of 14-3-3 with TREK were not indispensable for the effect of MARK2, suggesting that this kinase acts via the Ser274/Ser276/Ser279 cluster (Braun et al. 2011). These results are connected with two presently emerging fields, the cellular polarity and the microtubule dynamics, and suggested that their control is coupled to the regulation of the background K^+ current in the cells, which express TREK.

**Diseases refer to K_{2P} channels with treatments**

Cases of K_{2P} diseases with inherited or aquired mutations have only been detected in neuronal diseases. We have data for altered transcripts or protein levels of K_{2P} channels in a few diseases. Recently mutations and related channelopathies of K_{2P} have been discovered, as well as the regulation by small proteins associated to α-subunits of K_{2P} concerning brain or kidney diseases.

The dominant-negative mutation of TREK1 channel was discovered to be related to familial migraine with aura Lafreniere et al. 2010; Braun et al. 2011). TREK2 and THIK2 were shown also the cause of migraine (Nyholt et al. 2008).

 KCNK1 and KCNK2 coding genes for TWIK1 and TASK1 were both down-regulated at transcriptional and translational levels in dilated cardiomyopathy (DCM; Szűts et al. unpublished data) but we have no data if there were any mutations of K_{2P} channels.

First, the neuronal background of K_{2P} channels was focused on physiological studies targeting TASK channels and TREK1 for effective treatments in anaesthesia and neuronal diseases (Patel et al. 1999; Honore et al. 2007; Olschewski A 2010).

In cardiac Brugada syndrome there is an inherited sudden-death arrhythmia syndrome where the ion channels monitoring exhibited distinct clustering differences versus the control non-diseased and other ventricular-tachyarrhythmia groups (Gaborit et al. 2010) among 14 of 77 genes encoding important ion-channel/ion-transporter subunits. Among these K^+-channel genes, Brugada patients showed greater expression of the two-pore domain gene KCNK1/TWIK1 and reduced expression of Kv4.3 involved in the transient-outward K^+-current I_{to} versus I_{to} in Brugada patients than in other groups. In humans, the 2P-domain K^+-channels TWIK1 and TASK2 are more strongly expressed in Purkinje fibres than in the right ventricle, with higher level of expression of TWIK1 than TASK2 (Gaborit et al. 2007). Preferential expression of a 2P-channel in Purkinje fibres also occurs in mice. Increased 2P-channel expression might also compensate for reduced Na^+-channel function.

The neuronal two-pore domain potassium-channels and their modulators have been proposed as potential drug targets and have importance in anaesthesia, neuronal diseases, pain, migraine, Alzheimer disease etc. Recently the TREK and TRESK ion channels were examined and highlighted as novel therapeutic targets in the treatment of pain (Mathie et al. 2007; Mathie A 2010). K_{2P} channels in other organs, e.g. in the heart, should also be considered as potential targets.

Nowadays, the cardiac arrhythmia is one of the most widespread diseases not only in the elderly, but in the middle and younger ages too. A great number of people suffer also from depression. K_{2P} channels are targeted in current antidepressant (AD) treatments. One of the potential candidate drugs, spadin, blocks TREK1s, which are considered as new targets for AD but blocking these channels may result in del-
eterious side effects. Moha et al. (2012) showed that spadin did not interfere with other TREK1 controlled functions such as pain, epilepsy and ischemia. Spadin was unable to inhibit currents generated by TREK2, TRAAK, TASK and TRESK and, spadin also did not induce cardiac dysfunctions.

In the treatment of cardiac arrhythmia, one of the selective targets is the K\textsubscript{p}3.1 (TASK-1) potassium leak channel using amiodarone, a class III antiarrhythmic drug (Mathie et al. 2007; Giersten et al. 2010). Its therapeutic potential in neuronal anaesthesia was reported by Yoo et al. (2009) and in cardiology (Sear JW 2010).

Nowadays, ion channels as novel therapeutic targets are of increasing importance in the treatment of pain and migraine targeting K\textsubscript{p}2.1/TREK, K\textsubscript{p}18.1/TRESK channels (Mathie et al. 2007; Mathie 2010).

For effective treatment in cardiac, renal and cerebral diseases we need to know the exact mechanism of the action of the drugs as well as their metabolic pathways and their possible side effects. Targeting the K\textsubscript{p} channels may have a wide perspective in various diseases. Recognizing new effects on these channels by drugs already in use is also important. Further development can be expected from the application of agents targeting K\textsubscript{p} channels, e.g. antiarrhythmic drugs, with new mechanism of action.

Conclusions

K\textsubscript{p} channels conduct potassium-selective “leak” currents, which are time- and voltage-independent, and play pivotal role setting the resting membrane potential and controlling the potassium homeostasis and the cell volume. Furthermore, they modulate various physiological functions related to altered membrane potential, an consequent i.e. muscular and neuronal excitability, as well as neurotransmitter and hormone secretion. These channels serve as the molecular targets for certain volatile and local anaesthetics yet. We have to improve our knowledge in order to understand the exact mechanisms and regulations by binding partners of K\textsubscript{p} channels in the heart. That could considerably speed up the development of future therapies directed towards local regions of the heart in cardiac diseases.

Materials and Methods

Homology modelling

Homology modelling was performed by the program package MODELLER 9v8) (Šali et al. 1993) running under Linux Kubuntu (http://www.kubuntu.org) 11.4, Mandriva 2011 (http://www.mandriva.com/eu/linux). The model building algorithm of MODELLER includes an automatic alignment followed by modelling the backbone, side chains and disulfide bonds in accordance with the sequence alignment and other statistical potential related restraints embedded in the program. Finally, MODELLER finishes the model structures by a short simulated annealing molecular modelling using the Charmm22 molecular force field (http://salilab.org/modeller/9v7/manual/node442.html). Additional features of MODELLER were utilized as follows: using multiple chains, involvement of symmetry constraint forcing identical geometry for each chain and applying the “dopehr_loopmodel” module to improve the flexible loop regions. In the preparation of the template PDB files for modelling homotetramers the “MakeMultimer.py” (http://watcut.uwaterloo.ca/cgi-bin/makemultimer) python program was used to restore the symmetrical dimer coordinates where only the asymmetric unit was given together with the symmetry operator. Visualization of the structures was performed by the Visual Molecular Dynamics (VMD) program package (Humphrey et al. 1996). The quality assessment of the structures obtained by homology modelling was performed by the program PROCHECK (Laskowski et al. 1993). The disulfide bridge between the Cys69 residues of the chains was then reconstructed using VMD followed by a 1000 step energy minimization by the NAMD molecular dynamics program (Phillips et al. 2005) in vacuo.

Human tissue samples

All protocols for human heart sample procurement and handling were approved for cardiac material from non-diseased donor hearts (Fig. 3). The human ventricle (female; age 32 years) consisted of non-diseased endomyocardial tissue from hearts explanted to collect pulmonary and aortic valves for transplant surgery at the University of Szeged, Hungary. Samples from whom hearts were explanted received no medication except dobutamine, furosemide and plasma expanders. All experimental protocols for sample procurement and handling were approved by the Ethical Review Board of the Medical Center of the University of Szeged (No. 51-57/1997 OEJ) and conformed with the Helsinki Declaration of the World Medical Association. Cardiac tissue was stored in cardioplegic solution at 4°C for 4-8 h. After tissue preservation and dissection procedures, the samples were frozen in liquid nitrogen and stored until immunohistochemistry.

Left-ventricular (LV) subendocardial tissue-slices (1-mm thickness) were obtained at the cardiac base. This heart was histologically normal and had normal expression of atrial natriuretic protein-levels. Contamination by vascular, neuronal and fibrous tissue was excluded by the absent expression of calponin 1 (CNN1), ubiquitin thiolesterase (UCHL1) and collagen type-VI 1 (COL6A1).

Immunofluorescence

Cryostat sections (10 µm) of non-diseased heart ventricle biopsies were treated with 0.5% collagenase, blocked with 2% BSA in PBS and incubated for 2 h at room temperature with rabbit anti-TWIK1 and anti-TASK1 (Alomone; diluted 1:100). Sections were then washed and incubated with Alexa
488-labeled anti rabbit IgG (1:400, Molecular Probes) for 1 h at room temperature. Nuclei were stained with 1 µg/ml Hoechst 33258 in PBS for 10 min (method was modified after Pichca et al. 1999). After rinsing, the sections were mounted with fluorescent mounting medium (Dako), viewed and photographed with confocal laser scanning microscope using an Olympus Fluoview FV1000 (Olympus Life Science Europe GmbH, Hamburg, Germany). For each specimen (n=3 for each antibody staining) 60-80x field images were selected from the zones that presented the greatest variability in the diameters of the tissues.

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Structure and function of two-pore potassium channels in the heart


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