BJP British Journal of Pharmacology

DOI:10.1111/bph.12407
www.brjpharmacol.org

REVIEW

The role of ATP-sensitive potassium channels in cellular function and protection in the cardiovascular system

Andrew Tinker, Qadeer Aziz and Alison Thomas

William Harvey Heart Centre, Barts and The London School of Medicine and Dentistry, London, UK

Correspondence
Andrew Tinker, William Harvey Heart Centre, Barts and The London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, UK. E-mail: a.tinker@qmul.ac.uk

Keywords
ATP-sensitive potassium channel; cardiac myocyte; smooth muscle; physiology; pathophysiology

Received
23 May 2013
Revised
30 July 2013
Accepted
26 August 2013

ATP-sensitive potassium channels (K_{ATP}) are widely distributed and present in a number of tissues including muscle, pancreatic beta cells and the brain. Their activity is regulated by adenine nucleotides, characteristically being activated by falling ATP and rising ADP levels. Thus, they link cellular metabolism with membrane excitability. Recent studies using genetically modified mice and genomic studies in patients have implicated K_{ATP} channels in a number of physiological and pathological processes. In this review, we focus on their role in cellular function and protection particularly in the cardiovascular system.

Abbreviations

ABC, ATP binding cassette; AP, action potential; K_{ATP}, ATP-sensitive potassium channel; K_{CO}, ATP-sensitive potassium channel opening drug; PIP_2, phosphatidyl 4,5-bisphosphate; SUR, sulphonylurea receptor; VSM, vascular smooth muscle

Introduction

Two independent laboratories can lay claim to having first described the ATP-sensitive potassium channels (K_{ATP}; channel nomenclature follows Alexander *et al.*, 2013). Noma (1983) observed the appearance of an outward K^+ current in heart muscle cells when treated with metabolic poisons or hypoxia. This was reversed by ATP injected into the cell. Similar observations were made by another group (Trube and Hescheler, 1984). Such channels were subsequently described in pancreatic beta cells (Ashcroft *et al.*, 1984), skeletal muscle (Spruce *et al.*, 1985), smooth muscle (Standen *et al.*, 1989) and neurones (Ashford *et al.*, 1988). During this period, the basic electrophysiological and pharmacological properties of the channel were elucidated (Ashcroft, 1988; Noma and Takano, 1991). In inside-out patches in ~140 mM symmetrical K^+ concentrations, the single-channel conductance is ohmic with a conductance of 70–80 pS. The lower values sometimes noted in the literature generally have lower and asymmetric K^+ concentrations. The channel is highly selective for potassium (P_{Na}/P_K~0.01). Activity is inhibited by the application of ATP with a K_i of 10–500 μM with a Hill coefficient of more than 1 (generally around 2) depending on the tissue and recording configuration. The ATP inhibition is not dependent on ATP hydrolysis: it is not reliant on Mg^{2+} and ATP can be substituted by non-hydrolysable derivatives. In the absence of magnesium other adenine nucleotides can inhibit channel activity but they are less potent. However, in the presence of Mg^{2+} and ATP, ADP is stimulatory.

Even at the beginning of the 1990s the channels were known to have a rich pharmacology (see Edwards and Weston, 1993). Sulphonylureas were discovered accidentally when it was noted that the anti-microbial sulphonamides caused hypoglycaemia in animals. It became apparent that stimulation of insulin release from pancreatic beta cells occurred because of inhibition of K_{ATP} channels. There is a family of these drugs: the most widely known are the first-generation agents (e.g. tolbutamide, chlorpropamide) and the more potent second-generation agents (e.g. glibenclamide, gliclazide, glipizide). These agents still have a place in the management of type 2 diabetes mellitus. There are also agents that are able to open K_{ATP} channels [K_{ATP} channel opening drugs (K_{CO}s)]. Intriguingly, not only are some of these agents selective for K_{ATP} but they also exhibit a very broad range of chemical structures: for example, diazoxide is a benzothiadiazine, pinacidil a cyanoguanidine and

nicorandil a pyridyl nitrate (Mannhold, 2006). Agents known to block other K^+ channels, for example, Ba^{2+} and 4-aminopyridine, are also active on K_{ATP} channels.

Molecular cloning

The inwardly rectifying family of potassium channels (K_{IR}) resisted cloning even after the elucidation of the primary structure of voltage-gated potassium channels. It was not until expression cloning techniques were employed that the first cDNAs were isolated (Ho *et al.*, 1993; Kubo *et al.*, 1993) and a substantial gene family with seven subfamilies was revealed (see Nichols and Lopatin, 1997). The pore-forming α subunits have two transmembrane domains with an intracellular N- and C-terminus. The only significant area of homology with the voltage-gated family was the pore-forming H5 segment responsible for potassium selectivity. Initially, however, homology cloning approaches did not elucidate an obvious candidate for the K_{ATP} channel that functioned as expected in a heterologous expression system. A critical missing component was revealed as the sulphonylurea receptor (SUR) (Aguilar Bryan *et al.*, 1995). Co-expression of the $K_{IR}6.0$ family of inwardly rectifying potassium channels with the SUR reconstituted the K_{ATP} channel (Inagaki *et al.*, 1995a,c). It became apparent that there were two isoforms of $K_{IR}6.0$ ($K_{IR}6.1$ and $K_{IR}6.2$) and two variants of SUR (SUR1 and SUR2 with two splice variants SUR2A and SUR2B) (Inagaki *et al.*, 1996; Isomoto *et al.*, 1996; Yamada *et al.*, 1997). SUR is a member of the ATP-binding cassette (ABC) family of protein (Linton and Higgins, 2007). It is most closely related to the multidrug resistant-related proteins and they are now all classified in the ABCC family (Toyoda *et al.*, 2008). Characteristically, SUR has 17 transmembrane segments grouped into three domains comprised of five (TMD0), six (TMD1) and six (TMD2) helices respectively. The N-terminus is extracellular and each of these domains is connected by intracellular linkers and finally an intracellular C-terminus. The TMD1-TMD2 and C-terminus contain nucleotide-binding domains (NBDs) with Walker A and Walker B motifs and linker regions indicative of ATP binding and hydrolysis (Linton and Higgins, 2007). This topology is well supported by experimental data (Conti *et al.*, 2001). At the genomic level, the genes encoding SUR1 and $K_{IR}6.2$ and SUR2 and $K_{IR}6.1$ are adjacent to one another on 11p15.1 and 12p12.1, respectively, and this arrangement suggests a coordinated regulation of the SUR and $K_{IR}6.0$ subunit (Inagaki *et al.*, 1995a,b; Chutkow *et al.*, 1996). The mature K_{ATP} channel complex is a hetero-octamer of four $K_{IR}6.0$ subunits and four SUR subunits (Clement *et al.*, 1997; Shyng and Nichols, 1997). A diagram of K_{ATP} channel assembly is shown in Figure 1.

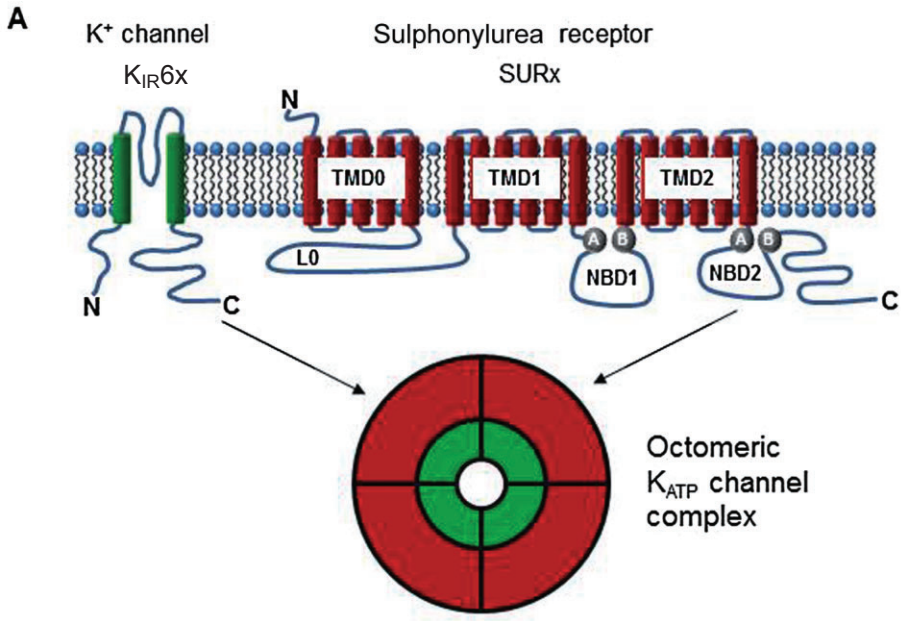
SUR2A\ $K_{IR}6.2$ underlies the cardiac K_{ATP} channel present in ventricular muscle and SUR2B\ $K_{IR}6.1$ that in smooth muscle. However, there are qualifications to this simplified picture. $K_{IR}6.1$ is ubiquitously expressed and thus there exists the potential for heteromultimerization with $K_{IR}6.2$. Indeed, this can be demonstrated with heterologous expression (Cui *et al.*, 2001) and it might occur potentially in the cardiac conduction system (Yoshida *et al.*, 2004; Bao *et al.*, 2011b). The issue of whether different SUR subunits can heteromultimerize is more controversial but practically there are not many occasions where more than one isoform is expressed and the evidence favours homomultimers (Giblin *et al.*, 2002; Tricarico *et al.*, 2006; Cheng *et al.*, 2008). Secondly, the composition of the channels may show subtle but important anatomical variations. For example, K_{ATP} in atrial cardiac myocytes is constituted by SUR1\$K_{IR}6.2$ and this differential tissue distribution may open the door for selective pharmacological manipulation in the heart in atrial fibrillation (Flagg *et al.*, 2008). Finally, most smooth muscle K_{ATP} channels have been known to be functionally different for some time. Some of these channels have a lower single-channel conductance (~35 pS), an absolute dependence on the provision of nucleotide diphosphates for activity ('K_{NDP}') and are less sensitive to ATP inhibition (Beech *et al.*, 1993). These properties were reproduced by the co-expression of SUR2B and $K_{IR}6.1$ subunits in heterologous systems (Yamada *et al.*, 1997; Cui *et al.*, 2002). However, $K_{IR}6.2$ alone or together with $K_{IR}6.1$ and SUR2B might participate in some vascular beds and in non-vascular smooth muscle (Teramoto *et al.*, 2009).

The octameric nature of the channel complex has led to interesting structure-function questions. This area has been reviewed extensively (Rodrigo and Standen, 2005; Flagg *et al.*, 2010). The continuing crystallization of K^+ channels is likely to enrich and supersede much of this work. A high resolution structure of the K_{ATP} channel complex or that of the $K_{IR}6.0$ pore-forming subunit alone has not yet been reported. A low resolution cryoEM study was possible and showed a compact structure with the four SUR1 subunits interacting with $K_{IR}6.0$ in the cytosolic and membrane domains (Mikhailov *et al.*, 2005). One interesting feature was a cleft between the SUR1 subunits by which ATP could access its binding site. The crystal structure of $K_{IR}2.2$, with and without phosphatidyl 4,5-bisphosphate (PIP_2), offers insight into potential gating mechanisms that might be revealed by high-resolution K_{ATP} channel structures (Hansen *et al.*, 2011). The most recent structure of the $K_{IR}3.0$ family of channels shows that these channels possess two gates, one as described for $K_{IR}2.2$ and another in the C-terminus, potentially gated by G-protein βγ subunits. Both gates need to be open to exhibit channel activity (Whorton and MacKinnon, 2011).

Metabolic regulation and mitochondrial K_{ATP} channels

One of the defining features of K_{ATP} channels is their sensitivity to metabolic changes. The inhibition by ATP is determined by the $K_{IR}6.0$ subunit and site-directed mutagenesis has identified key residues in the C- and N-terminus of the $K_{IR}6.2$, in particular R50, C166, I167, T171 and K185 (Tucker *et al.*, 1997; 1998), and this work underpins a detailed structural model (Antcliff *et al.*, 2005). $K_{IR}6.1$ may also have more substantial ATP sensitivity than is generally appreciated (Babenko and Bryan, 2001), though it may depend on the recording configuration and cellular environment (Cui *et al.*, 2002). It is certainly true that both $K_{IR}6.1$ and $K_{IR}6.2$ containing channel complexes are sensitive to metabolic poisoning (Farzaneh and Tinker, 2008).

The issue of how SUR interacts with nucleotides is complex and not fully resolved. Early work showed that the SUR subunit endows the channel complex with sensitivity to



B

Subunit composition	Tissue	Reference
SUR2A/K_{IR}6.2	Cardiac (ventricles)	Inagaki et., 1996
SUR1/K_{IR}6.2	Cardiac (atria)	Flagg *et al*., 2008
SUR2B/K_{IR}6.1/K_{IR}6.2	Cardiac conduction system	Bao *et al*., 2011b
SUR2B/K_{IR}6.1	Vascular smooth muscle	Yamada et al., 1997

Figure 1

Molecular basis of the K_{ATP} channel. (A) K_{ATP} channels are composed of K_{IR}6x (6.1 or 6.2) and SUR subunits. A tetrameric arrangement of K_{IR}6x subunits forms the channel pore, with each subunit comprised of two transmembrane domains (M1 and M2) with intracellular N- and C-terminus and a pore-forming H5 region with the K+ selectivity sequence. SUR has 17 transmembrane segments split into three domains, TMD0-2. TMD0 and L0 interact and modulate gating of K_{IR}6. TMD1-2 and the C-terminus contain the NBD1 and NBD2 with Walker A and B motifs where ATP binding and hydrolysis take place. SUR is also the pharmacological target of K_{CO} compounds such as pinacidil and diazoxide, and sulphonylurea drugs, such as glibenclamide and tolbutamide. The mature K_{ATP} channel is a hetero-octameric structure of K_{IR}6x and SURx subunits. (B) Tissue-specific composition of K_{ATP} channels in the cardiovascular system.

activation by MgADP, and this is a function of the NBDs (Gribble *et al*., 1997; Shyng *et al*., 1997). NBDs are asymmetric in function with NBD2 binding and hydrolysing MgATP rapidly while NBD1 binds ATP even in the absence of Mg^{2+} and hydrolyses it more slowly (Ueda *et al*., 1997; Bienengraeber *et al*., 2000). Furthermore, experiments using vanadate and beryllium which mimic the post- and pre-hydrolytic states, respectively, support the idea that ATP hydrolysis at NBD2 is needed for channel activation (Zingman *et al*., 2001). However, in inside-out patches, MgADP potently activates the channel, suggesting the activated state is directly accessible without prior hydrolysis. More recent work supports the idea that hydrolysis at NBD2 may not be necessary for activation (Ortiz *et al*., 2013). Another feature that has not been resolved is the potential dimerization of the NBDs during this cycle. In other ABC transporters, dimerization is a necessary prerequisite for ATP hydrolysis (Linton and Higgins, 2007). Thus, there are some outstanding questions in this complex mechanism. In the intact cell, studies have revealed the interaction of K_{ATP} channels with enzymes involved in cell metabolism. The cardiac channel complex (K_{IR}6.2\SUR2A) is able to directly interact with adenylate kinase, creatinine kinase and lactate dehydrogenase (Carrasco *et al*., 2001; Crawford *et al*., 2002a,b). These interactions may make the channel sensitive to small changes in cytoplasmic ATP within the cell and to ATP derived from glycolysis (Weiss and Lamp, 1987).

There have been suggestions that K_{ATP} channels are also present in mitochondria ('mitoK_{ATP}') (Inoue *et al*., 1991; Paucek *et al*., 1992). In the first study, channel activity was directly assayed to demonstrate a 10 pS channel inhibited by ATP and glibenclamide. Subsequent work revealed a possibly unique pharmacology in that mitoK_{ATP} was inhibited by 5-hydroxydecanoate and activated by diazoxide, properties not shared by the cardiac sarcolemmal channel (Grover and Garlid, 2000). However, these distinguishing features are no longer so clear cut (Li *et al*., 2010). The most convincing approach would be to directly clone the subunits. Are the known K_{IR}6.0 and\or SUR subunits the molecular equivalent of mitoK_{ATP}? A number of studies have proposed that K_{IR}6.1 might be a mitoK_{ATP} subunit specifically showing a mitochondrial localization or comparable pharmacology (Suzuki *et al*.,

1997; Liu *et al.*, 2001); however, the commercial antibodies may be detecting other unrelated proteins in mitochondria (Foster *et al.*, 2008). If $K_{IR}6.1$ does not underlie mitoK_{ATP} channels what are the other possibilities? One group isolated a complex of a succinate dehydrogenase, mitochondrial ABC protein 1, phosphate carrier, adenine nucleotide translocator and ATP synthase (Ardehali *et al.*, 2004). However, none of these proteins contain the canonical elements in the H5 segment of the established potassium channels and structures. Furthermore, some of the drugs used seem to have effects or are metabolized in other pathways (Das *et al.*, 2003; Hanley *et al.*, 2005). The most recent work in this area may address some of these issues (Foster *et al.*, 2012). These authors isolated $K_{IR}1.2$ from the inner mitochondrial membranes of bovine heart and cell imaging confirmed the mitochondrial localization of $K_{IR}1.2$, albeit after heterologous expression. In pharmacological studies, overexpression of $K_{IR}1.2$ along with the use of shRNA to silence the protein, implicated this channel subunit as underlying mitoK_{ATP} and having a role in cellular protection. These are potentially exciting findings but await confirmation and validation using more *in vivo* approaches.

Regulation through cell signalling pathways

K_{ATP} channels have a tendency to run down in ATP-free solutions and the channel activity can be 'refreshed' with low concentrations of MgATP. This dependence was not understood until channel activity was shown to be absolutely dependent on membrane phosphoinositides, in particular PIP_2 (Hilgemann and Ball, 1996; Fan and Makielski, 1997; Shyng and Nichols, 1998). The ATP is needed for the synthesis of plasma membrane PIP_2 via PI kinases and the PIP_2 antagonizes ATP inhibition and leads to channel opening (Shyng and Nichols, 1998; Pratt *et al.*, 2011). Direct evidence for the involvement of membrane PIP_2 has been obtained using an elegant binary membrane recruitment system and this works for K_{ATP} channel regulation also (Suh *et al.*, 2006; Quinn *et al.*, 2008). There is likely to be a direct interaction with the protein as the channel, $K_{IR}2.1$ and $K_{IR}2.2$ in this case, can be purified and reconstituted in liposomes of fixed lipid composition (D'Avanzo *et al.*, 2010). Furthermore, recent crystal structures show PIP_2 binding in the homologous $K_{IR}2.2$ leads to C-terminal domain translocation to interact with the transmembrane domain and this change opens the helix bundle gate which occludes the lower pore.

In smooth muscle, downstream activation of PKA through various receptors coupled to the stimulatory G-protein G_s, such as adenosine A_2, β adrenoreceptors, calcitonin gene-related peptide and prostacyclin, leads to vasodilatation. A major contribution to the vascular smooth muscle (VSM) cell hyperpolarization is the opening of K_{ATP} channels (Standen *et al.*, 1989; Nelson *et al.*, 1990; Rodrigo and Standen, 2005). Subsequent molecular studies on $K_{IR}6.1$\SUR2B revealed that this was likely due to the direct phosphorylation of both channel subunits (T633, S1387 and S1465 on SUR2B; S385 on $K_{IR}6.1$) (Quinn *et al.*, 2004; Shi *et al.*, 2007). Further regulation may occur through dephosphorylation of these residues via the Ca^{2+}-dependent phosphatase calcineurin (Wilson *et al.*, 2000; Orie *et al.*, 2009). The receptor\PKA\K_{ATP} axis may be selectively localized within membrane compartments of the cell. Thus, there is evidence that PKA is largely present in a particulate fashion through interaction with A-kinase anchoring proteins (Hayabuchi *et al.*, 2001a). Furthermore, the channel complex may be localized to caveolae and this may be important for signalling (Sampson *et al.*, 2004; 2007; Davies *et al.*, 2010).

Vasoconstrictors, such as angiotensin II and endothelin-1, activate PKC and there is evidence that K_{ATP} channel activity can be modulated through such pathways (Kubo *et al.*, 1997; Cole *et al.*, 2000; Hayabuchi *et al.*, 2001b; Thorneloe *et al.*, 2002; Quinn *et al.*, 2003; Sampson *et al.*, 2007). The regulation is Ca^{2+} independent and mediated by PKCε (Hayabuchi *et al.*, 2001b; Quinn *et al.*, 2003). Direct channel phosphorylation of $K_{IR}6.1$ is likely responsible with a cluster of serine residues in the distal C-terminus playing a key role (Quinn *et al.*, 2003; Shi *et al.*, 2008). There may also be effects on channel internalization and recycling perhaps via caveolae (Jiao *et al.*, 2008). Furthermore, vasoconstrictors may inhibit PKA as this would act as an additional inhibitory input to K_{ATP} channels (Hayabuchi *et al.*, 2001b). One mechanism that has been little explored is the role of PIP_2 depletion in addition to PKC activation. However, it should be noted that the modulation is essentially abolished by PKC inhibitors and that $K_{IR}6.1$ seems to have a relatively high affinity for PIP_2, suggesting channel activity may be maintained in the face of substantial depletion (Quinn *et al.*, 2003; Sampson *et al.*, 2007). It should be emphasized that PKC-dependent modulation of VSM K_{ATP} channel activity is only likely to account for a part of the action of vasoconstrictors.

A group of endothelial mediators – the so-called gasotransmitters – may also influence VSM K_{ATP} channel function (Zhao *et al.*, 2001; Mustafa *et al.*, 2009b). The action of NO is of course well known but the endothelium also generates two other gasotransmiters, CO and H_2S. H_2S is produced largely by cystathionine γ-lyase and production is regulated by the activation of Ca^{2+}-calmodulin, in a manner analogous to that of NO (Yang *et al.*, 2008). The effector mechanism is thought to be the activation of K_{ATP} channels in VSMs through direct modification of channel cysteines, though other mechanisms are possible (Zhao *et al.*, 2001; Cheng *et al.*, 2004; Mustafa *et al.*, 2009a).

Thus, the vascular K_{ATP} channel and the cloned equivalent $K_{IR}6.1$\SUR2B are subject to prominent hormonal regulation through direct subunit phosphorylation. What is known about the regulation of $K_{IR}6.2$ complexes particularly in cardiac muscle? This issue is particularly pertinent for PKC modulation as this has been implicated as being central in cellular protection and preconditioning in cardiac cells (Yellon and Downey, 2003). In early studies, PKC modulation was thought to activate sarcolemmal cardiac K_{ATP}; however, it now appears that there is a biphasic regulation with activation followed by a slower inhibitory response corresponding to channel internalization (Light *et al.*, 1996; Hu *et al.*, 2003). The phenomena are critically dependent on the prevailing conditions of study. We thought for some time that PKC activation did not modulate $K_{IR}6.2$ (Quinn *et al.*, 2003). However, in intact cells and with higher pipette Ca^{2+} in whole cell recordings, we did see biphasic modulation. The inhibitory response was due to channel internalization and

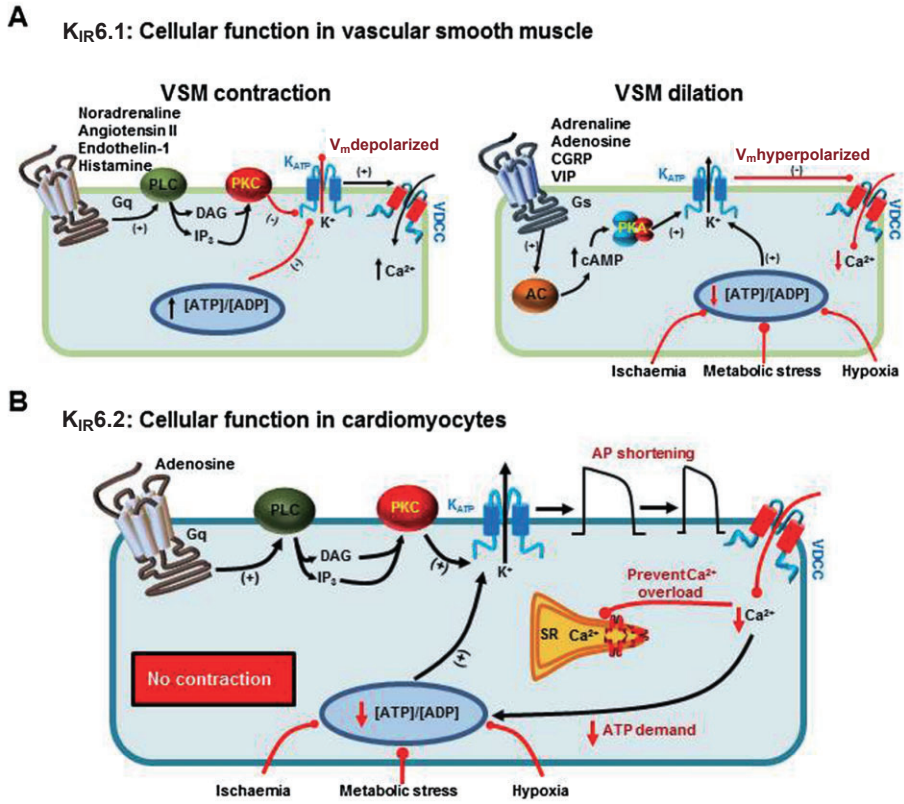


Figure 2

Functional roles of $K_{IR}6.1$ and $K_{IR}6.2$ in the cardiovascular system. (A) K_{ATP} channels comprising $K_{IR}6.1$ in vascular smooth muscle (VSM) cells regulate vascular tone by controlling the membrane potential and subsequently the influx of Ca^{2+} through L-type voltage-dependent Ca^{2+} channels. K_{ATP} channel activity in VSM can be modulated by the PKC (inhibitory) and PKA (activation) signalling pathways and metabolic stress such as hypoxia and ischaemia. (B) $K_{IR}6.2$-containing K_{ATP} channels are predominant in cardiomyocytes, where they are involved in AP repolarization. Activation of K_{ATP} by PKC or metabolic insults such as ischaemia and/or hypoxia leads to shortening of AP duration, decreased influx of Ca^{2+} and reduced contractility, thus preventing Ca^{2+} overload and ATP preservation.

occurred because of phosphorylation of S372 in $K_{IR}6.2$ (Aziz *et al.*, 2012). The regulation of sarcolemmal K_{ATP} channels by PKA has been little studied. With $K_{IR}6.2$\SUR1, PKA phosphorylation leads to increased channel activity through residues in SUR1 and $K_{IR}6.2$ that are homologous to those in SUR2B and $K_{IR}6.1$ (Gonoi *et al.*, 1999; Lin *et al.*, 2000; Quinn *et al.*, 2004). Indeed, a plausible *a priori* case could be made for a contribution of sarcolemmal cardiac K_{ATP} in the action potential (AP) shortening occurring with the increased heart rate in exercise, in addition to the slowly activating component of the delayed rectifier current. One interesting feature is the subcellular localization of K_{ATP} channels that appear to be concentrated at the neck of the T-tubule, suggesting activation could have a significant influence on excitation-contraction coupling (Korchev *et al.*, 2000).

Pathophysiological function of K_{ATP} channels in the cardiovascular system

Generally, three types of study contribute to the understanding of the physiological role of K_{ATP} channels: *ex vivo* and *in vivo* pharmacological studies, functional studies in genetically engineered mice, and human genetics (Figure 2).

Cardiac protection

Paradoxically, there has been substantial focus on the role of the cardiac sarcolemmal K_{ATP} channel in pathobiology (see below), without really addressing the issue of what physiological role the channel might perform in ventricular myocytes. The use of mice with global genetic deletion of $K_{IR}6.2$ ($K_{IR}6.2$ null) has suggested some intriguing possibilities. These animals have an attenuated ability to perform high-intensity exercise and are predisposed to catecholamine cardiotoxicity (Zingman *et al.*, 2002). However, it is not yet clear if it is the deletion in cardiac myocytes that is critical. Channel function will also be impaired in skeletal muscle, pancreatic beta cells and in central neurons, and all of these could have an influence on the integrated physiological function. Mice with cardiac overexpression of a dominant negative $K_{IR}6.2$ subunit had a proarrhythmic phenotype and impaired exercise tolerance (Tong *et al.*, 2006). The study of mice with conditional deletion of $K_{IR}6.2$. (and SUR2) in cardiac myocytes is likely to be highly informative.

Sarcolemmal K_{ATP} channels are essentially closed under normal metabolic conditions and hence are thought not to contribute towards the coupling of membrane excitation and contraction in the basal physiological state. In keeping with this, studies in ventricular myocytes from $K_{IR}6.2$ null mice show that AP duration and contractile function are normal (Suzuki *et al.*, 2001). However, exposure to severe metabolic insults such as during hypoxia and ischaemia leads to opening of cardiac K_{ATP} channels. Metabolic stress leads to substantial shortening of the AP and attenuated contraction (Lederer *et al.*, 1989; Venkatesh *et al.*, 1991), and these effects are absent when $K_{IR}6.2$ is not present (Li *et al.*, 2000), or in the presence of a K_{ATP} channel blocker (Venkatesh *et al.*, 1991). Primarily, opening of K_{ATP} channels is likely to be a protective mechanism because the increase in K^+ conductance stabilizes the resting membrane potential, shortens the AP and reduces Ca^{2+} influx, resulting in the conservation of intracellular energy stores and preventing calcium overload. In support of this proposal, application of the K_{CO} pinacidil enhanced ischaemia-induced AP shortening, early contractile failure and preserved ATP levels (McPherson *et al.*, 1993), and in hearts from $K_{IR}6.2$ null mice, contraction was prolonged and AP duration unaffected during ischaemia (Suzuki *et al.*, 2002). In addition, pinacidil activation of sarcolemmal K_{ATP} channels reduced reperfusion-induced Ca^{2+} overload in cardiac myocytes, and ablation of K_{ATP} channels *in vivo* gives rise to a greater susceptibility to Ca^{2+} overload and impairs contractile recovery. Moreover, exercise causes significant remodelling of cardiac K_{ATP} channels. Specifically, exercise induces an increase in K_{ATP} channel expression (~40%) in mouse ventricles promoting AP shortening in response to an increased heart rate, and these effects are abolished when non-functional K_{ATP} channels are transgenically expressed (Zingman *et al.*, 2011). Interestingly, overexpression of SUR2A in cardiac tissue leads to a phenotype protected from ischaemia (Du *et al.*, 2006). An intracellular pool of K_{ATP} channels may serve as a reservoir to modulate membrane surface density in stress conditions (Bao *et al.*, 2011a).

There is a substantial body of work proposing that mitoK_{ATP} may also have a role in cardioprotection and this has been reviewed in detail elsewhere (Yellon and Downey, 2003). It is worth stating that there are persuasive data favouring the involvement of sarcolemmal cardiac K_{ATP} channels in these phenomena, at least in mice. In $K_{IR}6.2$ null mice, the protective effect of ischaemic preconditioning was abolished and recovery of contractile function was compromised (Suzuki *et al.*, 2002; 2003; Gumina *et al.*, 2003). Moreover, the preconditioning effect of diazoxide was also absent (Suzuki *et al.*, 2003).

Cardiac arrhythmia

The initial opening of K_{ATP} channels in response to a metabolic insult is cardioprotective; however, activation of K_{ATP} channels also induces early repolarization, thereby shortening the QT interval and reducing the refractory period, hence predisposing to re-entrant arrhythmias. Typical ECG changes observed during ischaemic insults include ST elevation or depression suggesting changes in repolarization. These ischaemia-induced changes in ECG characteristics are ameliorated by the K_{ATP} channel blocker glibenclamide and induced by the K_{CO} pinacidil (in the absence of ischaemia) consistent with K_{ATP} channel activation underlying these features (Kubota *et al.*, 1993). In support, $K_{IR}6.2$ null mice are not prone to ST elevation in response to ischaemia (Li *et al.*, 2000). However, ischaemia-induced ST elevation was observed in SUR2 null mice (Chutkow *et al.*, 2002). $K_{IR}6.1$ null mice also have episodes of cardiac ischaemia accompanied by ST elevation (Miki *et al.*, 2002). This was originally postulated to be due to the absence of VSM K_{ATP} channels; however, it is likely this is not strictly the case as when SUR2B was selectively re-introduced to smooth muscle in SUR2 null mice, the phenotype persisted (Kakkar *et al.*, 2006). These data suggest that $K_{IR}6.1$ in heart may also be involved in early repolarization and ischaemia-induced arrhythmia and is consistent with recent studies showing heterogeneity of K_{ATP} subunit composition in different regions of the heart (Flagg *et al.*, 2008; Bao *et al.*, 2011b). Early repolarization patterns in the ECG has historically been considered to be of little consequence and is commonly observed in healthy males and athletes. However, there is now evidence to suggest that the early repolarization pattern ('J wave syndromes') may be associated with increased risk of ventricular fibrillation (Antzelevitch, 2012). A clear association of K_{ATP} channels with early repolarization syndromes was made when Haissaguerre *et al.* (2009) found a rare variant of KCNJ8 ($K_{IR}6.1$) in a patient with idiopathic ventricular fibrillation and prominent early repolarization. In further independent studies, the same missense mutation of a highly conserved serine to leucine (S422L) was discovered in five more patients (Medeiros-Domingo *et al.*, 2010; Barajas-Martinez *et al.*, 2012). Both studies showed an increased current density when $K_{IR}6.1$-S422L was co-expressed heterologously with SUR2A. Mechanistically, this gain of function in the mutant channel can be explained by the decreased ATP sensitivity of $K_{IR}6.1$-S422L channels compared with wild-type (WT) channels (Barajas-Martinez *et al.*, 2012).

Most studies of cardiac arrhythmias resulting from myocardial ischaemia have been focused predominantly on the abnormalities of ventricular rhythm and relatively little is known about the role of K_{ATP} channels in abnormal atrial rhythm. A recent study has shown that activation of K_{ATP} channels by β-adrenoceptor-induced metabolic stress provides a substrate for atrial tachyarrhythmias in mouse isolated heart (Kim *et al.*, 2012). In support, pinacidil shortens atrial AP duration and increases arrhythmia inducibility in human right atrial and right ventricular wall (Fedorov *et al.*, 2011). Furthermore, atrial electrical remodelling and increased arrhythmia inducibility in a murine model of salt-induced hypertension have been shown to be associated with increased K_{ATP} current and SUR1 expression (Lader *et al.*, 2011). A recent screening of patients with atrial fibrillation found two patients with the $K_{IR}6.1$-S422L variant (Delaney *et al.*, 2012).

Heart failure, hypertrophy and cell swelling

Cardiac hypertrophy is triggered by a prolonged increase in cardiac workload. When transverse aortic constriction was applied in $K_{IR}6.2$ null mice or in mice with cardiac specific overexpression of SUR1 which paradoxically disrupts cardiac sarcolemmal K_{ATP} channel function, increased left ventricular hypertrophy was observed (Yamada *et al.*, 2006; Hu *et al.*, 2008). Interestingly, there seems to be an interaction between

cardiac K_{ATP} channel expression and the activity of the PPAR-γ coactivator, PGC-1α. Decreased channel function leads to decreased activity at the PGC-1α promoter partly via FOXO-1 repression (Hu *et al.*, 2008). Remodelled ventricular cardiomyocytes from rats subjected to coronary occlusion show up-regulation of $K_{IR}6.1$, especially around the infarct zone (Isidoro *et al.*, 2007). Congestive heart failure or infarction in human hearts leads to an increased AP duration and sensitivity to potassium channel openers in both atria and ventricles (Fedorov *et al.*, 2011). There are a variety of other molecules/enzymes that have been shown to be involved in the progression from compensated hypertrophy to heart failure possibly by their interactions with K_{ATP} channels. Angiotensin II and TNF-α expression is positively correlated to that of $K_{IR}6.1$ in failing rat myocardium or cultured cardiomyocytes and negatively correlated with $K_{IR}6.2$ (Isidoro *et al.*, 2009). Furthermore, cardiomyocytes treated with these were responsive to diazoxide, indicating increased expression of $K_{IR}6.1$/SUR2B in these cells as part of the progression to hypertrophy (Isidoro *et al.*, 2007; 2009).

Various mutations have been identified within K_{ATP} channel subunits which confer susceptibility to cardiomyopathy, hypertrophy and heart failure. A cohort of patients with dilated cardiomyopathy allowed the identification of a frameshift mutation leading to a premature stop codon at Leu1524 and a missense mutation A1513T in SUR2A. Both mutations are located in the NBD2 and compromise the ability of ATP to be hydrolysed (Bienengraeber *et al.*, 2004). In $K_{IR}6.2$, a non-synonymous polymorphism leading to the coding change, E23K, was identified in 18% of heart failure patients (Reyes *et al.*, 2009) and is also known to lead to an increased risk of type 2 diabetes (Gloyn *et al.*, 2003). Both heterozygous and homozygous patients have the same resting heart rates and show similar degrees of left ventricular dysfunction and remodelling. When these homozygous patients are exercised, they show a reduced heart rate, oxygen consumption and peak VO_2. Other missense mutations in SUR2A within transmembrane domains have also been identified to cause activation of the channel in the rare Cantu syndrome, characterised by cardiac hypertrophy and cardiomegaly (Harakalova *et al.*, 2012). Although the human and murine experimental data are very different in nature, there seems to be inconsistency in that both reduction and increases in K_{ATP} channel activity, can result in cardiac hypertrophy.

Excessive changes in cell volume in the heart can result in the alteration of the structural integrity of the cells affecting cellular functions and cell death. These changes can arise as a result of an intracellular accumulation of metabolites that increase cellular osmolality, allow water to enter the cell, increase the cell volume and alter ion channel function. Reduction of cardiomyocyte swelling during myocardial ischaemia may be a potential mechanism of cardioprotection (Shi *et al.*, 2009). K_{ATP} has been shown to be regulated during cell volume changes with atrial K_{ATP} channels opening in response to cell swelling leading to AP shortening (Saegusa *et al.*, 2005). The absence of $K_{IR}6.2$ in cardiac myocytes isolated from $K_{IR}6.2$ null mice prevents cell swelling from occurring whereas in WT mice there is exaggerated cell swelling which can be disrupted by the addition of diazoxide (Prasad *et al.*, 2006). The use of diazoxide in some of the studies appears to be more complicated. Although cell swelling could be diminished by the addition of diazoxide, the addition of HMR1098 and 5-hydroxydecanoate did not reverse the events initially suggesting that diazoxide may be acting via a mechanism separate from the activation of K_{ATP} channels (Maffit *et al.*, 2012).

Vascular reactivity and hypertension

The modulation of VSM K_{ATP} currents by vasoactive agents suggests the channel may be important for blood pressure control. In VSM cells from $K_{IR}6.1$ and SUR2 null mice K_{ATP} currents were absent, whereas cells from $K_{IR}6.2$ null mice exhibited normal K_{ATP} currents (Suzuki *et al.*, 2001; Chutkow *et al.*, 2002; Miki *et al.*, 2002). As well as providing direct evidence for the molecular composition of the VSM K_{ATP} channel, $K_{IR}6.1$ and SUR2 null mice exhibit hypercontractility of the coronary vasculature and are prone to early sudden death due to coronary artery spasm (Chutkow *et al.*, 2002; Miki *et al.*, 2002). SUR2 null mice also show focal narrowing of the coronary arteries and have significantly elevated blood pressure (Chutkow *et al.*, 2002). Interestingly, restoration of the vascular K_{ATP} channel in SUR2 null mice does not protect against a rise in baseline coronary artery perfusion pressure suggesting a role for K_{ATP} channels from tissues other than VSM (Kakkar *et al.*, 2006). Specifically, SUR2B RNA has been detected in endothelium and it is believed that heteromeric $K_{IR}6.1$/ $K_{IR}6.2$ in combination with SUR2B could form an endothelial K_{ATP} channel (Yoshida *et al.*, 2004). In mice expressing endothelium-specific dominant-negative $K_{IR}6.1$ subunits, basal coronary perfusion pressure and ET-1 concentrations were substantially elevated suggesting a role for endothelial K_{ATP} channels in the regulation of vascular tone (Malester *et al.*, 2007).

Further evidence for the role of VSM K_{ATP} channels in vascular tone regulation comes from studies of hypertensive animal models where there is substantial remodelling of K_{ATP} channels in vascular beds (Blanco-Rivero *et al.*, 2008; Tajada *et al.*, 2012). K_{ATP} channels in hypertensive phenotypes show altered vascular reactivity probably as a result of impaired and fewer K_{ATP} channels. VSM cells from hypertensive animals are significantly depolarized and K_{CO} compounds have little effect on membrane potential compared with normotensive animals (Tajada *et al.*, 2012). The K_{CO} iptakalim has been put forward as a promising anti-hypertensive agent for mild to moderate essential hypertension (Sikka *et al.*, 2012). Interestingly, on a mechanistic level iptakalim has also been shown to inhibit ET-1 release and synthesis and increase NO release and NOS activity in aortic endothelial cells (Gao *et al.*, 2009). Additionally, there are data to suggest that K_{CO} compounds such as iptakalim have therapeutic potential as a treatment for pulmonary hypertension (Sikka *et al.*, 2012).

Sepsis

The role of K_{ATP} channels in sepsis is complex (Buckley *et al.*, 2006). There is pharmacological evidence that channel activation occurs in septic shock leading to hypotension and this can be reversed by glibenclamide (Matsuda and Hattori, 2007). There is also evidence for increased activity of these channels but that their pharmacology alters such that they becomes unresponsive to sulphonylureas and only direct

pore blockers can inhibit activity (O'Brien *et al.*, 2009), and this is consistent with the lack of clinical efficacy (Warrillow *et al.*, 2006; Morelli *et al.*, 2007). However, animals, and also flies, with global genetic deletion of the channel are predisposed to an early and substantial survival disadvantage in sepsis (Kane *et al.*, 2006; Croker *et al.*, 2007). Furthermore, the expression of $K_{IR}6.1$ is regulated via Toll-like receptors and NF-κB and the increase in expression of the current is postulated to underlie the poor response to vasoconstrictors in septic shock (Shi *et al.*, 2010). The exact mechanism for the survival disadvantage is unclear but inappropriate coronary artery vasoconstriction during increased cardiac demand is one proposal. However, the pathophysiological circulatory changes in severe sepsis are actually profound and widespread. These include hypotension, hyporesponsiveness to vasoconstrictors, microvascular dysfunction, endothelial dysfunction, and increased vascular and capillary permeability (Matsuda and Hattori, 2007). The absence of $K_{IR}6.1$ in both smooth muscle and endothelium may promote these adaptations.

Conclusions

The physiological role of K_{ATP} channels is well defined in the pancreatic beta cell. Recent work has begun to reveal similar pathophysiological importance in the function of cardiac muscle, specialized conduction tissues in the heart and of VSM (Figure 2). These channels have a rich existing pharmacology that could be exploited to develop novel therapeutic agents for the treatment of cardiovascular disease.

Acknowledgements

This work was supported by the British Heart Foundation, Wellcome Trust, Medical Research Council and the National Institute for Health Research Barts Cardiovascular Biomedical Research.

Conflict of interest

The authors have no conflicts of interest to declare.

References

Aguilar Bryan L, Nichols CG, Wechsler SW, Clement JP, Boyd AE, Gonzalez G *et al.* (1995). Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 268: 423–426.

Alexander SPH *et al.* (2013). The Concise Guide to PHARMACOLOGY 2013/14: Overview. Br J Pharmacol 170: 1449–1867.

Antcliff JF, Haidar S, Proks P, Sansom MS, Ashcroft FM (2005). Functional analysis of a structural model of the ATP-binding site of the KATP channel Kir6.2 subunit. EMBO J 24: 229–239.

Antzelevitch C (2012). Genetic, molecular and cellular mechanisms underlying the J wave syndromes. Circ J 76: 1054–1065.

Ardehali H, Chen Z, Ko Y, Mejia-Alvarez R, Marban E (2004). Multiprotein complex containing succinate dehydrogenase confers mitochondrial ATP-sensitive K+ channel activity. Proc Natl Acad Sci U S A 101: 11880–11885.

Ashcroft FM (1988). Adenosine 5′-triphosphate-sensitive potassium channels. Annu Rev Neurosci 11: 97–118.

Ashcroft FM, Harrison DE, Ashcroft SJ (1984). Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. Nature 312: 446–448.

Ashford ML, Sturgess NC, Trout NJ, Gardner NJ, Hales CN (1988). Adenosine-5′-triphosphate-sensitive ion channels in neonatal rat cultured central neurones. Pflugers Arch 412: 297–304.

Aziz Q, Thomas AM, Khambra T, Tinker A (2012). Regulation of the ATP-sensitive potassium channel subunit, Kir6.2, by a Ca2+-dependent protein kinase C. J Biol Chem 287: 6196–6207.

Babenko AP, Bryan J (2001). A conserved inhibitory and differential stimulatory action of nucleotides on K(IR)6.0/SUR complexes is essential for excitation- metabolism coupling by K(ATP) channels. J Biol Chem 276: 49083–49092.

Bao L, Hadjiolova K, Coetzee WA, Rindler MJ (2011a). Endosomal KATP channels as a reservoir after myocardial ischemia: a role for SUR2 subunits. Am J Physiol Heart Circ Physiol 300: H262–H270.

Bao L, Kefaloyianni E, Lader J, Hong M, Morley G, Fishman GI *et al.* (2011b). Unique properties of the ATP-sensitive K(+) channel in the mouse ventricular cardiac conduction system. Circ Arrhythm Electrophysiol 4: 926–935.

Barajas-Martinez H, Hu D, Ferrer T, Onetti CG, Wu Y, Burashnikov E *et al.* (2012). Molecular genetic and functional association of Brugada and early repolarization syndromes with S422L missense mutation in KCNJ8. Heart Rhythm 9: 548–555.

Beech DJ, Zhang H, Nakao K, Bolton TB (1993). K channel activation by nucleotide diphosphates and its inhibition by glibenclamide in vascular smooth muscle cells. Br J Pharmacol 110: 573–582.

Bienengraeber M, Alekseev AE, Abraham MR, Carrasco AJ, Moreau C, Vivaudou M *et al.* (2000). ATPase activity of the sulfonylurea receptor: a catalytic function for the KATP channel complex. FASEB J 14: 1943–1952.

Bienengraeber M, Olson TM, Selivanov VA, Kathmann EC, O'Cochlain F, Gao F *et al.* (2004). ABCC9 mutations identified in human dilated cardiomyopathy disrupt catalytic KATP channel gating. Nat Genet 36: 382–387.

Blanco-Rivero J, Gamallo C, Aras-Lopez R, Cobeno L, Cogolludo A, Perez-Vizcaino F *et al.* (2008). Decreased expression of aortic KIR6.1 and SUR2B in hypertension does not correlate with changes in the functional role of K(ATP) channels. Eur J Pharmacol 587: 204–208.

Buckley JF, Singer M, Clapp LH (2006). Role of KATP channels in sepsis. Cardiovasc Res 72: 220–230.

Carrasco AJ, Dzeja PP, Alekseev AE, Pucar D, Zingman LV, Abraham MR *et al.* (2001). Adenylate kinase phosphotransfer communicates cellular energetic signals to ATP-sensitive potassium channels. Proc Natl Acad Sci U S A 98: 7623–7628.

Cheng WW, Tong A, Flagg TP, Nichols CG (2008). Random assembly of SUR subunits in K(ATP) channel complexes. Channels (Austin) 2: 34–38.

Cheng Y, Ndisang JF, Tang G, Cao K, Wang R (2004). Hydrogen sulfide-induced relaxation of resistance mesenteric artery beds of rats. Am J Physiol Heart Circ Physiol 287: H2316–H2323.

Chutkow WA, Simon MC, Le Beau MM, Burant CF (1996). Cloning, tissue expression, and chromosomal localization of SUR2, the putative drug-binding subunit of cardiac, skeletal muscle, and vascular KATP channels. Diabetes 45: 1439–1445.

Chutkow WA, Pu J, Wheeler MT, Wada T, Makielski JC, Burant CF *et al*. (2002). Episodic coronary artery vasospasm and hypertension develop in the absence of Sur2 K(ATP) channels. J Clin Invest 110: 203–208.

Clement JP, Kunjilwar K, Gonzalez G, Schwanstecher M, Panten U, Aguilar Bryan L *et al*. (1997). Association and stoichiometry of K(ATP) channel subunits. Neuron 18: 827–838.

Cole WC, Malcolm T, Walsh MP, Light PE (2000). Inhibition by protein kinase C of the K(NDP) subtype of vascular smooth muscle ATP-sensitive potassium channel. Circ Res 87: 112–117.

Conti LR, Radeke CM, Shyng SL, Vandenberg CA (2001). Transmembrane topology of the sulfonylurea receptor SUR1. J Biol Chem 276: 41270–41278.

Crawford RM, Budas GR, Jovanovic S, Ranki HJ, Wilson TJ, Davies AM *et al*. (2002a). M-LDH serves as a sarcolemmal K(ATP) channel subunit essential for cell protection against ischemia. EMBO J 21: 3936–3948.

Crawford RM, Ranki HJ, Botting CH, Budas GR, Jovanovic A (2002b). Creatine kinase is physically associated with the cardiac ATP-sensitive K+ channel in vivo. FASEB J 16: 102–104.

Croker B, Crozat K, Berger M, Xia Y, Sovath S, Schaffer L *et al*. (2007). ATP-sensitive potassium channels mediate survival during infection in mammals and insects. Nat Genet 39: 1453–1460.

Cui Y, Giblin JP, Clapp LH, Tinker A (2001). A mechanism for ATP-sensitive potassium channel diversity: functional coassembly of two pore forming subunits. Proc Natl Acad Sci U S A 98: 729–734.

Cui Y, Tran S, Tinker A, Clapp LH (2002). The molecular composition of K(ATP) channels in human pulmonary artery smooth muscle cells and their modulation by growth. Am J Respir Cell Mol Biol 26: 135–143.

D'Avanzo N, Cheng WW, Doyle DA, Nichols CG (2010). Direct and specific activation of human inward rectifier K+ channels by membrane phosphatidylinositol 4,5-bisphosphate. J Biol Chem 285: 37129–37132.

Das M, Parker JE, Halestrap AP (2003). Matrix volume measurements challenge the existence of diazoxide/glibencamide-sensitive KATP channels in rat mitochondria. J Physiol 547: 893–902.

Davies LM, Purves GI, Barrett-Jolley R, Dart C (2010). Interaction with caveolin-1 modulates vascular ATP-sensitive potassium (KATP) channel activity. J Physiol 588: 3255–3266.

Delaney JT, Muhammad R, Blair MA, Kor K, Fish FA, Roden DM *et al*. (2012). A KCNJ8 mutation associated with early repolarization and atrial fibrillation. Europace 14: 1428–1432.

Du Q, Jovanovic S, Clelland A, Sukhodub A, Budas G, Phelan K *et al*. (2006). Overexpression of SUR2A generates a cardiac phenotype resistant to ischemia. FASEB J 20: 1131–1141.

Edwards G, Weston AH (1993). The pharmacology of ATP-sensitive potassium channels. Annu Rev Pharmacol Toxicol 33: 597–637.

Fan Z, Makielski JC (1997). Anionic phospholipids activate ATP-sensitive potassium channels. J Biol Chem 272: 5388–5395.

Farzaneh T, Tinker A (2008). Differences in the mechanism of metabolic regulation of ATP-sensitive K+ channels containing Kir6.1 and Kir6.2 subunits. Cardiovasc Res 79: 621–631.

Fedorov VV, Glukhov AV, Ambrosi CM, Kostecki G, Chang R, Janks D *et al*. (2011). Effects of KATP channel openers diazoxide and pinacidil in coronary-perfused atria and ventricles from failing and non-failing human hearts. J Mol Cell Cardiol 51: 215–225.

Flagg TP, Kurata HT, Masia R, Caputa G, Magnuson MA, Lefer DJ *et al*. (2008). Differential structure of atrial and ventricular KATP: atrial KATP channels require SUR1. Circ Res 103: 1458–1465.

Flagg TP, Enkvetchakul D, Koster JC, Nichols CG (2010). Muscle KATP channels: recent insights to energy sensing and myoprotection. Physiol Rev 90: 799–829.

Foster DB, Rucker JJ, Marban E (2008). Is Kir6.1 a subunit of mitoK(ATP)? Biochem Biophys Res Commun 366: 649–656.

Foster DB, Ho AS, Rucker J, Garlid AO, Chen L, Sidor A *et al*. (2012). Mitochondrial ROMK channel is a molecular component of MitoKATP. Circ Res 111: 446–454.

Gao S, Long CL, Wang RH, Wang H (2009). K(ATP) activation prevents progression of cardiac hypertrophy to failure induced by pressure overload via protecting endothelial function. Cardiovasc Res 83: 444–456.

Giblin JP, Cui Y, Clapp LH, Tinker A (2002). Assembly limits the pharmacological complexity of ATP-sensitive potassium channels. J Biol Chem 277: 13717–13723.

Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G *et al*. (2003). Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. Diabetes 52: 568–572.

Gonoi T, Nagashima K, Nishimura M, Seino S (1999). PKA-mediated phosphorylation of the human K_{ATP} channel: separate roles of Kir6.2 and SUR1 subunit phosphorylation. EMBO J 18: 4722–4732.

Gribble FM, Tucker SJ, Ashcroft FM (1997). The essential role of the Walker A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide. EMBO J 16: 1145–1152.

Grover GJ, Garlid KD (2000). ATP-Sensitive potassium channels: a review of their cardioprotective pharmacology. J Mol Cell Cardiol 32: 677–695.

Gumina RJ, Pucar D, Bast P, Hodgson DM, Kurtz CE, Dzeja PP *et al*. (2003). Knockout of Kir6.2 negates ischemic preconditioning-induced protection of myocardial energetics. Am J Physiol Heart Circ Physiol 284: H2106–H2113.

Haissaguerre M, Chatel S, Sacher F, Weerasooriya R, Probst V, Loussouarn G *et al*. (2009). Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/KATP channel. J Cardiovasc Electrophysiol 20: 93–98.

Hanley PJ, Drose S, Brandt U, Lareau RA, Banerjee AL, Srivastava DK *et al*. (2005). 5-Hydroxydecanoate is metabolised in mitochondria and creates a rate-limiting bottleneck for beta-oxidation of fatty acids. J Physiol 562: 307–318.

Hansen SB, Tao X, MacKinnon R (2011). Structural basis of PIP2 activation of the classical inward rectifier K+ channel Kir2.2. Nature 477: 495–498.

Harakalova M, van Harssel JJ, Terhal PA, van Lieshout S, Duran K, Renkens I *et al*. (2012). Dominant missense mutations in ABCC9 cause Cantu syndrome. Nat Genet 44: 793–796.

Hayabuchi Y, Dart C, Standen NB (2001a). Evidence for involvement of A-kinase anchoring protein in activation of rat arterial K(ATP) channels by protein kinase A. J Physiol 536: 421–427.

Hayabuchi Y, Davies NW, Standen NB (2001b). Angiotensin II inhibits rat arterial KATP channels by inhibiting steady-state protein kinase A activity and activating protein kinase Ce. J Physiol 530: 193–205.

Hilgemann DW, Ball R (1996). Regulation of cardiac Na+,Ca2+ exchange and KATP potassium channels by PIP2. Science 273: 956–959.

Ho K, Nichols CG, Lederer WJ, Lytton J, Vassilev PM, Kanazirska MV *et al.* (1993). Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. Nature 362: 31–38.

Hu K, Huang CS, Jan YN, Jan LY (2003). ATP-sensitive potassium channel traffic regulation by adenosine and protein kinase C. Neuron 38: 417–432.

Hu X, Xu X, Huang Y, Fassett J, Flagg TP, Zhang Y *et al.* (2008). Disruption of sarcolemmal ATP-sensitive potassium channel activity impairs the cardiac response to systolic overload. Circ Res 103: 1009–1017.

Inagaki N, Gonoi T, Clement JP, Namba N, Inazawa J, Gonzalez G *et al.* (1995a). Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. Science 270: 1166–1170.

Inagaki N, Inazawa J, Seino S (1995b). cDNA sequence, gene structure, and chromosomal localization of the human ATP-sensitive potassium channel, uKATP-1, gene (KCNJ8). Genomics 30: 102–104.

Inagaki N, Tsuura Y, Namba N, Masuda K, Gonoi T, Horie M *et al.* (1995c). Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart. J Biol Chem 270: 5691–5694.

Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar Bryan L, Bryan J *et al.* (1996). A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K+ channels. Neuron 16: 1011–1017.

Inoue I, Nagase H, Kishi K, Higuti T (1991). ATP-sensitive K+ channel in the mitochondrial inner membrane. Nature 352: 244–247.

Isidoro TN, Philip-Couderc P, Papageorgiou I, Baertschi AJ, Lerch R, Montessuit C (2007). Expression and function of ATP-dependent potassium channels in late post-infarction remodeling. J Mol Cell Cardiol 42: 1016–1025.

Isidoro TN, Philip-Couderc P, Baertschi AJ, Lerch R, Montessuit C (2009). Angiotensin II and tumour necrosis factor alpha as mediators of ATP-dependent potassium channel remodelling in post-infarction heart failure. Cardiovasc Res 83: 726–736.

Isomoto S, Kondo C, Yamada M, Matsumoto S, Higashiguchi O, Horio Y *et al.* (1996). A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K+ channel. J Biol Chem 271: 24321–24324.

Jiao J, Garg V, Yang B, Elton TS, Hu K (2008). Protein kinase C-epsilon induces caveolin-dependent internalization of vascular adenosine 5′-triphosphate-sensitive K+ channels. Hypertension 52: 499–506.

Kakkar R, Ye B, Stoller DA, Smelley M, Shi NQ, Galles K *et al.* (2006). Spontaneous coronary vasospasm in KATP mutant mice arises from a smooth muscle-extrinsic process. Circ Res 98: 682–689.

Kane GC, Lam CF, O'Cochlain F, Hodgson DM, Reyes S, Liu XY *et al.* (2006). Gene knockout of the KCNJ8-encoded Kir6.1 K(ATP) channel imparts fatal susceptibility to endotoxemia. FASEB J 20: 2271–2280.

Kim SJ, Zhang H, Khaliulin I, Choisy SC, Bond R, Lin H *et al.* (2012). Activation of glibenclamide-sensitive ATP-sensitive K+ channels during beta-adrenergically induced metabolic stress produces a substrate for atrial tachyarrhythmia. Circ Arrhythm Electrophysiol 5: 1184–1192.

Korchev YE, Negulyaev YA, Edwards CR, Vodyanoy I, Lab MJ (2000). Functional localization of single active ion channels on the surface of a living cell. Nat Cell Biol 2: 616–619.

Kubo M, Quayle JM, Standen NB (1997). Angiotensin II inhibition of ATP-sensitive K+ currents in rat arterial smooth muscle cells through protein kinase C. J Physiol 503 (Pt 3): 489–496.

Kubo Y, Baldwin TJ, Jan YN, Jan LY (1993). Primary structure and functional expression of a mouse inward rectifier potassium channel. Nature 362: 127–133.

Kubota I, Yamaki M, Shibata T, Ikeno E, Hosoya Y, Tomoike H (1993). Role of ATP-sensitive K+ channel on ECG ST segment elevation during a bout of myocardial ischemia. A study on epicardial mapping in dogs. Circulation 88: 1845–1851.

Lader JM, Vasquez C, Bao L, Maass K, Qu J, Kefalogianni E *et al.* (2011). Remodeling of atrial ATP-sensitive K(+) channels in a model of salt-induced elevated blood pressure. Am J Physiol Heart Circ Physiol 301: H964–H974.

Lederer WJ, Nichols CG, Smith GL (1989). The mechanism of early contractile failure of isolated rat ventricular myocytes subjected to complete metabolic inhibition. J Physiol 413: 329–349.

Li RA, Leppo M, Miki T, Seino S, Marban E (2000). Molecular basis of electrocardiographic ST-segment elevation. Circ Res 87: 837–839.

Li X, Rapedius M, Baukrowitz T, Liu GX, Srivastava DK, Daut J *et al.* (2010). 5-Hydroxydecanoate and coenzyme A are inhibitors of native sarcolemmal KATP channels in inside-out patches. Biochim Biophys Acta 1800: 385–391.

Light PE, Sabir AA, Allen BG, Walsh MP, French RJ (1996). Protein kinase C-induced changes in the stoichiometry of ATP binding activate cardiac ATP-sensitive K+ channels. A possible mechanistic link to ischemic preconditioning. Circ Res 79: 399–406.

Lin YF, Jan YN, Jan LY (2000). Regulation of ATP-sensitive potassium channel function by protein kinase A-mediated phosphorylation in transfected HEK293 cells. EMBO J 19: 942–955.

Linton KJ, Higgins CF (2007). Structure and function of ABC transporters: the ATP switch provides flexible control. Pflugers Arch 453: 555–567.

Liu Y, Ren G, O'Rourke B, Marban E, Seharaseyon J (2001). Pharmacological comparison of native mitochondrial K(ATP) channels with molecularly defined surface K(ATP) channels. Mol Pharmacol 59: 225–230.

McPherson CD, Pierce GN, Cole WC (1993). Ischemic cardioprotection by ATP-sensitive K+ channels involves high-energy phosphate preservation. Am J Physiol 265: H1809–H1818.

Maffit SK, Sellitto AD, Al-Dadah AS, Schuessler RB, Damiano RJ, Jr, Lawton JS (2012). Diazoxide maintains human myocyte volume homeostasis during stress. J Am Heart Assoc 1: jah3-e000778.

Malester B, Tong X, Ghiu I, Kontogeorgis A, Gutstein DE, Xu J *et al.* (2007). Transgenic expression of a dominant negative K(ATP) channel subunit in the mouse endothelium: effects on coronary flow and endothelin-1 secretion. FASEB J 21: 2162–2172.

Mannhold R (2006). Structure-activity relationships of K(ATP) channel openers. Curr Top Med Chem 6: 1031–1047.

Matsuda N, Hattori Y (2007). Vascular biology in sepsis: pathophysiological and therapeutic significance of vascular dysfunction. J Smooth Muscle Res 43: 117–137.

Medeiros-Domingo A, Tan BH, Crotti L, Tester DJ, Eckhardt L, Cuoretti A *et al*. (2010). Gain-of-function mutation S422L in the KCNJ8-encoded cardiac K(ATP) channel Kir6.1 as a pathogenic substrate for J-wave syndromes. Heart Rhythm 7: 1466–1471.

Mikhailov MV, Campbell JD, de Wet H, Shimomura K, Zadek B, Collins RF *et al*. (2005). 3-D structural and functional characterization of the purified KATP channel complex Kir6.2-SUR1. EMBO J 24: 4166–4175.

Miki T, Suzuki M, Shibasaki T, Uemura H, Sato T, Yamaguchi K *et al*. (2002). Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. Nat Med 8: 466–472.

Morelli A, Lange M, Ertmer C, Broeking K, Van AH, Orecchioni A *et al*. (2007). Glibenclamide dose response in patients with septic shock: effects on norepinephrine requirements, cardiopulmonary performance, and global oxygen transport. Shock 28: 530–535.

Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK *et al*. (2009a). H2S signals through protein S-sulfhydration. Sci Signal 2: ra72.

Mustafa AK, Gadalla MM, Snyder SH (2009b). Signaling by gasotransmitters. Sci Signal 2: re2.

Nelson MT, Huang Y, Brayden JE, Hescheler J, Standen NB (1990). Arterial dilations in response to calcitonin gene-related peptide involve activation of K+ channels. Nature 344: 770–773.

Nichols CG, Lopatin AN (1997). Inward rectifier potassium channels. Annu Rev Physiol 59: 171–191.

Noma A (1983). ATP-regulated K+ channels in cardiac muscle. Nature 305: 147–148.

Noma A, Takano M (1991). The ATP-sensitive K+ channel. Jpn J Physiol 41: 177–187.

O'Brien A, Stidwill RP, Clapp LH, Singer M (2009). Variable effects of inhibiting iNOS and closing the vascular ATP-sensitive potassium channel (via its pore-forming and sulfonylurea receptor subunits) in endotoxic shock. Shock 31: 535–541.

Orie NN, Thomas AM, Perrino BA, Tinker A, Clapp LH (2009). Ca2+/calcineurin regulation of cloned vascular K ATP channels: crosstalk with the protein kinase A pathway. Br J Pharmacol 157: 554–564.

Ortiz D, Gossack L, Quast U, Bryan J (2013). Reinterpreting the action of ATP analogs on KATP channels. J Biol Chem 288: 18894–18902.

Paucek P, Mironova G, Mahdi F, Beavis AD, Woldegiorgis G, Garlid KD (1992). Reconstitution and partial purification of the glibenclamide-sensitive, ATP-dependent K+ channel from rat liver and beef heart mitochondria. J Biol Chem 267: 26062–26069.

Prasad SM, Al-Dadah AS, Byrd GD, Flagg TP, Gomes J, Damiano RJ, Jr *et al*. (2006). Role of the sarcolemmal adenosine triphosphate-sensitive potassium channel in hyperkalemic cardioplegia-induced myocyte swelling and reduced contractility. Ann Thorac Surg 81: 148–153.

Pratt EB, Tewson P, Bruederle CE, Skach WR, Shyng SL (2011). N-terminal transmembrane domain of SUR1 controls gating of Kir6.2 by modulating channel sensitivity to PIP2. J Gen Physiol 137: 299–314.

Quinn KV, Cui Y, Giblin JP, Clapp LH, Tinker A (2003). Do anionic phospholipids serve as cofactors or second messengers for the regulation of activity of cloned ATP-sensitive K+ channels? Circ Res 93: 646–655.

Quinn KV, Giblin JP, Tinker A (2004). Multisite phosphorylation mechanism for protein kinase A activation of the smooth muscle ATP-sensitive K+ channel. Circ Res 94: 1359–1366.

Quinn KV, Behe P, Tinker A (2008). Monitoring changes in membrane phosphatidylinositol 4,5-bisphosphate in living cells using a domain from the transcription factor tubby. J Physiol 586: 2855–2871.

Reyes S, Park S, Johnson BD, Terzic A, Olson TM (2009). KATP channel Kir6.2 E23K variant overrepresented in human heart failure is associated with impaired exercise stress response. Hum Genet 126: 779–789.

Rodrigo GC, Standen NB (2005). ATP-sensitive potassium channels. Curr Pharm Des 11: 1915–1940.

Saegusa N, Sato T, Saito T, Tamagawa M, Komuro I, Nakaya H (2005). Kir6.2-deficient mice are susceptible to stimulated ANP secretion: K(ATP) channel acts as a negative feedback mechanism? Cardiovasc Res 67: 60–68.

Sampson LJ, Hayabuchi Y, Standen NB, Dart C (2004). Caveolae localize protein kinase A signaling to arterial ATP-sensitive potassium channels. Circ Res 95: 1012–1018.

Sampson LJ, Davies LM, Barrett-Jolley R, Standen NB, Dart C (2007). Angiotensin II-activated protein kinase C targets caveolae to inhibit aortic ATP-sensitive potassium channels. Cardiovasc Res 76: 61–70.

Shi L, Xu M, Liu J, Zhang Z, Bao Z, Wang Y *et al*. (2009). K(ATP) channels are involved in regulatory volume decrease in rat cardiac myocytes. Physiol Res 58: 645–652.

Shi W, Cui N, Wu Z, Yang Y, Zhang S, Gai H *et al*. (2010). Lipopolysaccharides up-regulate Kir6.1/SUR2B channel expression and enhance vascular KATP channel activity via NF-kappaB-dependent signaling. J Biol Chem 285: 3021–3029.

Shi Y, Wu Z, Cui N, Shi W, Yang Y, Zhang X *et al*. (2007). PKA phosphorylation of SUR2B subunit underscores vascular KATP channel activation by beta-adrenergic receptors. Am J Physiol Regul Integr Comp Physiol 293: R1205–R1214.

Shi Y, Cui N, Shi W, Jiang C (2008). A short motif in Kir6.1 consisting of four phosphorylation repeats underlies the vascular KATP channel inhibition by protein kinase C. J Biol Chem 283: 2488–2494.

Shyng S, Nichols CG (1998). Membrane phospholipid control of nucleotide sensitivity of K_{ATP} channels. Science 282: 1138–1141.

Shyng S, Ferrigni T, Nichols CG (1997). Regulation of KATP channel activity by diazoxide and MgADP. Distinct functions of the two nucleotide binding folds of the sulfonylurea receptor. J Gen Physiol 110: 643–654.

Shyng SL, Nichols CG (1997). Octameric stoichiometry of the K-ATP channel complex. J Gen Physiol 110: 655–664.

Sikka P, Kapoor S, Bindra VK, Saini M, Saxena KK (2012). Iptakalim: a novel multi-utility potassium channel opener. J Pharmacol Pharmacother 3: 12–14.

Spruce AE, Standen NB, Stanfield PR (1985). Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. Nature 316: 736–738.

Standen NB, Quayle JM, Davies NW, Brayden JE, Huang Y, Nelson MT (1989). Hyperpolarizing vasodilators activate ATP-sensitive K+ channels in arterial smooth muscle. Science 245: 177–180.

Suh BC, Inoue T, Meyer T, Hille B (2006). Rapid chemically induced changes of PtdIns(4,5)P2 gate KCNQ ion channels. Science 314: 1454–1457.

Suzuki M, Kotake K, Fujikura K, Inagaki N, Suzuki T, Gonoi T *et al*. (1997). Kir6.1: a possible subunit of ATP-sensitive K+ channels in mitochondria. Biochem Biophys Res Commun 241: 693–697.

Suzuki M, Li RA, Miki T, Uemura H, Sakamoto N, Ohmoto-Sekine Y *et al.* (2001). Functional roles of cardiac and vascular ATP-sensitive potassium channels clarified by Kir6.2-knockout mice. Circ Res 88: 570–577.

Suzuki M, Sasaki N, Miki T, Sakamoto N, Ohmoto-Sekine Y, Tamagawa M *et al.* (2002). Role of sarcolemmal K(ATP) channels in cardioprotection against ischemia/reperfusion injury in mice. J Clin Invest 109: 509–516.

Suzuki M, Saito T, Sato T, Tamagawa M, Miki T, Seino S *et al.* (2003). Cardioprotective effect of diazoxide is mediated by activation of sarcolemmal but not mitochondrial ATP-sensitive potassium channels in mice. Circulation 107: 682–685.

Tajada S, Cidad P, Moreno-Dominguez A, Perez-Garcia MT, Lopez-Lopez JR (2012). High blood pressure associates with the remodelling of inward rectifier K+ channels in mice mesenteric vascular smooth muscle cells. J Physiol 590: 6075–6091.

Teramoto N, Zhu HL, Shibata A, Aishima M, Walsh EJ, Nagao M *et al.* (2009). ATP-sensitive K+ channels in pig urethral smooth muscle cells are heteromultimers of Kir6.1 and Kir6.2. Am J Physiol Renal Physiol 296: F107–F117.

Thorneloe KS, Maruyama Y, Malcolm AT, Light PE, Walsh MP, Cole WC (2002). Protein kinase C modulation of recombinant ATP-sensitive K(+) channels composed of Kir6.1 and/or Kir6.2 expressed with SUR2B. J Physiol 541: 65–80.

Tong X, Porter LM, Liu G, Dhar-Chowdhury P, Srivastava S, Pountney DJ *et al.* (2006). Consequences of cardiac myocyte-specific ablation of KATP channels in transgenic mice expressing dominant negative Kir6 subunits. Am J Physiol Heart Circ Physiol 291: H543–H551.

Toyoda Y, Hagiya Y, Adachi T, Hoshijima K, Kuo MT, Ishikawa T (2008). MRP class of human ATP binding cassette (ABC) transporters: historical background and new research directions. Xenobiotica 38: 833–862.

Tricarico D, Mele A, Lundquist AL, Desai RR, George AL, Jr, Conte CD (2006). Hybrid assemblies of ATP-sensitive K+ channels determine their muscle-type-dependent biophysical and pharmacological properties. Proc Natl Acad Sci U S A 103: 1118–1123.

Trube G, Hescheler J (1984). Inward-rectifying channels in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell-attached patches. Pflugers Arch 401: 178–184.

Tucker SJ, Gribble FM, Zhao C, Trapp S, Ashcroft FM (1997). Truncation of Kir6.2 produces ATP-sensitive K+ channels in the absence of the sulphonylurea receptor. Nature 387: 179–183.

Tucker SJ, Gribble FM, Proks P, Trapp S, Ryder TJ, Haug T *et al.* (1998). Molecular determinants of KATP channel inhibition by ATP. EMBO J 17: 3290–3296.

Ueda K, Inagaki N, Seino S (1997). MgADP antagonism to Mg2+-independent ATP binding of the sulfonylurea receptor SUR1. J Biol Chem 272: 22983–22986.

Venkatesh N, Lamp ST, Weiss JN (1991). Sulfonylureas, ATP-sensitive K+ channels, and cellular K+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. Circ Res 69: 623–637.

Warrillow S, Egi M, Bellomo R (2006). Randomized, double-blind, placebo-controlled crossover pilot study of a potassium channel blocker in patients with septic shock. Crit Care Med 34: 980–985.

Weiss JN, Lamp ST (1987). Glycolysis preferentially inhibits ATP-sensitive K+ channels in isolated guinea pig cardiac myocytes. Science 238: 67–69.

Whorton MR, MacKinnon R (2011). Crystal structure of the mammalian GIRK2 K+ channel and gating regulation by G proteins, PIP2, and sodium. Cell 147: 199–208.

Wilson AJ, Jabr RI, Clapp LH (2000). Calcium modulation of vascular smooth muscle ATP-sensitive K(+) channels: role of protein phosphatase-2B. Circ Res 87: 1019–1025.

Yamada M, Isomoto S, Matsumoto S, Kondo C, Shindo T, Horio Y *et al.* (1997). Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea- sensitive but ATP-insensitive K+ channel. J Physiol Lond 499: 715–720.

Yamada S, Kane GC, Behfar A, Liu XK, Dyer RB, Faustino RS *et al.* (2006). Protection conferred by myocardial ATP-sensitive K+ channels in pressure overload-induced congestive heart failure revealed in KCNJ11 Kir6.2-null mutant. J Physiol 577: 1053–1065.

Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K *et al.* (2008). H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. Science 322: 587–590.

Yellon DM, Downey JM (2003). Preconditioning the myocardium: from cellular physiology to clinical cardiology. Physiol Rev 83: 1113–1151.

Yoshida H, Feig JE, Morrissey A, Ghiu IA, Artman M, Coetzee WA (2004). K ATP channels of primary human coronary artery endothelial cells consist of a heteromultimeric complex of Kir6.1, Kir6.2, and SUR2B subunits. J Mol Cell Cardiol 37: 857–869.

Zhao W, Zhang J, Lu Y, Wang R (2001). The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. EMBO J 20: 6008–6016.

Zingman LV, Alekseev AE, Bienengraeber M, Hodgson D, Karger AB, Dzeja PP *et al.* (2001). Signaling in channel/enzyme multimers: ATPase transitions in SUR module gate ATP-sensitive K+ conductance. Neuron 31: 233–245.

Zingman LV, Hodgson DM, Bast PH, Kane GC, Perez-Terzic C, Gumina RJ *et al.* (2002). Kir6.2 is required for adaptation to stress. Proc Natl Acad Sci U S A 99: 13278–13283.

Zingman LV, Zhu Z, Sierra A, Stepniak E, Burnett CM, Maksymov G *et al.* (2011). Exercise-induced expression of cardiac ATP-sensitive potassium channels promotes action potential shortening and energy conservation. J Mol Cell Cardiol 51: 72–81.