A touching case of channel regulation: the ATP-sensitive K^+ channel

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The classical type of K_{ATP} channel is an octameric (4:4) complex of two structurally unrelated subunits, Kir6.2 and SUR. The former serves as an ATP-inhibitable pore, while SUR is a regulatory subunit endowing sensitivity to sulphonylurea and K^+ channel opener drugs, and the potentiatory action of MgADP. Both subunits are required to form a functional channel.

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Abbreviations

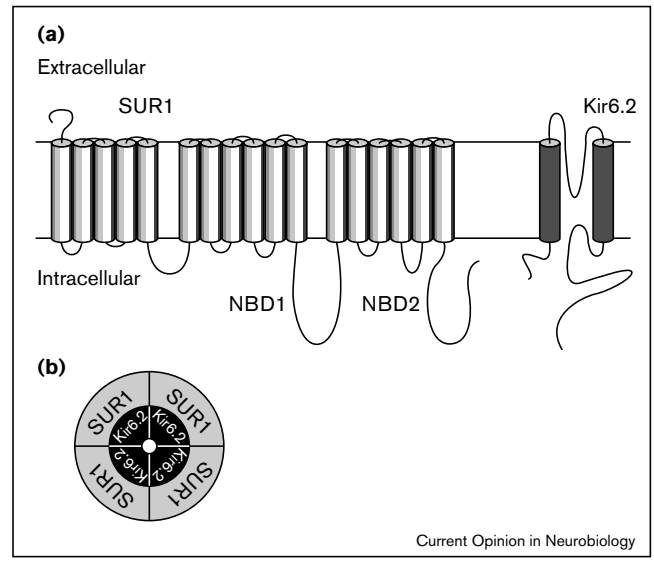
NBD	nucleotide-binding domain
PHHI	persistent hyperinsulinaemic hypoglycaemia of infancy
SUR	sulphonylurea receptor

Introduction

Potassium channels inhibited by adenosine 5′-triphosphate (K_{ATP} channels) are found in a wide variety of tissues, where their primary role is to couple cell metabolism to electrical activity and K^+ fluxes. They are involved in the response to cerebral and cardiac ischaemia, the regulation of vascular smooth muscle tone, epithelial K^+ transport and the electrical activity of several different types of neurone [1–3]. However, their physiological role is best understood in the pancreatic β-cell, where they link changes in blood glucose concentration to insulin secretion [4]. Under normal conditions, the K_{ATP} channel is open and sets the β-cell resting membrane potential. Elevation of blood glucose concentration results in increased glucose uptake and metabolism by the β-cell. This closes the K_{ATP} channel, producing a depolarisation that activates voltage-gated Ca^{2+} channels and thereby induces a rise in intracellular Ca^{2+} which stimulates insulin release. K_{ATP} channels play a similar role in glucose-sensing in ventromedial hypothalamic neurones. How metabolism influences K_{ATP} channel activity remains controversial, although it is widely believed that metabolically generated changes in adenine nucleotide concentrations are involved, as ATP inhibits, whereas MgADP potentiates, K_{ATP} channel activity [1–4].

This review summarises the major findings of the past year on the relationship between K_{ATP} channel structure and function. It is important to emphasise that K_{ATP} channels in different tissues may have markedly different biophysical properties, ATP sensitivity and pharmacology. Recent studies have revealed that this diversity results from differences in the molecular composition of K_{ATP} channels. In this review, we focus primarily on those K_{ATP} channels that are blocked with high affinity by ATP (K_i 10–100 μM), activated by MgADP and MgGDP, and modulated by many additional cytosolic factors (e.g. H^+) [1–3]. In addition, they are blocked by sulphonylurea drugs, which are used to treat the symptoms of non-insulin dependent diabetes mellitus, and activated by a group of unrelated drugs collectively known as K-channel openers [5]. We refer to these as 'classical' K_{ATP} channels.

Figure 1



(a) Putative membrane topology of SUR1 and Kir6.2. **(b)** Schematic showing the stoichiometry of the K_{ATP} channel.

Molecular identity

The first clue to the structure of the K_{ATP} channel came from studies with glibenclamide, a sulphonylurea that inhibits the channel at nanomolar concentrations. This property was exploited by Aguilar-Bryan and colleagues [6] to purify and subsequently clone a high-affinity sulphonylurea-binding protein from β-cell membranes. The sulphonylurea receptor they isolated (SUR1) turned out to be a member of the ABC-transporter superfamily, which includes the cystic fibrosis gene product (CFTR) and the multidrug-resistance protein (MDR) [7]. These proteins are characterised by multiple transmembrane domains and two intracellular nucleotide-binding domains (NBDs) (Figure 1a). The precise membrane topology is unknown, but the most recent model suggests that the transmembrane domains are arranged in two groups of 11 and 6 [8].

Although initially an attractive candidate gene for the K_{ATP} channel, expression of SUR1 produced high-affinity sulphonylurea binding but no channel activity [6]. The missing subunit proved to be an inwardly rectifying K^+-channel subunit, Kir6.2 (Figure 1a). Coexpression of Kir6.2 with SUR1 in both mammalian cells and *Xenopus* oocytes resulted in K_{ATP} currents with properties identical to those of the native β-cell K_{ATP} channel [9–11]. Subsequently, a second sulphonylurea receptor (SUR2) was identified, which is produced in two forms (SUR2A, SUR2B) as a result of alternative splicing [12–14]. Currently, most 'classical' K_{ATP} channels are thought to consist of Kir6.2 in combination with an SUR subunit. Thus, cardiac channels are composed of Kir6.2 and SUR2A [12,13], whereas smooth muscle K_{ATP} channels may be formed from Kir6.2 and SUR2B [13,14]. Single-cell PCR studies have identified both SUR1/Kir6.2 and SUR2A/Kir6.2 combinations in different types of substantia nigra neurones, suggesting that multiple types of K_{ATP} channel may exist in the brain [15]. Overlapping distributions of SUR1 and Kir6.2 have been observed in several other regions of the brain, including the hippocampus and cerebellum [16]. The identity of the K_{ATP} channels in ventromedial hypothalamic neurones is, however, far from clear since they show very different single-channel properties, ATP sensitivity, and pharmacology to the 'classical' type of K_{ATP} channel [17,18].

The question of whether other Kir subunits can couple functionally to SUR to form ATP-sensitive K^+ channels has also received attention. Recent experiments suggest that the closely related Kir6.1 subunit associates with SUR2B to form a MgADP-activated, but ATP-insensitive, K^+ channel [19•]. A channel with these properties is observed in vascular smooth muscle. Coexpression of Kir6.1 and SUR1 also produces a metabolically sensitive K^+ channel [20]: whether this channel is sensitive to ATP has not been directly addressed. Most other Kir subunits do not appear to couple to SUR1 [11,21••].

Although it is tempting to regard Kir6.2 as the primary α-subunit and SUR1 as an accessory β-subunit of the K_{ATP} channel, similar to the α- and β-subunits of voltage-gated Na^+ and K^+ channels [22], this analogy is not strictly correct. The two K_{ATP} channel subunits are more intimately linked, as both must be coexpressed to obtain functional channel activity [9–11]. Unlike other Kir subunits, Kir6.2 and Kir6.1 do not show channel activity when expressed alone.

K_{ATP} channel stoichiometry

The direct physical association of Kir6.2 and SUR1 was demonstrated by the ability of [^{125}I]azidoglibenclamide to co-photolabel both SUR1 and Kir6.2, and by the purification of a high-molecular-weight complex containing both Kir6.2 and SUR1 [21••]. Physical association of SUR1 with Kir6.1, but not with Kir1.1 or Kir3.4, was also observed [21••].

One question that has received much attention during the past year has been the stoichiometry of the K_{ATP} channel. Several groups showed that a 1:1 relative stoichiometry was sufficient to form a functional channel by demonstrating that a dimeric SUR1–Kir6.2 fusion construct was capable of forming functional channels [21••,23•,24•]. Since channel activity was reduced when this construct was coexpressed with wild-type Kir6.2, but was restored by supplementation with wild-type SUR1, it appears that each Kir 6.2 subunit requires one SUR1 subunit in order to generate a functional channel. Additionally, because four Kir6.2 subunits are required to form the K_{ATP} channel pore [21••,23•], this means that the K_{ATP} channel is an octameric complex with a 4:4 stoichiometry (Figure 1b).

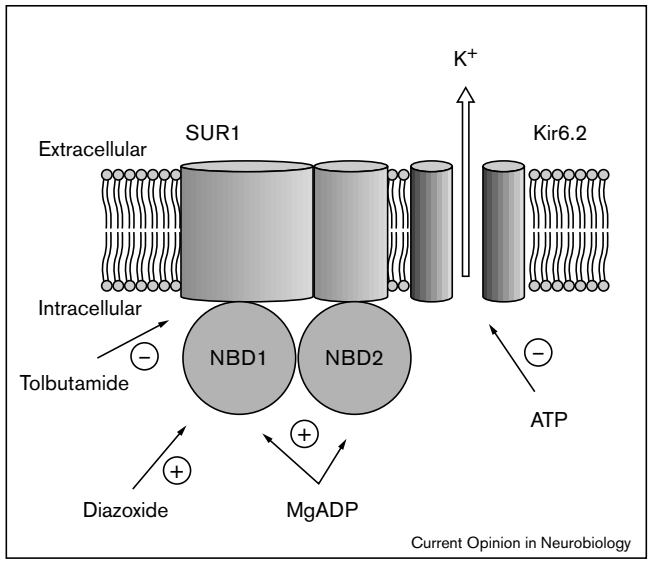
Which subunit does what?

The question of which K_{ATP} channel properties are intrinsic to Kir6.2 and which are conferred by association with SUR has been complicated by the inability to obtain functional expression of Kir6.2 independently of the sulphonylurea receptor. This issue has now been resolved, using a variety of approaches. First, sulphonylurea-binding studies showed that SUR1 endows the K_{ATP} channel with sensitivity to these drugs [6]. Second, the very different sensitivities of Kir6.2/SUR1 channels and Kir6.2/SUR2 channels to K-channel openers argued that K-channel openers also interact with the SUR subunit [12,14,19•]. Third, site-directed mutations implicated the NBDs of SUR1 in the stimulatory action of MgADP, MgGDP and the K-channel opener diazoxide [25–28]. Finally, and most directly, it was found that isoforms of Kir6.2, in which either the last 26 (Kir6.2ΔC26) or 36 (Kir6.2ΔC36) amino acids had been deleted, produced functional channels in the absence of SUR1 [29••]. As predicted from earlier studies, these channels were insensitive to sulphonylureas, diazoxide and the potentiatory action of MgADP, but coexpression with SUR1 restored sensitivity to these agents. Remarkably, the truncated Kir6.2 isoforms were also blocked by ATP ($K_i \sim 100\,\mu M$), despite the lack of an obvious consensus motif for ATP binding in the sequence of Kir6.2. This experiment therefore demonstrates that the primary site for ATP inhibition does not reside on SUR1. Figure 2 summarises the sites of interaction of the main modulatory agents with the K_{ATP} channel.

Intrinsic properties of Kir6.2

The pore of the K_{ATP} channel appears to be composed principally of Kir6.2 subunits, as the single-channel conductance and rectification properties of Kir6.2ΔC26 channels are identical to those of wild-type channels [29••,30]. Moreover, mutations in this subunit affect the rectification and gating properties of the channel [31•]. The observations that truncated Kir6.2 isoforms are inhibited by adenine nucelotides (ATP and ADP) and that mutations in this subunit alter the channel ATP sensitivity, are consistent with the hypothesis that ATP interacts directly with Kir6.2 [29••,31•]. However, ATP-binding

Figure 2



Schematic showing the sites of interaction of modulatory agents with Kir6.2 and SUR1.

experiments are required to confirm this idea and to exclude the possibility that ATP binds to a third subunit, which is endogenously expressed. Imidazoline drugs such as phentolamine appear to mediate their inhibitory effects on the K_{ATP} channel via Kir6.2, rather than SUR1 [30]. Kir6.2ΔC26 currents are also blocked by sulphonylureas, albeit with very low affinity ($K_i \sim 100\,\mu M$ for glibenclamide) [32•]. Although of little clinical significance, this finding is important for interpreting experiments in which glibenclamide is tested at high concentration, as has often been the case in brain slice preparations.

Regulation by SUR

In addition to endowing Kir6.2 with sensitivity to sulphonylureas, K-channel openers and the stimulatory action of MgADP (and MgGDP), SUR1 also enhances the channel open probability [30] and its sensitivity to ATP (shifting the K_i from ~100 μM to ~10 μM [29••]). As yet, it is not known which domains of SUR1 or Kir6.2 interact with each other to transduce these effects. The binding site for sulphonylureas and K-channel openers on SUR has also not been identified. However, there is accumulating evidence that the NBDs of SUR1 are involved in the stimulation of channel activity by MgADP (and GDP). Mutations in these domains abolish nucleotide activation of the K_{ATP} channel [25–28] and interfere with nucleotide binding to SUR1 [33••].

It is well established that MgADP enhances the apparent sensitivity of the β-cell K_{ATP} channel to sulphonylureas. Recent studies demonstrate that this effect results from the ability of sulphonylureas to prevent the stimulatory action of the nucleotide: this unmasks the inhibitory effect of MgADP (mediated by interaction with Kir6.2), thereby producing a further reduction in current amplitude [32•]. Such interactions complicate analysis of the molecular mechanism of action of sulphonylureas.

Physiological consequences of impaired K_{ATP} channel regulation

The physiological importance of the regulatory role of the SUR1 subunit is demonstrated by the fact that mutations in this subunit have been found in patients with persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI), a serious, but rare, disorder characterised by excessive and unregulated insulin secretion [34]. Some of these mutations abolish the ability of MgADP to enhance channel activity [25]. That they also prevent channel activation in response to metabolic inhibition, supports the idea that MgADP may provide the link between cell metabolism and channel activity. Other PHHI mutations cause premature truncation of SUR1, or occur in Kir6.2 [35]. Both types of mutation lead to a loss of K_{ATP} channel activity in the intact cell, and are expected to cause the maintained β-cell depolarisation and continuous insulin secretion that characterises the disease. Indeed, no K_{ATP} channel activity is observed in either cell-attached or excised patches from PHHI β-cells [36]. It remains unclear whether the brain damage found in some PHHI patients results simply from the low blood glucose level or whether it reflects the effect of PHHI mutations on neuronal K_{ATP} channels (which may also comprise Kir6.2 and SUR1 subunits).

The effects of a loss of Kir6.2 function have also been explored using a transgenic mouse expressing a dominant-negative form of Kir6.2 [37]. While neonatal animals display symptoms of PHHI, surprisingly, those that survive develop hypoinsulinaemia and hyperglycaemia when adult.

Hormonal regulation of K_{ATP} channels

One well-established property of the K_{ATP} channel is its tissue-specific regulation by hormones and neurotransmitters. This year has seen leptin added to the list of K_{ATP} channel modulators. Leptin is the product of the *ob* gene, mutations in which lead to severe obesity and diabetes [38]. Leptin activates both the β-cell K_{ATP} channel [39•] and that of ventromedial hypothalamic neurones [40•]. The mechanism of K_{ATP} channel activation remains to be elucidated, but leptin is known to activate the JAK/STAT pathway so tyrosine kinase phosphorylation is a possibility. Whatever the mechanism, these results raise the intriguing possibility that K_{ATP} channels may be involved in the control of body weight.

Some native K_{ATP} channels are regulated by G proteins, which mediate the effects of hormones and transmitters (this regulation is distinct from the potentiatory effects of guanine nucleotides produced by interaction with the NBDs of SUR1). Recent studies have shown that purified G proteins may directly modulate both Kir6.2/SUR1 and Kir6.2/SUR2A channels [41].

Novel K_{ATP} channels and sulphonylurea receptors

Sulphonylurea receptors and sulphonylurea-sensitive K_{ATP} channels are not only found in the plasma membrane; they have also been reported in the membranes of secretory granules [42,43] and mitochondria [44]. The demonstration that Kir6.1 is expressed in mitochondria suggests that it may be a subunit of the mitochondrial K_{ATP} channel [45]. It is still unclear which sulphonylurea receptor partner(s) are part of intracellular K_{ATP} channels. However, a low-affinity sulphonylurea receptor of 65 kDa (K_d 6 μM for glibenclamide) was recently demonstrated in pancreatic zymogen granule membranes, and may be a subunit of the K_{ATP} channel in these membranes [46]. In this respect, it is interesting that a 65 kDa sulphonylurea receptor has also been reported in β-cell membranes [47]. Finally, a protein sharing sequence homology with SUR1 was identified this year in plants [48].

Future directions

Studies during the past year have revealed that the 'classical' type of K_{ATP} is an octameric complex formed by the physical association of two subunits, Kir6.2 and SUR, which have distinct functional roles. The regions of each subunit that participate in this interaction now need to be identified, as does the mechanism by which drug or nucleotide binding to SUR regulates the activity of Kir6.2. The locations of the binding sites for drugs and nucleotides on SUR1, and Kir6.2, also await discovery. The ability to express truncated forms of Kir6.2 independently of SUR1 may facilitate such studies, but given the complex regulation of K_{ATP} channel activity, sorting out exactly how Kir6.2 and SUR interact may take some time. Other important avenues of research include how the K_{ATP} channel complex is assembled, whether Kir6.x (or SUR) subunits can form heteromultimers, and the molecular identity of the non-classical types of K_{ATP} channel.

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