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Review article

Cardiac sarcolemmal K_{ATP} channels: Latest twists in a questing tale!

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ABSTRACT

Reconstitution of K_{ATP} channel activity from coexpression of members of the pore-forming inward rectifier gene family (Kir6.1, *KCNJ8*, and Kir6.2 *KCNJ11*) with sulfonylurea receptors (SUR1, *ABCC8*, and SUR2, *ABCC9*) of the ABCC protein sub-family, has led to the elucidation of many details of channel gating and pore properties, as well as the essential roles of Kir6.2 and SUR2 subunits in generating cardiac ventricular K_{ATP}. However, despite this extensive body of knowledge, there remain significant holes in our understanding of the physiological role of the cardiac K_{ATP} channel, and surprising new findings keep emerging. Recent findings from genetically modified animals include the apparent insensitivity of cardiac sarcolemmal channels to nucleotide levels, and unenvisioned complexities of the subunit make-up of the cardiac channels. This topical review focuses on these new findings and considers their implications.

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1. Introduction

From earlier indications of a K conductance activated by metabolic inhibition, the tale of the cardiac K_{ATP} channel really began in 1983 with Akinori Noma's first description of "specific K^+ channels which are depressed by intracellular ATP (ATP_i) at levels greater than 1 mM"[1]. Because this K_{ATP} channel is gated directly by intracellular ATP and ADP, it is therefore a strong candidate for coupling the metabolic state of the cell with its electrical activity and hence contractility. Reconstitution of K_{ATP} channel activity by coexpression of members of the pore-forming inward rectifier gene family (Kir6.1, *KCNJ8*, and Kir6.2 *KCNJ11*) with sulfonylurea receptors (SUR1, *ABCC8*, and SUR2, *ABCC9*) of the ABCC protein sub-family, has led to the elucidation of many details of channel gating and pore properties[2], as well as essential roles of Kir6.2 and SUR2 subunits in generating cardiac ventricular K_{ATP} [3] and the detrimental consequences of knocking them out on the whole organism [4]. However, despite this extensive body of knowledge, there remain significant holes in our understanding of the physiological role of the cardiac K_{ATP} channel, and surprising new findings keep emerging. Our purpose in this topical review is to focus on these new findings and to consider their implications.

2. Nucleotide-dependent regulation *in vivo*: are all bets off?

Several reviews have covered in-depth the molecular basis of cardiac K_{ATP} channel activity and the accumulated understanding of nucleotide regulation of channel activity [2,5,6]. K_{ATP} channels are typically half-maximally inhibited by ~10–50 μM ATP, which act by binding directly to the regulatory Kir6.2 subunit, with or without Mg^{2+}. However, they are also activated by MgATP and MgADP, which

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are hydrolysed (MgATP) or maintain an activated post-hydrolytic state (MgADP) that counters ATP inhibition, and leads to the prediction that higher [ATP] (in the range of 0.1–1 mM) would be necessary to cause half-maximal inhibition under physiological conditions.

The discovery that application of the phospholipid PIP_2 to the cytoplasmic side of inside-out patches causes a decrease in sensitivity to ATP [7,8] demonstrates that nucleotide sensitivity is not fixed, and could be modulated by other cellular factors. This realization means that the underlying question of when K_{ATP} channels become active will not simply be a question of what is the cellular phosphorylation potential (i.e. the [ATP]:[ADP] ratio), but the integration of this with the signals that regulate channel responsivity. Phosphorylation of recombinant K_{ATP} channels by protein kinase A or C [9–11] modulates nucleotide sensitivity, as do fatty acyl CoA esters [12], and minute-to-minute modulation of lipid content in the cells may also regulate the channel *in vivo* [13]. Interestingly, an early study of ATP inhibition of cardiac K_{ATP} channels revealed $K_{1/2}$ values ranging from 9 to 580 μM in 102 individually excised patches [14], illustrating the very marked patch-to-patch variability that is present. This variability is likely to reflect at least in part the complex regulation of nucleotide sensitivity by ambient factors, including the level of PIP2 and other activatory lipids [7,15]. However, it is also possible that the physical make-up of the individual channels varies, and that, as discussed below, there could be varying numbers of SUR subunits associated with each channel [16,17].

In experiments where K_{ATP} was activated in isolated cardiac myocytes by anoxia, the duration of channel activity was short-lived declining in parallel with a fall in the levels of PIP and PIP_2, suggesting that the levels of these two phospholipids act in concert with the intracellular nucleotides to control channel function [18]. Such data illustrate the lability of nucleotide inhibition in the intact cell, but even the relevance of nucleotide sensitivity to physiological activation of the channels might be questioned in light of recent findings regarding the *in vivo* consequences of channel mutations. Many mutations in the pore-forming Kir6.2 subunit have now been identified as causal in human neonatal diabetes mellitus (NDM), a very severe form of diabetes that typically occurs within the first days or weeks of life [19,20]. All of the identified Kir6.2 mutations result in a reduced channel sensitivity to ATP inhibition, in recombinant channels, leading to channel activation at elevated [glucose], maintained hyperpolarization of pancreatic islet β-cells, and electrical inexcitability, with consequent inhibition of insulin secretion [20]. The same pore-forming Kir6.2 subunit is present in the heart and pancreas, and hence cardiac K_{ATP} channels should also be ATP-insensitive, yet there are no reports of any cardiac abnormalities in NDM patients. Moreover, in transgenic mice that express an ATP-insensitive Kir6.2 subunit (Kir6.2 [ΔN30,K185Q]) in the heart, sarcolemmal K_{ATP} channels are extremely insensitive to ATP-dependent inhibition ($K_{1/2} = 1.4$ mM c.f. 25 μM in WT), yet still remain essentially closed in intact cells [21,22]. As K_{ATP} channels have a higher density than other sarcolemmal K^+ channels, opening of as few as 1% of K_{ATP} channels is expected to shorten cardiac action potential by about 50%, and multiple studies [23–27] predict that this dramatic reduction of ATP sensitivity should ensure that channels are active enough to significantly shorten the action potential in normal conditions, yet the action potential duration is unaffected [21]. Clearly something other than nucleotide sensitivity is at play.

3. Channel subunit expression in the heart: could it get more complicated?

In order to probe the significance of channel composition in K_{ATP} function in cardiac myocytes, not only the ATP-insensitive Kir6.2 subunit (Kir6.2[ΔN30,K185Q] [22], but also SUR2A or SUR1 [28] subunits have been overexpressed under cardiac alpha-MHC control. In each case, cardiac function is only minimally affected; in the Kir6.2 [ΔN30,K185Q] transgenics there is a very small increase in background K_{ATP}, although this is balanced by a 'pre-stimulated' Ca current [21,22]. Conceivably this reflects an intrinsic compensatory mechanism; i.e. shortening of the action potential being compensated by enhanced Ca current, although underlying signaling processes are unknown. Overexpression of either SUR1 or SUR2A is without effect on the ECG, other than a consistent P-R prolongation in SUR1-overexpressing hearts [28]. Surprisingly, overexpression of any single one of these subunits also significantly suppresses sarcolemmal K_{ATP} channel density [22,28]. One possible explanation is that overexpressed SUR1, SUR2A or Kir6.2 subunits interact with endogenous subunits, and thereby disrupt the stoichiometry of the channel [29] and then may not reach the plasma membrane [16,30]. A simple resolution to this would seem to be that co-overexpression of both subunits (i.e. by crossing the two animals) should restore appropriate stoichiometry of expression, leading to a high density of ATP-insensitive channels in the sarcolemma. The dramatic result, however, is an even greater suppression of total channel density [31], and a whole constellation of arrhythmias, leading to sudden death. Not only does this surprising result tell us that altered K_{ATP} channels can have profound—but unexplained—effects on electrical activity, but that there is something very specific required for 'correct' expression of the two subunits. A clue may be found in the fact that endogenous *Kir6* and *SUR* genes are immediately adjacent. Although perhaps heretical to suggest, it may be that common elements of gene regulation ensure that both are normally transcribed with temporal or spatial coordination, leading to correct assembly at a very early stage of synthesis, and that this coordination is absent when transgenes are exogenously expressed under alpha-MHC promoter control.

Another question is exactly which subunits are expressed in different regions of the heart. The pancreatic β-cell K_{ATP} channel is formed as a complex of four Kir6.2 subunits each associated with one SUR1 subunit [29,32]. Together with studies of gene knockout mice (which show that *Kir6.2* and *SUR2* genes are essential for normal ventricular K_{ATP} currents, whereas K_{ATP} currents are unaffected in ventricular myocytes from SUR1 or Kir6.1 knockout animals [6]), early studies of recombinant channel pharmacology [33,34] led to the widely accepted notion that the sarcolemmal K_{ATP} channel is a heteromultimer of Kir6.2 and SUR2A, presumably with the same octameric arrangement. However, several studies have demonstrated that both Kir6 subunits (Kir6.1 and Kir6.2) and both SUR subunits (SUR1 and SUR2A) are expressed in the heart [35–37]. Dominant-negative coexpression strategies demonstrate that Kir6.1 and Kir6.2 may assemble into functional channel complexes [38], and in some studies [36], dominant-negative Kir6.1 subunits suppress sarcolemmal K_{ATP} currents [39]. The idea that SUR2 is essential for ventricular K_{ATP} is also muddied by the finding that some K_{ATP} channels are still present in $SUR2^{-/-}$ myocytes [40]. In this case, it is noteworthy that Kir6.2 channels have been shown to be present at the surface membrane in recombinant cells, in the complete absence of expressed SUR subunits [16,17,41], albeit at lower levels than are found with SUR subunits expressed. These channels are less sensitive to ATP inhibition than channels associated with SUR subunits, but lack Mg-nucleotide activation [41] It remains a possibility that an octameric arrangement is not absolutely obligatory, and that variations in channel structure at this level may influence channel activation. However, antisense oligonucleotides specific for either SUR1 or SUR2A/B suppress K_{ATP} current in neonatal rat ventricular myocytes, which suggests that SUR1 might also participate in forming the channel, either alone or in conjunction with SUR2A [42], and recombinant channel studies demonstrate that within a single channel, more than one Kir6.x or SURx subunit can clearly co-exist [38,43–46].

Definitive proof that SUR1 is a significant component of sarcolemmal K_{ATP} channels comes from a recent study of SUR1 knockout ($SUR1^{-/-}$) animals [47]. A key finding is that while K_{ATP} currents