

Metabolic Modulation of Potassium Channels

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(Published 18 May 2004)

Potassium ion (K^+) channels play a crucial role in basic cellular functions such as excitability, secretion, proliferation, and volume regulation (1, 2). Occurring in virtually every eukaryotic and prokaryotic cell, they are among the most conserved structures of life. With the enormous advances recently made in the elucidation of their architecture and function, K^+ channels have arguably become the best-understood ion channels (3–6). Despite extensive studies, however, they are still full of surprises.

In neurons, K^+ channels are involved in maintaining the resting potential, repolarizing the membrane after action potentials, and controlling the timing of repetitive firing. Because activation of K^+ channels leads to hyperpolarization, regulation of their activity by cellular metabolites may be a particularly important homeostatic mechanism for suppressing electrical activity during periods of overstimulation, cellular damage, hypoxia, or stress. However, it should not be assumed that K^+ channels are always the good guys. Recent studies have revealed that K^+ channel activity is essential for the proliferation of several types of tumor cells (7, 8).

K^+ channels are activated by changes in transmembrane potential (voltage gating), the concentration of intracellular ligands (ligand gating), or both. Their activity can be further modulated by various cytoplasmic ligands, as well as by phosphorylation. So-called inward-rectifying K^+ channels, in particular, are subject to intracellular regulation. Inward-rectifying K^+ channels stabilize the resting potential near the K^+ equilibrium potential and thus control cellular excitability. Their name is derived from the fact that inward currents evoked by hyperpolarization are greater than currents elicited by depolarization of the same amplitude. In addition to heterotrimeric guanine nucleotide-binding proteins (G proteins) and protons, they have been found to respond to certain small molecules that reflect the metabolic state of a cell, such as adenosine 5'-triphosphate (ATP) (1).

Indeed, the role of inward-rectifying ATP-sensitive K^+ channels (K_{ATP} channels) in coupling metabolic state to excitability is now well established (1). Pancreatic β cells, for example, release insulin in response to increased ATP concentration associated with rising glucose levels. This process is mediated by K_{ATP} channels that are activated by Mg-ADP (Mg-adenosine 5'-diphosphate) and close upon binding to ATP. The depolarization that results from their binding ATP triggers calcium ion (Ca^{2+}) influx and ultimately results in insulin release from secretory granules.

Ca^{2+} -activated K^+ channels, on the other hand, begin to activate and hyperpolarize cells in response to the high levels of intracellular Ca^{2+} that accompanies hypoxia (9). This may act as an "excitatory brake" for additional Ca^{2+} influx, such as would occur if additional action-potential firing continued unabated. Hence, it is not surprising that Ca^{2+} -activated K^+ channels are positioned in cellular domains sensitive to stress-induced Ca^{2+} elevation, such as in presynaptic

nerve terminals (10). Indeed, Ca^{2+} -activated K^+ channels are often colocalized with voltage-gated Ca^{2+} channels, at least in part to prevent excessive Ca^{2+} influx.

Recent investigations have expanded the range of small molecules known to interact with K^+ channels, assigning new potential roles to these channels in signal transduction cascades and linking them more directly to cellular metabolism. Among others, hydrogen peroxide (H_2O_2) and heme [iron(III) protoporphyrin IX] have been shown to affect K^+ channels. Although the functional significance of these results remains to be fully elucidated, it is already clear that K^+ channels interface in a more varied way with the intracellular environment than previously thought.

Avshalumov and Rice recently showed that inward-rectifying H_2O_2 -sensitive K_{ATP} channels play a crucial role in inhibiting dopamine release in the central nervous system (CNS) (11). Using fast-scan cyclic voltametry to measure dopamine release, and selective blockers to probe the involvement of different channels, the authors showed that H_2O_2 -sensitive K_{ATP} channels are opened by endogenous H_2O_2 generated downstream of activation of the AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) glutamate receptor. The exact mechanism whereby H_2O_2 activates striatal K^+ channels—or even whether this is a direct effect—remains unclear. The resulting hyperpolarization prevents the discharge of dopamine from neurons in the dorsal striatum.

K_{ATP} channels had previously been found to play a neuroprotective role in CNS neurons—for example, silencing activity triggered by anoxia-elicited decreases in cytoplasmic ATP concentration (12). This study showed that by mediating the inhibition of dopamine release by glutamate, K_{ATP} channels also contribute to the normal physiology of dopaminergic neurons. Dopaminergic neurons play important roles in movement control, cognitive processing, motivation, and reward. Accordingly, dysfunctions of this system have been implicated in Parkinson's disease, schizophrenia, and substance abuse (13–17). However, other CNS neurons that possess K_{ATP} channels are more closely linked to metabolic regulation. These include glucose-sensitive neurons in the hypothalamus, vagal neurons that regulate heart rate, and medullary neurons that regulate respiratory rate. Activation of K_{ATP} channels by H_2O_2 in these cells might help to re-

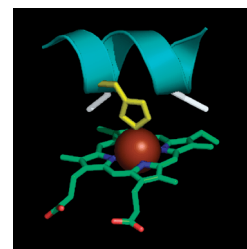


Fig. 1. Heme bound to the CKACH binding motif in cytochrome *c* from the bacterium *Rhodospseudomonas palustris* (Protein Data Bank ID code 1A7V). The histidine residue shown in yellow coordinates to the iron atom, whereas the two cysteine residues (white), projecting from the same side of the α helix, contact the porphyrin ring. An analogous structural motif is presumably present in Slo1 BK channels upon heme binding.

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strain overexcitation by AMPA receptors, providing an important homeostatic feedback function.

Hoshi and co-workers recently identified a heme-binding site in the large-conductance, Ca^{2+} -activated Slo1 BK channel (18). Activation of this K^+ channel results in hyperpolarization restricting Ca^{2+} influx and excitability, which is important for cytoprotection from ischemia and for mitochondrial homeostasis. Through analysis of its amino acid sequence, the channel was found to contain a heme-binding motif. This conserved binding sequence, CXXCH (where X is any amino acid, C is cysteine, and H is histidine), is typical of c-type cytochromes. Heme is a key biological cofactor, occurring in hemoglobin and many cytochrome P450 enzymes, where it mediates oxygen transport and redox reactions.

Using electrophysiological methods, the authors showed that heme binds to the intracellular side of Slo1 BK with high affinity (median inhibitory concentration of 45 to 120 nM) and decreased the opening frequency of the channel. Structural studies performed with short peptides containing the binding motif and site-directed mutagenesis of the native channels, which resulted in loss of sensitivity toward heme, further corroborated the proposed interaction. A three-dimensional representation of the heme-binding motif CKACH found in Slo1 BK and some cytochromes is shown in Fig. 1.

Given the high affinity of heme for the channel, which is comparable to the dissociation constant for hemoproteins, these results appear to be physiologically relevant and suggest that heme functions as an acute cell-signaling molecule. Slo1 BK channels have been demonstrated in mitochondria, where heme biosynthesis takes place (19). It is well known that heme is released from hemoproteins after cellular injury, hypoxia, or stress (20), and that extracellular heme released during trauma can be transported across cell membranes (21).

The fact that heme released under these conditions closes Slo1 BK channels and prevents them from exerting their cytoprotective function seems somewhat counterintuitive, and the full physiological significance of this effect remains to be determined. However, the inhibition of Slo1 BK channels by heme may be of clinical importance because these channels are opened by certain pharmacological agents administered after trauma or ischemia (22, 23). Whether these agents directly interfere with heme binding and counterbalance its negative effect is currently unknown.

Another potential link between the metabolic state of a cell and its transmembrane potential involves the β subunits of certain voltage-gated K^+ channels (2). These intracellular tetrameric protein assemblies are typically associated with the transmembrane α subunits and presumably are in close contact with their voltage sensor. Efforts to determine the functional role of β subunits have so far been inconclusive. In certain cases, they have been shown to provide the "ball-and-chain" that rapidly inactivates K^+ channels. Interestingly, in Shaker, the archetypical K^+ channel from *Drosophila melanogaster*, this inactivation mechanism is provided by the α subunit itself (N-type inactivation gate). Other studies suggested that β subunits act as molecular chaperones to α subunits (24).

The crystal structure of a mammalian β subunit elucidated by MacKinnon, Gulbis, and co-workers suggests a potential third role for these subunits (25, 26). An NADP⁺ (nicotinamide adenine dinucleotide phosphate) molecule was found residing in a binding pocket of each protomer. Structurally, this binding

pocket resembles the active site of the enzyme aldose reductase, suggesting that the β subunit may function as an oxido-reductase. Thus far, no endogenous substrates appear to have been identified, and the enzymatic activity of the β subunit remains shrouded in mystery. Nevertheless, the work of MacKinnon *et al.* suggests that the voltage sensor on the α subunit is influenced by a redox-active β subunit and that membrane excitability is thus directly linked to the metabolic state of a cell. Conversely, the β subunit could function as a "voltage-gated enzyme."

Although the K^+ channels discussed here fall into three distinct categories, they all appear to be modulated by intracellular ligands that reflect the metabolic state of a cell. Chances are, other K^+ channels will be found to be regulated by similar or identical small molecules. Searches in genomic databases could identify the binding motifs for many other potential ligands in genes corresponding to K^+ channels or their auxiliary subunits. It will be interesting to establish the functional significance of these discoveries, as well as their pharmacological and clinical relevance.

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- Citation:** D. Trauner, R. H. Kramer, Metabolic modulation of potassium channels. *Sci. STKE* **2004**, pe20 (2004).