ATP-Sensitive K⁺ Channels in the Brain: Sensors of Hypoxic Conditions

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Rapid minimization of energy consumption in excitable tissues is effective protection from lethal effects of extreme metabolic stress. The ATP-sensitive K⁺ channels in the brain respond in ATP-depleted metabolic states such as hypoxia and may be involved in the protection mechanism against energy-consuming generalized seizure.

The brain is an unresting assembly of cells continually receiving and routing information to maintain the integrity of the individual organism. The aerobic metabolism of glucose is critical in this process. Indeed, although the brain represents only ~2% of body weight, it accounts for ~20% of total body resting O₂ consumption. Because of the high metabolic rate and limited energy stores, interruption of the O₂ or glucose supply by stroke, global ischemia, or pulmonary failure readily causes loss of consciousness and, if unheeded, generalized convulsive seizure.

During seizure, the cerebral metabolic rates of O₂ and glucose uptake increase more than under any other circumstance (2). This massive energy demand causes a rapid fall in ATP that, if prolonged, leads ultimately to irreversible cell damage (5) due to intracellular ionic derangements such as Na⁺ and Ca²⁺ overload (2). To prevent the development in the brain of energy-demanding seizure during metabolic stress, the ATP-sensitive K⁺ (K_ATP) channel, the molecule that controls membrane potentials by sensing intracellular ATP levels, may play a pivotal role. In this brief review, recent progress in accord with this hypothesis is discussed, together with other views.

K_ATP channels, discovered in cardiac myocytes and then found in many other excitable cells, including hormone-secreting cells, skeletal and smooth muscle cells, and neurons, alter open probability as the cytosolic ATP concentration during ischemia or hypoxia promotes K⁺ efflux from the cells by activating the K_ATP channels, which rapidly dampens excitability by shortening the action potential duration.

K_ATP channels are also expressed in the brain, but their functional role is poorly understood (19). Binding studies using radiolabeled sulfonylureas show that most brain areas, including basal ganglia, thalamus, hippocampus, and cerebral cortex, express K_ATP channels with different affinities for sulfonylureas (12). In the CA1 area of the hippocampus in response to brief oxygen deprivation, the neurons are hyperpolarized by activation of K⁺ conductance (5, 9). The K_ATP channel was proposed to be directly responsible for this change in conductance (19). Indeed, intracellular ATP decreases to 15% after ~2 min of hypoxic challenge (15), and hypoxia-induced hyperpolarization is depressed by the sulfonylureas glibenclamide and tolbutamide in some CA1 neurons (19). However, in other CA1 neurons (for review, see Ref. 19) that are insensitive to K_ATP channel blockers, a rise in the intracellular Ca²⁺ concentration as well as a decrease in the cytosolic ATP concentration followed by activation of Ca²⁺-activated K⁺ (K_Ca) channels is reported (9). Yamamoto and coworkers (19) have suggested that both K_ATP and K_Ca channels contribute to hypoxia-induced hyperpolarization in CA1 neurons and that the ratio of the contribution of these two channels differs in individual CA1 neurons.

A key question remains from these extensive studies: what is the physiological role of brain K_ATP channels? Recent investigations of K_ATP channels by molecular approaches provide many insights. The structure of the K_ATP channel was initially determined in pancreatic ß-cells to be an octameric complex of two types of subunit (7): a pore-forming channel subunit (Kir6.2) and a regulatory subunit, the sulfonylurea receptor (SUR1), belonging to the ATP-binding cassette superfamily. Later, additional Kir and SUR subunits were identified that form complexes with different pharmacological properties: the cardiac and skeletal muscle types are composed of Kir6.2 and SUR2A, an isoform of SUR1 (8); the vascular smooth muscle type is composed of Kir6.1 and SUR2B, a splice variant of SUR2A (for review, see Ref. 14). SUR2A and SUR2B have low affinity for sulfonylurea, whereas SUR1 (ß-cell type) has high affinity.

The role of brain K_ATP channels during hypoxic challenge was investigated by using mutant mice lacking Kir6.2 [Kir6.2(−/−) mice] (18). The midbrain nucleus, called the substantia nigra pars reticulata (SNr), which consists mostly of GABAergic neurons, was selected as a focus partly because the nucleus expresses the highest binding densities for sulfonylureas (12). In the CA1 area of the hippocampus in response to brief oxygen deprivation, the neurons are hyperpolarized by activation of K⁺ conductance (5, 9). The K_ATP channel was proposed to be directly responsible for this change in conductance (19). Indeed, intracellular ATP decreases to 15% after ~2 min of hypoxic challenge (15), and hypoxia-induced hyperpolarization is depressed by the sulfonylureas glibenclamide and tolbutamide in some CA1 neurons (19). However, in other CA1 neurons (for review, see Ref. 19) that are insensitive to K_ATP channel blockers, a rise in the intracellular Ca²⁺ concentration as well as a decrease in the cytosolic ATP concentration followed by activation of Ca²⁺-activated K⁺ (K_Ca) channels is reported (9). Yamamoto and coworkers (19) have suggested that both K_ATP and K_Ca channels contribute to hypoxia-induced hyperpolarization in CA1 neurons and that the ratio of the contribution of these two channels differs in individual CA1 neurons.

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During brief hypoxia, the membrane potentials of wild-type mice were reversed in the hyperpolarized direction, whereas Kir6.2(/−/−) mice remained depolarized. However, during brief hypoxic challenge, the wild-type mice showed a marked decrease in the firing rate of SNr neurons, promoting K⁺ outflow, hyperpolarizing the membrane, and inactivating the neuronal spike activity to suppress generalized seizure.

How do KATP channels control the seizure threshold? To investigate the cellular and ionic mechanisms, single unit activities were recorded in the SNr by acute slice preparations in Kir6.2(+/−) and wild-type mice. The spontaneous firing rate of the SNr neurons under resting conditions was similar in both mice. However, during brief hypoxic challenge, the wild-type neurons showed a marked decrease in the firing rate to about one-third, whereas the firing rate of knockout neurons increased ~1.8-fold. In addition, the sulfonylurea tolbutamide reversed the hypoxia-induced inhibition of the firing of wild-type neurons to facilitation just as in Kir6.2(+/−) neurons, although tolbutamide had no effect on the firing rate or the membrane potential of SNr neurons under resting conditions. During brief hypoxia, the membrane potentials of wild-type SNr neurons were shifted in the hyperpolarized direction, whereas Kir6.2(+/−) SNr neurons showed no hyperpolarization but rather were depolarized in nystatin perforated-patch recordings using dissociated SNr neurons. These results indicate that the opening of the KATP channels exerts a strong suppressive effect on wild-type SNr neuronal activity during hypoxic challenge by shifting membrane potentials in the hyperpolarized direction sufficiently to reverse the facilitation of K⁺ outflow.

**FIGURE 1.** Simplified Kir6.2-containing ATP-sensitive K⁺ (KATP) channel alternative functions in insulin-secreting pancreatic β-cells and midbrain GABAergic substantia nigra pars reticulata (SNR) neurons. Left: in hyperglycemia, glucose metabolism increases the intracellular ATP/ADP ratio in pancreatic β-cells by oxidative phosphorylation and closes the KATP channels, which depolarizes the plasma membrane to allow cellular excitation to induce insulin secretion. Right: in hypoxia, decreased oxygen and the resultant decrease in cytosolic ATP/ADP ratio opens the KATP channels in SNR neurons, promoting K⁺ outflow, hyperpolarizing the membrane, and inactivating the neuronal spike activity to suppress generalized seizure.
of neuronal activity in the Kir6.2(−/−) neurons that is due to membrane depolarization (Fig. 1). The mechanism of the spike facilitation and membrane depolarization observed in Kir6.2(−/−) SNr neurons is currently unknown; depression of electrogenic Na⁺-K⁺ pump activity during hypoxic challenge is most likely, but other mechanisms such as inactivation of O₂-sensitive K⁺ channels by decreased Po₂ are possible (4).

As mentioned, presynaptic modulatory effects on neuronal firing by brain KATP channels have been suggested during metabolic stress (1, 16, 17). In the substantia nigra pars compacta (SNc), the KATP channels on the striatogniral terminals of SNc dopaminergic neurons might become active due to reduced intraterminal levels of ATP, and the resultant hyperpolarization of the terminal should lead to a reduction of GABA release and so facilitate SNc neuronal activity. However, as pointed out by Watts and colleagues (17), it is difficult to reconcile this hypothesis with the postsynaptic suppressive effects of KATP channel activation. The contribution of postsynaptic KATP channels in neuronal responses to hypoxic challenge was investigated in acute slice preparations (18). In the condition of isola-
tion from presynaptic effects by the presence of blockers of both excitatory and inhibitory fast neurotransmissions, the firing rates of both wild-type and Kir6.2(−/−) GABAergic SNr neurons increased ~35% in normoxia, indicating some facilitatory effect of blocked presynaptic GABAergic terminals on the firings of SNr neurons. However, the responses to hypoxic challenge in the presence and absence of blockers were similar; a striking contrast in the spontaneous firing rate of SNr neu-
rons and a net decrease in wild-type and a net increase in Kir6.2(−/−) neurons, indicating that the opening of the postsynaptic KATP channels is pivotal in the hypoxia-induced responses of wild-type SNr neurons, at least in this experimen-
tal condition. The physiological significance of the presynaptic KATP channels must be further investigated.

It is important to note that wild-type mice exhibited generalized convulsion in very severe hypoxic conditions, such as 4.3% O₂ for 150 s (18). In milder hypoxic conditions, such as 7.3% O₂, most of the Kir 6.2(−/−) mice showed no convulsion and none died. Secher and Wilhjelm (13) have reported that the tolerance of conscious animals to anoxia increases very rapidly when the O₂ concentration is >5%, whereas at <4% O₂ survival time is extremely short (<10 min). There is a rapid deflection in the O₂ concentration vs. survival time curve at ~4-5% O₂. Thus the KATP channel may fully exert its protective role only in limited severities of hypoxic challenge just above this critical transition. In addition, the EEG and EMG of Kir6.2(−/−) mice responded within several seconds after the hypoxic condition was achieved, suggesting involvement of the KATP channels in the initial stage of the response to hypoxic challenge.

To investigate the contribution of the KATP channels in other brain nuclei to the hypoxia-induced response is important. Although KATP channels are functionally expressed in various nuclei, such as cerebral cortex (11), hippocampus (19), hypothalamus, and SNc (reviewed in Ref. 10), the molecular makeup of neuronal KATP channels appears not to be homoge-
neous. Lis and colleagues (10), using a combined approach of patch-clamp and single-cell RT-PCR, reported that dopamin-
gic SNc neurons express different types of KATP channel with differing sensitivities to metabolic inhibition and proposed a novel mechanism of the selective vulnerability of some dopaminergic neurons in Parkinson's disease. They showed that neurons with β-cell-type KATP channels, which comprise Kir6.2 and SUR1, have the highest metabolic sensitivity and that these and not neurons with other types of KATP channels survive in weaver mice, suggesting that the β-cell-type KATP channels might have the strongest neuroprotective effect.

Zawar and colleagues (20) also reported heterogeneous expression profiles of KATP channels in the hippocampal CA1 area: functional KATP channels (Kir6.1 plus SUR1, Kir6.2 plus SUR1 or SUR2) are expressed in 17% of the pyramidal cells and 75% of the interneurons. Especially interesting, 58% of CA1 interneurons express β-cell-type KATP channels. Clarification of the involvement of these channels in energy-depleted conditions should provide clues to understanding why certain sets of pyramidal neurons are extremely vulnerable to ischemic stress but others are not.

What then is the specific role of the KATP channels in the SNr? It is widely known that the neurons of the SNr show the highest spontaneous activity (up to 100 Hz) in the brain, indicating a very high metabolic rate in these neurons in the normoxic condition. Indeed, SNr neurons are extremely sensitive to hypoxia (18). On the other hand, it has been reported that the potentials evoked by electrical stimulation in hippocampal (5) and cerebral cortical (18) neurons are not altered during brief hypoxia. Thus the KATP channels in SNr neurons are likely to act as the sensors in hypoxia, responding before the general self-defense reaction to hypoxic conditions in other neuron types (Fig. 1). In addition, SNr neurons innervate various dis-
tant nuclei in diverse motor-related functions, including the ventral thalamic nuclei, superior colliculus, and pedunculo-
pontine nucleus in the brain stem. Abrupt silence in the SNr GABAergic projection neurons during an early phase of brain metabolic emergency might well exert the nigral protection mechanism by conveying a signal of massive disinhibition to all of these targets simultaneously, which should protect the whole brain from generalized seizure. In addition, these studies suggest that KATP channels may be a target of site-specific treatment of brain disorders associated with ATP insufficiency such as stroke and metabolic encephalopathies.

References


