ATP-Sensitive Potassium Channels in Dopaminergic Neurons: Transducers of Mitochondrial Dysfunction

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ATP-sensitive potassium (K_{ATP}) channels directly couple the metabolic state of a cell to its electrical activity. Dopaminergic midbrain neurons express alternative types of K_{ATP} channels mediating their differential response to mitochondrial complex I inhibition. Because reduced complex I activity is present in Parkinson's Disease, differential K_{ATP} channel expression suggests a novel candidate mechanism for selective dopaminergic degeneration.

Dopaminergic neurons play a crucial role in a variety of brain functions such as reward, working memory, and voluntary movement. The latter is severely affected in Parkinson's Disease (PD), a common neurodegenerative movement disorder that affects >1% of the elderly population. The pathological hallmark of PD is the selective degeneration of a subpopulation of dopaminergic midbrain neurons, mainly in the substantia nigra pars compacta (SNpc). This highlights one of the enigmas of PD: why are some dopaminergic neurons highly vulnerable to the degenerative process but others remain largely unaffected? It suggests the presence of relevant differences in gene expression that determine the differential vulnerability of single dopaminergic neurons. In some rare familial forms of PD, the mutated genes have recently been identified (e.g., α-synuclein, parkin). However, for the common form of sporadic PD, the molecular disease mechanisms remain unclear. Metabolic stress has been identified as an important trigger factor for the neurodegenerative process of PD. In particular, reduced activity of the mitochondrial respiratory chain complex I (CXI, also known as NADH:ubiquinone reductase) of ~40% has been consistently found in PD patients. Cybrid studies indicate that the CXI defect in PD has a genetic component and may arise from mutations in the mitochondrial DNA. In these studies, mitochondria from PD patients were fused with carrier cells that possessed no mitochondria (15). However, the pathophysiological downstream events that are induced by CXI inhibition and lead to the selective dopaminergic degeneration are still unclear.
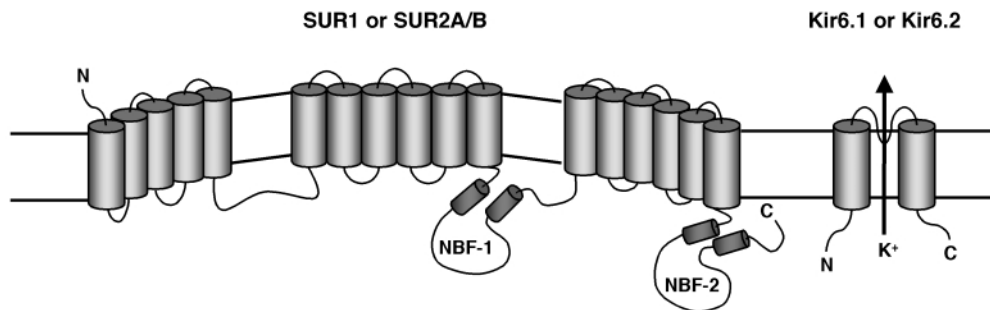
The K_{ATP} channel

In this context of metabolic dysfunction in PD, ATP-sensitive potassium (K_{ATP}) channels are of special interest, because their open probability directly depends on the metabolic state of a cell. K_{ATP} channels are closed at high ATP-to-ADP ratios and open in response to decreased ATP and increased ADP levels. By this mechanism, K_{ATP} channel activity exerts a powerful control mechanism of cellular excitability.

The K_{ATP} channel was first discovered by Akinori Noma in cardiac myocytes (12) and was subsequently found to be expressed in many other cell types, e.g., in cardiac and smooth muscle, pancreatic beta cells, and various brain regions. K_{ATP}

channels are octameric proteins consisting of two different types of subunits: members of the Kir6 inwardly rectifying potassium channel family and sulfonylurea receptor (SUR) subunits, which are members of the ATP-binding cassette transporter superfamily (Fig. 1). In functional channels, four pore-forming Kir6 subunits are joined together with four regulatory SUR subunits. At present, two members of the Kir6 family have been cloned, Kir6.1 and Kir6.2, and two SUR isoforms have been identified, SUR1 and SUR2. A variety of SUR1 and SUR2 splice variants have been described, with SUR2A and SUR2B being the most important. In heterologous expression systems, these different subunit combinations give rise to functional K_{ATP} channels with different biophysical, pharmacological, and metabolic properties. Kir6.2 in combination with SUR1 subunits form K_{ATP} channels with properties very similar to those described in pancreatic beta cells. Kir6.2 and SUR2A pairs resemble the K_{ATP} channels of cardiac and skeletal muscle, and SUR2B in combination with Kir6.1 subunits generates K_{ATP} channels that possess properties reminiscent of those studied in smooth muscle. Structure-and-function aspects of the K_{ATP} channel have been intensively studied (for recent reviews, see Refs. 1, 2, and 16).

K_{ATP} channel subunits are also widely expressed throughout different brain regions, as indicated by sulfonylurea binding studies and in situ hybridization data. Moreover, a variety of different neuronal populations possess somatodendritic K_{ATP} currents with diverging biophysical and pharmacological properties (e.g., in hippocampal pyramidal neurons, locus coerulus and dorsal vagal neurons, striatal interneurons, hypothalamic neurons, and GABAergic and dopaminergic substantia nigra neurons), and there is also evidence for functional presynaptic K_{ATP} channels. In accordance with the functional and pharmacological diversity, the molecular makeup of neuronal K_{ATP} channels also appears to be not homogeneous. With a combined approach of electrophysiological patch-clamp and single-cell mRNA expression profiling techniques, different combinations of coexpressed K_{ATP} channel subunits have been identified. This suggests the presence of molecularly distinct neuronal K_{ATP} channels that might reflect different functional roles in the brain. Here we would like to focus on the potential role of molecularly identified K_{ATP} channel subtypes expressed in dopaminergic SNpc neurons in the context of their selective vulnerability in PD.

FIGURE 1. ATP-sensitive potassium (K_{ATP}) channels are octameric proteins made up of 4 sulfonylurea receptor subunits (SUR1, SUR2A, or SUR2B) and 4 pore-forming inwardly rectifying potassium channel subunits (Kir6.1 or Kir6.2). Two intracellular nucleotide-binding folds (NBF-1 and NBF-2) are located on the SUR subunits.

K_{ATP} channels in dopaminergic neurons: function and molecular composition

The functional role of K_{ATP} channels is best understood for the pancreatic beta cells where these channels couple blood glucose levels to insulin secretion. Thus beta cell K_{ATP} channels are essential in peripheral glucose sensing. By analogy, neuronal K_{ATP} channels, in particular in the hypothalamus, are involved in central glucose sensing and neuroendocrine control of glucose homeostasis (7, 11). In a more general sense, neuronal K_{ATP} channels could act as energy control elements, adapting electrical activity and in turn neuronal ATP consumption to the delicate metabolic state of neurons. K_{ATP} channel-mediated membrane hyperpolarization will reduce neuronal activity and neurotransmitter release and thus could counteract calcium overload and excitotoxicity. This mechanism could be important in pathophysiological situations like ischemia or epilepsy. In addition, activation of K_{ATP} channels before an insult initiates adaptive responses (preconditioning) that have strong neuroprotective effects (5). The molecular mechanisms of preconditioning are unclear; however, protein kinase C-mediated phosphorylation of Kir6.2 has recently been proposed.

In this context, K_{ATP} channels in dopaminergic neurons might have an additional role. It has been shown that dopaminergic terminals directly innervate brain arterioles. Thus dopaminergic neurons might not only control neuronal activity but also the perfusion of their axonal target areas (6). Under physiological conditions, dopaminergic midbrain neurons show spontaneous action potential firing and, at least in in vitro brain slices, most K_{ATP} channels are closed (Fig. 2A). The activation of K_{ATP} channels leads to a hyperpolarization of the dopaminergic neurons, accompanied by a complete loss of their normal pacemaker activity (Fig. 2B). However, the K_{ATP} channel response to metabolic stress is not uniform within the population of dopaminergic SNpc neurons. In acute mouse brain slices, a partial inhibition of CXI by rotenone only activates K_{ATP} channels in a subpopulation of ~40% of dopaminergic neurons with a half-maximal effective concentration (EC_{50}) of 15 nM. Combined single-cell RT-PCR experiments demonstrated that these highly responsive dopaminergic neurons express the K_{ATP} channel subunits SUR1 and Kir6.2 (9). In contrast, the other population of dopaminergic SNpc neurons possess a >100-fold higher EC_{50} (2 µM) for rotenone-induced K_{ATP} channel activation, indicative of a significantly lower sen-

sitivity to CXI inhibition. These neurons, which maintain their pacemaker activity during partial CXI inhibition, express the other SUR isoform, SUR2B, in combination with Kir6.2 mRNA (9). It is important to note that the molecular mechanisms defining metabolic sensitivity of K_{ATP} channels are still not clear. However, recombinant SUR1/Kir6.2-containing K_{ATP} channels also show a higher sensitivity to metabolic stress compared with SUR2A/Kir6.2-mediated channels (2). This demonstrates that the alternative expression of SUR subunits is a major determinant of metabolic sensitivity of K_{ATP} channels. In addition, other intracellular factors like phosphoinositol phosphates (e.g., PIP_2), G proteins, or protein kinases could
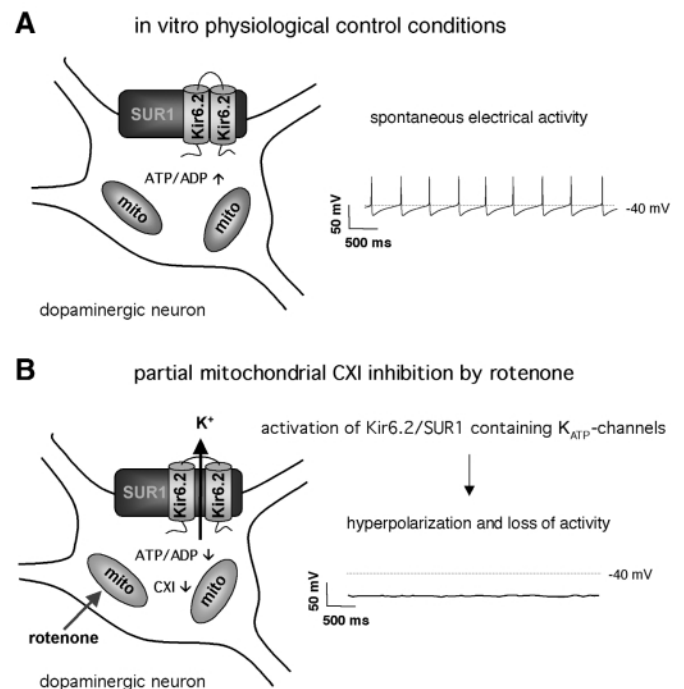
FIGURE 2. Schematic illustration of the effect of complex I (CXI) inhibition in single dopaminergic neurons. *A*: under physiological control conditions, dopaminergic neurons show pacemaker activity and most K_{ATP} channels are closed. A patch-clamp recording (current clamp) of spontaneous activity of a dopaminergic neuron in an in vitro mouse brain slice preparation is shown at *left*. *B*: in the presence of the CXI inhibitor rotenone, SUR1/Kir6.2-containing K_{ATP} channels activate. That leads to a membrane hyperpolarization of the dopaminergic neuron, which is accompanied by a complete loss of spontaneous activity. Current-clamp recording of the same neuron as in *A* but in the presence of the CXI blocker rotenone (100 nM) is shown at *left*.

modulate the metabolic sensitivity of K_{ATP} channels (3, 8, 17). The rotenone EC_{50} of 15 nM for activating SUR1-containing K_{ATP} channels and inducing complete loss of activity would correspond to a degree of CXI inhibition similar to that found in brains of PD patients (15) and suggests the possibility of K_{ATP} channel activation in PD. By analogy, the activity of SUR2B-expressing dopaminergic neurons might not be perturbed in PD.

K_{ATP} channels in the *weaver* mouse: a genetic model of dopaminergic degeneration

Studies of dopaminergic midbrain neurons in the *weaver* mouse, a genetic mouse model of dopaminergic degeneration similar to that in PD (13), support the idea of K_{ATP} channel activation as a neuroprotective strategy. The *weaver* mouse is characterized by a point mutation in the pore region of a G protein-coupled inwardly rectifying potassium channel (GIRK2). As a homomer, the mutated GIRK2 channel lost its potassium selectivity and can be constitutively active, resulting in a chronic, depolarizing sodium influx into dopaminergic SNpc neurons. The chronic sodium overload is expected to further enhance ATP consumption of the Na^+-K^+-ATPase, which already is the major single consumer of neuronal ATP. We demonstrated for dopaminergic neurons in homozygous *weaver* mice that SUR1/Kir6.2-containing K_{ATP} channels are tonically activated in direct consequence to the activity of mutated GIRK2 channels in the *weaver* mouse. This partially compensates for the chronic depolarization of the dopaminergic *weaver* neurons. Single-cell RT-PCR experiments revealed the complete absence of SUR2B-expressing dopaminergic neurons in *weaver* mice (9, 10). These results give the first evidence that alternative SUR1 expression and activation of K_{ATP} channels might be an important event in the process of dopaminergic neurodegeneration.

Selective dopaminergic degeneration by CXI inhibition

The status of CXI inhibition and its potential downstream consequences like K_{ATP} channel activation are still unresolved in PD. Here, neurotoxicological animal models of PD, in which the mitochondrial CXI is chronically inhibited, provide further insights. One classic neurotoxicological rodent model of PD is the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse (14). 1-Methyl-4-phenyl-pyridinium (MPP^+), the neurotoxic metabolite of MPTP, is selectively transported into dopaminergic neurons via the dopamine transporter (DAT). Once in the cell, MPP^+ blocks the CXI and finally triggers selective dopaminergic degeneration, similar to that found in PD. Alternatively, MPP^+ can be sequestered into intracellular vesicles via the vesicular monoamine transporter (VMAT2). Because the toxic intracellular MPP^+ concentration depends on the relative expression levels of these two dopamine transporters, the pattern of selective vulnerability might simply reflect differential DAT/VMAT2 expression ratios in the dopaminergic midbrain system. Thus the MPTP model can not fully address two central questions: *1*) are dopaminergic neurons more vulnerable to CXI inhibition per se compared with

other neurons? and *2*) is there differential vulnerability to CXI inhibition within the dopaminergic midbrain population, and is this correlated with the pattern of neurodegeneration? In this context, a new neurotoxicological PD model, developed by Bertabet et al. (4), is of high relevance. Chronic brain infusion of low doses of the CXI inhibitor rotenone induced Parkinsonism and dopaminergic degeneration similar to that of PD in rats. Because there is no selective mechanism for rotenone uptake, all neurons throughout the brain are expected to be exposed to the same degree of partial CXI inhibition. Importantly, this rotenone model is highly selective for dopaminergic neurons. Moreover, dopaminergic neurons showed a pattern of differential sensitivity to rotenone-induced cell death, similar to that found in PD. This rotenone model of PD demonstrates that dopaminergic neurons possess important intrinsic differences in responses to CXI inhibition that are crucial for death or survival. Differential gene expression between dopaminergic subpopulations is likely to determine these critical differences in the pathophysiological response to CXI inhibition. One promising candidate that is differentially expressed in dopaminergic neurons and directly senses CXI inhibition is the K_{ATP} channel. In the rotenone model, the free toxin concentration is in the range of 20–30 nM. This corresponds to the degree of CXI inhibition found in PD (15) but might not substantially impair ATP synthesis in brain mitochondria. It is, however, sufficient to activate SUR1/Kir6.2-containing K_{ATP} channels in dopaminergic neurons. Consequently, a subpopulation of dopaminergic neurons might tonically hyperpolarize and reduce their physiological spontaneous activity (Fig. 2).

If and how K_{ATP} channel-mediated changes in electrical activity finally decide about death or survival of dopaminergic neurons is unknown. However, one can speculate that in PD a chronic reduction of neuronal activity might not be primarily neuroprotective by reducing ATP consumption but might lead to a reduced expression of activity-dependent genes that promote survival, such as neurotrophins. In this scenario, transient K_{ATP} channel activation is a short-term neuroprotective response to metabolic stress, but chronic K_{ATP} channel activity could have fatal consequences for the dopaminergic neuron.

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Surviving Anoxia With the Brain Turned On

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Crucian carp is one of few vertebrates that tolerate anoxia. It maintains brain ATP during anoxia partially by reducing ATP consumption. However, unlike turtles, which become comatose during anoxia, this fish remains physically active. This striking difference in anoxic survival strategy is reflected all the way down to the cellular level.

Anoxia-related diseases are major causes of death in the western world, and the success of medical science in counteracting the devastating effects of anoxia has been limited. Still, evolution solved the problem of anoxic survival millions of years ago by giving rise to anoxia-tolerant vertebrates such as some freshwater turtles and carp fish.

During most of the last millennium, until aquarium air pumps came into use, virtually the only fish species that was kept as an indoor pet was the goldfish (*Carassius auratus*), the reason being its extraordinary ability to survive with little or no oxygen. To achieve diversity in the aquarium, elaborate breeding of this small carp species started in eastern Asia and resulted in varieties of the most bizarre forms and shapes. In fact, the natural goldfish is hardly golden at all and virtually indistinguishable from its close European relative, the crucian carp (*Carassius carassius*). Possibly, wild goldfish and crucian carp are just the easternmost and westernmost forms of one and the same species, and both are exceptionally anoxia tolerant. At room temperature, they can tolerate anoxia for one or two days, and, at temperatures close to 0°C, the crucian carp has been found to survive anoxia for several months.

The anoxic brain

The principal problem for an anoxic cell is to maintain its ATP levels. The stop in oxidative ATP production (giving up to 36 mol ATP/mol glucose) leaves the cell with glycolysis (2 mol ATP/mol glucose) as the only route for ATP production. As a result of the brain's high rate of ATP use, mainly associated with the ion pumping needed to sustain electrical activity, brain ATP levels fall drastically within minutes of anoxia in "normal" anoxia-sensitive vertebrates. Consequently, the ATP-demanding Na+-K+ pump slows down or stops, initially leading to a net outflux of K+. Soon, extracellular K+ reaches a concentration high enough to depolarize the brain. At this point, Na+ and Ca^{2+} flood into the cells, a process also stimulated by a concomitant release of excitatory neurotransmitters like glutamate. Indeed, a major route for Ca^{2+} entry is the glutamate-activated *N*-methyl-D-aspartate receptors. Neuronal death appears largely to be initiated by the uncontrolled rise in intracellular Ca^{2+}, which activates various degenerative and lytic processes.

Anoxia-tolerant vertebrates

Just like the American freshwater turtles of the genera *Trachemys* and *Chrysemys*, anoxia tolerance in *Carassius* has evolved to allow overwintering in an anoxic freshwater habitat. In northern Europe, many crucian carp populations inhabit small ponds, where the ice and snow cover in the winter block photosynthesis, rendering the water anoxic for several months. Like the freshwater turtles, the *Carassius* species have become model organisms for the study of anoxia tolerance. When comparing these fishes with the turtles, the emerging picture is that of contrasting strategies of anoxia tolerance (6). The most obvious difference is already revealed on the behavioral level: