Molecular Membrane Biology, 2001, 18, 117–127

Molecular physiology of neuronal K-ATP channels (Review)

Birgit Liss and Jochen Roeper*

MRC Anatomical Neuropharmacology Unit, University of Oxford, Mansfield Road, Oxford OX1 3TH, UK

Summary

ATP sensitive potassium (K-ATP) channels are widely expressed in many cell types including neurons. K-ATP channels are heteromeric membrane proteins that consist of two very different subunits: the pore-forming, two-transmembrane spanning potassium channel subunit (Kir6) and the regulatory, 17 transmembrane spanning sulphonylurea receptor (SUR). This ensemble — joined together in a 4:4 stoichiometry — endows this channel with a unique combination of functional properties. The open probability of K-ATP channels directly depends on the intracellular ATP/ADP levels allowing the channels to directly couple the metabolic state of a cell to its electrical activity. Here, recent progress on the molecular composition and functional diversity of neuronal K-ATP channels is reviewed. One is particular concerned with single-cell mRNA expression studies that give insight to the coexpression patterns of Kir6 and SUR isoforms in identified neurons. In addition, the physiological roles of neuronal K-ATP channels in glucose sensing and adapting neuronal activity to metabolic demands are discussed, as well as their emerging pathophysiological functions in acute brain ischemia and chronic neurodegenerative diseases.

Keywords: K-ATP channels; single-cell RT-PCR; glucose sensing; ischemia; Parkinson's disease

Abbreviations: CXI, complex I of the respiratory chain; ER, endoplasmatic reticulum; Girk2, G-protein coupled inwardly rectifying potassium channel 2; GR, glucose responsive; K-ATP, ATP sensitive potassium channel; bp, basepairs; Kir, inwardly rectifying potassium channel; NBF, nucleotide binding folds; RT-PCR, reverse-transcriptase polymerase chain reaction; MPTP, 1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine; NTS, nucleus tractus solitaris; SNpc, substantia nigra pars compacta; SUR, sulphonylurea receptor; TPEA, three prime end amplification; VMH ventromedial hypothalamus.

Introduction

ATP sensitive potassium (K-ATP) channels were first discovered by Noma (1983) in cardiac myocytes and were subsequently found in many other excitable celltypes, like cardiac and smooth muscle cells, pancreatic islet cells, and various brain regions (Ashcroft 1988). K-ATP channels are unique in their molecular makeup and their cellular function: Functional channels are octameric proteins formed by two different types of subunits, members of the Kir6 inwardly rectifying potassium channel family (Reimann and Ashcroft 1999) and sulphonylurea receptor (SUR) subunits, members of the ABC transporter superfamily (Higgins 1995). As the open probability of K-ATP channels directly depends on the intracellular ATP/ADP levels, the channels directly couple the metabolic state of a cell to its electrical activity. The physiological role of K-ATP channels is best understood in pancreatic beta cells, where they couple blood glucose levels to insulin secretion. In cardiac myocytes, their activation during ischemia leads to shortening of the action potential. In vascular smooth muscle cells, they are involved in controlling the vessel tone and in skeletal muscle they contribute to the exercise induced K+ efflux (Quayle *et al.* 1997, Ashcroft and Gribble 1999). This review will focus on the differential expression and functional roles of neuronal K-ATP channels in the brain.

Molecular composition of K-ATP channels

Functional K-ATP channels are octameric complexes, consisting of four Kir6 subunits joined together with four regulatory SUR subunits (Clement *et al.* 1997, Inagaki *et al.* 1997, Shyng and Nichols 1997) (Figure 1). The K-ATP channel pore is formed by the Kir6 subunits. Like other members of the inwardly rectifying potassium channel family, the Kir6 subunit consists of two transmembrane domains (TMs), linked by a pore loop, and intracellular amino- and carboxyterminal ends. An endoplasmatic reticulum (ER) retention signal is located in the C-terminus of Kir6.2, preventing the delivery of functional K-ATP channels to the plasmamembrane in the absence of SUR subunits. However, a C-terminally truncated form of Kir6.2 (Kir6.2ΔC26 or Kir6.2ΔC36) gives rise to functional, ATP-sensitive potassium channels that reach the plasmamembrane without the SUR subunits (Tucker *et al.* 1997, Zerangue *et al.* 1999). The SUR is believed to contain 17 TMs, with an extracellular N- and an intracellular C-terminus. The TMs are arranged in three groups (5, 6, 6), connected by cytoplasmatic linkers, and two intracellular SUR domains contain a nucleotide-binding domain (NBD). These NBDs contain the classical Walker A and B motive for ATP binding and hydrolysis. Surprisingly, the ATP sensitivity of K-ATP channels is not located on the SUR subunits, but on the Kir6 subunit and is decreased by phosphoinositolphosphates like PIP2 (Baukrowitz *et al.* 1998, Shyng and Nichols 1998). The ATP binding site of Kir6.2 has not yet been identified, although several important C and N-terminal residues have been described (Tucker *et al.* 1997, 1998, Aguilar-Bryan *et al.* 1998). The regulatory SUR subunits mask the Kir6.2 ER retention signal and transfer the sensitivity to K-ATP channel blockers (e.g. sulphonylureas like tolbutamide and glibenclamide) and activators (e.g. diazoxide, pinacidil and nicorandil). The high-affinity binding site for sulphonylureas is located on the SUR1 subunit; a low affinity site is located on the Kir6.2 subunit (Gribble *et al.* 1997). In addition, SUR is critically involved in the metabolic sensitivity of K-ATP channels as the binding of magnesium nucleosides and nucleotides leads to an increase of channel activity. The pharmacological diversity of native and recombinant K-ATP channels has

*To whom correspondence should be addressed.
e-mail: jochen.roeper@ pharm.ox.ac.uk

Molecular Membrane Biology
ISSN 0968-7688 print/ISSN 1464-5203 online
http://www.tandf.co.uk/journals
DOI: 10.1080/09687680110047373

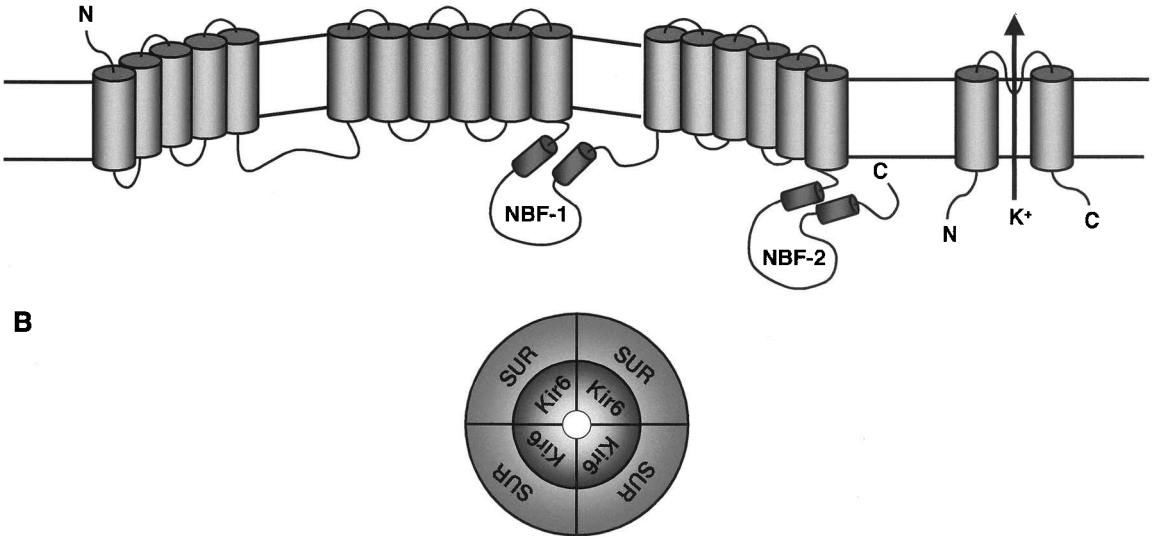

Figure 1. Subunit composition and stoichiometry of K-ATP channels. (*a*) K-ATP channels are made up by inwardly rectifying potassium channel subunits (Kir6.1 or Kir6.2) and sulphonylurea receptor subunits (SUR1, SUR2A or SUR2B). Two intracellular nucleotide-binding folds (NBF-1 and NBD-2) are located intracellularly on the SUR subunit. (*b*) In functional K-ATP channels, four pore forming Kir6 subunits are joined together with four regulatory SUR subunits.

recently been reviewed (Bryan and Aguilar-Bryan 1999, Seino 1999, Ashcroft and Gribble 2000).

Up until now, two members of the Kir6 family have been cloned, Kir6.2 and Kir6.1 (Inagaki *et al.* 1995a, c, Sakura *et al.* 1995), and two SUR isoforms have been identified, SUR1 and SUR2 (Aguilar-Bryan *et al.* 1995). Furthermore, a variety of SUR1 and SUR2 splice variants have been described, with SUR2A and SUR2B being the most important (Sakura *et al.* 1995, Chutkow *et al.* 1996, 1999, Inagaki *et al.* 1996, Isomoto *et al.* 1996). The clustered localization of Kir6.2 and SUR1 on the human chromosome 11p15.1 (Inagaki *et al.* 1995a) and of Kir6.1 and SUR2 at 12p11 (Inagaki *et al.* 1995b, Chutkow *et al.* 1996) indicate that they might have been separated from a single ancestral gene earlier in evolution. In this context, it is noteworthy that a recently cloned *Drosophilia* SUR could form functional sulphonylurea sensitive potassium channels in the absence of Kir6 subunits (Nasonkin *et al.* 1999). Recently, an additional player has been cloned. Alpha-endosulphine is a peptide highly expressed in the brain that acts like an endogenous sulphonylurea on the SUR and might be involved in the control of K-ATP channel activity. However, its precise functional role remains unclear at present (Heron *et al.* 1998).

Diversity of K-ATP channels

In heterologous expression systems, different combinations of the possible K-ATP channel subunits give rise to functional K-ATP channels with different biophysical, pharmacological and metabolic properties (Yokoshiki *et al.* 1998). Kir6.2, in combination with SUR1, forms highly diazoxide-sensitive K-ATP channels with single-channel conductances of 60–80 pS and properties very similar to those present in pancreatic beta cells (Inagaki *et al.* 1995a, Sakura *et al.* 1995). Kir6.2 and SUR2A resemble the K-ATP channels of cardiac and skeletal muscle, relatively insensitive to diazoxide with similar single-channel conductances (Inagaki *et al.* 1996, Okuyama *et al.* 1998). SUR2B, in combination with Kir6.1 or Kir6.2 subunits, generates diazoxide-sensitive K-ATP channels that possess properties reminiscent of those studied in smooth muscle. K-ATP channels, with Kir6.1 as pore forming subunit, display a significantly smaller single-channel conductance of ~30 pS (Isomoto *et al.* 1996, Yamada *et al.* 1997). The molecular composition of the mitochondrial K-ATP channels remain unclear; however, its single channel conductance of 10–30 pS would be consistent with Kir6.1 subunits forming the channel pore of mitochondrial K-ATP channels (Inoue *et al.* 1991, Mironova *et al.* 1999). In addition, Kir6.1 protein has been immunocytochemically localized at mitochondria (Suzuki *et al.* 1997, Zhou *et al.* 1999).

Neuronal K-ATP channels

K-ATP channel subunits are also widely expressed throughout different brain regions, as indicated by sulphonlyurea binding studies (Mourre *et al.* 1990, Treherne and Ashford 1991, Zini *et al.* 1991, Hicks *et al.* 1994, Dunn-Meynell *et al.* 1997), *in situ* hybridization and immunohistochemistry data (Karschin *et al.* 1997, Dunn-Meynell *et al.* 1998, Zhou *et al.* 1999). Moreover, a variety of different neuronal populations possess somatodenditic K-ATP currents with diverging

biophysical and pharmacological properties, indicating that the K-ATP channels in the brain do not belong to a homogeneous group. K-ATP channels have been described in cortical and hippocampal pyramidal- and interneurons (Ohno-Shosaku and Yamamoto 1992, Hyllienmark and Brismar 1996, Fujimura *et al*. 1997, Zawar *et al*. 1999, Pelletier *et al*. 2000), striatal neurons (Schwanstecher and Panten 1994, Schwanstecher and Bassen 1997, Lee *et al*. 1998), various nuclei of the hypothalamus (Ashford *et al*. 1990, Spanswick *et al*. 1997, 2000, Lee *et al*. 1999), GABAergic and dopaminergic substantia nigra neurons (Schwanstecher and Panten 1993, Roper *et al*. 1995, Stanford and Lacey 1995, 1996, Watts *et al*. 1995, Guatteo *et al*. 1998, Liss *et al*. 1999a), locus coeruleus neurons (Finta *et al*. 1993, Illes *et al*. 1994, Koyama *et al*. 1999), dorsal vagal (Trapp and Ballanyi 1995, Karschin *et al*. 1998), and other brainstem neurons (Dallaporta *et al*. 2000). Also, there is evidence for functional presynaptic K-ATP channels (Amoroso *et al*. 1990, Watts *et al*. 1995, Ye *et al*. 1997).

In accordance with the described high and low sulphonylurea binding sites and the functional and pharmacological diversity of K-ATP currents, the molecular makeup of neuronal K-ATP channels appears to be not homogeneous. However, it seems that Kir6.2 is the pore forming subunit in most neuronal populations and Kir6.1 may form the pore of glial and mitochondrial K-ATP channels in the brain (Karschin *et al*. 1997, Suzuki *et al*. 1997, Dunn-Meynell *et al*. 1998, Zawar *et al*. 1999, Zhou *et al*. 1999).

A combined approach of electrophysiological patch-clamp and single-cell mRNA expression profiling techniques (RT-PCR) (Monyer and Lambolez 1995) was used to study the coexpression patterns of K-ATP channel subunits in individual neurons. As illustrated in Figure 2, in this method, the cytoplasm of an electrophysiologically characterized neuron is harvested and the cellular mRNA is subsequently reverse transcribed into cDNA. After an optional amplification step of the single-cell cDNA pool, the expression of K-ATP channel subunits is detected by PCR amplification. So far, three variants of the single-cell method have been described: multiplex nested RT-PCR (Lambolez *et al*. 1992), antisense RNA (aRNA) amplification (Eberwine *et al*. 1992) and three prime and amplification (TPEA) PCR (Dixon *et al*. 1998).

Single-cell RT-PCR studies detected heterogeneous expression profiles of K-ATP channel subunits between and also within different neuronal populations. Table 1 summarizes the results of these studies. All known K-ATP channel subunits (except SUR2A) have been detected in different combinations in individual neurons. This suggests the presence of molecularly distinct neuronal K-ATP channels that might reflect different functional roles in the brain. However, very little is known about the plasticity and regulation of gene expression of neuronal K-ATP channels in the brain and, thus, any firm conclusions might be premature.

Function of neuronal K-ATP channels

Similar to peripheral tissues, two functional roles for neuronal K-ATP channels have been proposed. Inspired by the key role of K-ATP channels in beta cells, it is assumed that in certain specialized neurons, e.g. in the hypothalamus, K-ATP channels might serve a similar role in glucose sensing and control of glucose homeostasis (Levin *et al*. 1999). As K-ATP channels are very widely expressed in the CNS, they are likely to serve a different functional role in most neuronal populations that are not involved in glucose-sensing; presumably in adapting electrical activity of neurons and synapses to their intrinsic energy metabolism.

Essential role of hypothalamic K-ATP channels in central glucose sensing

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. In beta cells, K-ATP channels close in response to increased extracellular glucose concentrations that arise after food intake. To induce K-ATP channel closure, glucose has to be taken up and metabolized by the beta cells. Therefore, proteins like the low affinity glucose carrier GLUT2 and the glucose phosphorylating enzyme glucokinase are also essential components of glucose sensing. Furthermore, the NADH shuttle links cytosolic glycolysis with the activation of mitochondrial energy metabolism that increases the ATP/ADP ratio of the cell (Eto *et al*. 1999). This increased ATP/ADP ratio induces closure of K-ATP channels and subsequent membrane depolarization, which in turn initiates electrical activity and calcium-mediated exocytosis of insulin (Ashcroft and Gribble 1999). Recent dominant-negative transgenic and K-ATP knockout models have given explicit genetic evidence for the key role of Kir6.2/SUR1 containing K-ATP channels in glucose-induced insulin secretion (Miki *et al*. 1997, 1998, Seghers *et al*. 2000, Seino *et al*. 2000). Furthermore, targeting mutated K-ATP channels with reduced ATP-sensitivity to beta cells severely disrupts glucose-induced insulin secretion directly, demonstrating that accurate tuning of the ATP/ADP sensing function of the K-ATP channel is an essential molecular mechanism of peripheral glucose sensing *in vivo* (Koster *et al*. 2000).

Do some neuronal K-ATP channels serve an analogous function in central glucose sensing? In the hypothalamus, especially in the ventromedial hypothalamus (VMH) and the arcuate nucleus, a subset of neurons, called glucose-responsive (GR) neurons, are known to enhance their rate of action potential firing in response to physiological increases in glucose levels. These neurons are known to be involved in the neuroendocrine control of glucose homeostasis (Minami *et al*. 1986, Silver and Erecinska 1998, Yang *et al*. 1999). In this context, it is important to note that hypothalamic neurons also express glucokinase and GLUT2, other essential proteins for glucose sensing (Rayner *et al*. 1994, Nualart *et al*. 1999, Lynch *et al*. 2000). Further interesting parallels between beta cell and hypothalamic glucose sensors have been described (Yang *et al*. 1999, Schuit *et al*. 2001). GR neurons not only sense glucose concentrations like beta cells, but their electrical activities are also modulated by important hormones like leptin and insulin (Spanswick *et al*. 1997, 2000, Shiraishi *et al*. 2000). Although the existence of K-ATP channels has been demonstrated in GR neurons, the direct evidence for their involvement in the physiological

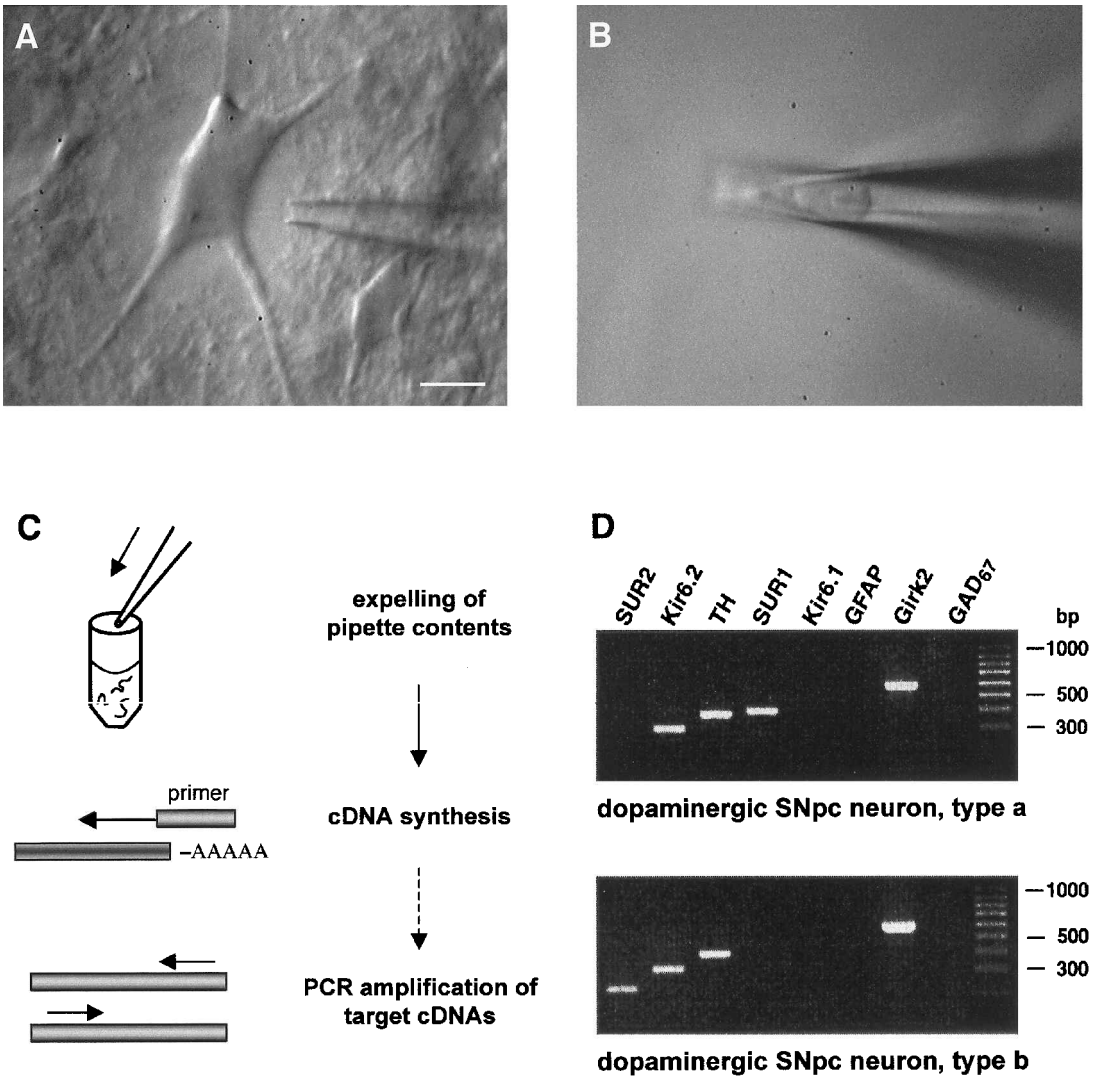

Figure 2. Principle of the single-cell RT-PCR technique to study K-ATP channel subunit expression in individual neurons. (*a*) A dopaminergic neuron in an acute mouse brain slice preparation before patch clamp recording visualized by IR-DIC videomicroscopy (scale bar: 15 μm). (*b*) After electrophysiological characterization, the cytoplasmic contents of the neuron, containing the mRNA and the nucleus, are harvested with the patch pipette without loosing the gigaseal. Picture shows the pipette after aspiration with the nucleus visible inside. (*c*) The contents of the patch pipette are subsequently expelled into a PCR reaction tube containing the contents for reverse transcription of the mRNA. After cDNA synthesis with random hexamer- or oligo-dT-primers, the cDNA is directly used for PCR amplification of target genes. Alternatively, the cDNA pool could be amplified prior to PCR. (*d*) After PCR amplification with gene-specific primers, the single-cell expression profiles are analysed via gelelectrophoresis. Picture shows the multiplex nested PCR results for two dopaminergic SNpc neurons. In addition to the four K-ATP channel subunits, it is probed in parallel for the expression of four markergenes (TH-tyrosine hydroxylase, GFAP-glial fibrillaric acidic protein, Girk2-G-protein coupled inwardly rectifying potassium channel, GAD67-glutamic acidic decarboxylase). A band indicates single-cell expression of the respective gene.

glucose-dependent regulation of excitability of GR neurons was missing. We could, recently, show the complete absence of both, functional K-ATP channels and glucose sensing in VMH neurons in a Kir6.2 knockout mouse (Liss *et al.* 2000). These findings demonstrate that the Kir6.2 subunit of K-ATP channels is not only a necessary molecular component for native VMH plasmamembrane K-ATP channels, it is also essential for glucose sensing in GR neurons. Furthermore, single-cell mRNA expression profiling and quantitative pharmacological data indicate that SUR1, in combination with Kir6.2, builds the native beta cell-like K-ATP channel in GR neurons in the VMH (Miki *et al.* 2001). In contrast to the beta cell, glucose-induced activity in GR neurons does not induce direct secretion of neuronal hormones. It controls, via brainstem nuclei, the release of peripheral hormones, e.g. glucagons, that are mediators of glucose homeostasis (Steffens *et al.* 1990, Levin *et al.* 1999, Miki *et al.* 2001). How many other types of neurons, e.g. in the nucleus tractus solitaris (NTS), are involved in central glucose sensing is unclear, as well as the molecular identities

Table 1. Single-cell RT-PCR expression profiling of K-ATP channel subunits in different neuronal populations.

Brain region	Neuronal type	Single channel conductance	Reference electrophysiology	K-ATP subunit expression	Reference single-cell RT-PCR
Hippocampus (rat)	CA1 pyramidal cells	?	Hyllienmark and Brismar (1996) Fujimura et al. (1997)	SUR1/Kir6.1 SUR2/Kir6.2 SUR1/Kir6.2 SUR2/Kir6.1	Zawar et al. (1997)*
Hippocampus (rat)	Stratum radiatum interneurons	?	Zawar et al. (1997)	SUR1/Kir6.1 SUR2/Kir6.2 SUR1/Kir6.2 SUR2/Kir6.1	Zawar et al. (1997)*
Striatum (rat)	Cholingeric interneurons	44 pS	Lee et al. (1998)	SUR1/Kir6.1	Lee et al. (1998)
Hypothalamus (rat)	Ventromedial hypothalamic neurons	65 and 140 pS	Ashford et al. (1990) Spanswick et al. (1997) Liss et al. (2000)	SUR1/Kir6.1 SUR1/Kir6.2	Lee et al. (1999) Miki et al. (2001)
Substantia nigra pars compacta (mouse, guinea-pig)	Dopaminergic neurons	64 pS	Roeper et al. (1990) Stanford and Lacey (1996)	SUR1/Kir6.2 SUR2B/Kir6.2	Liss et al. (1999a)
Substantia nigra pars reticulata (mouse)	GABAergic neurons	71 pS	Schwanstecher and Panten (1993) Stanford and Lacey (1996)	SUR1/Kir6.2	Liss et al. (1999a)
Brainstem (rat)	Dorsal vagal neurons	73 pS	Trapp and Ballanyi (1995)	SUR1/Kir6.2	Karschin et al. (1998)

Note that three different variants of the single-cell RT-PCR method are used. Liss and Zawar: multiplex nested RT-PCR, Lee: three prime end amplification (TPEA) PCR, Karschin: antisense RNA amplification. *Primers did not discriminate between SUR2A and SUR2B splice variants.

and functional roles of K-ATP channels in these neurons (Dallaporta *et al.* 2000). A different aspect of the important role of K-ATP channels for glucose sensing was illustrated by the recent finding that the Kir6.2 and SUR1 transcription rates are decreased with increasing glucose levels in isolated beta cells (Moritz *et al.* 2001). The glucose-dependent regulation of K-ATP subunit expression might also be operative in the brain, as neurons and beta cells possess the same K-ATP subunit promotor (Hernandez-Sanchez *et al.* 1999). In addition to central neurons, peripheral enteric neurons have also been shown to be glucose sensing and to express the K-ATP channel subunits SUR1 and Kir6.2 (Liu *et al.* 1999).

Neuronal K-ATP channels as metabolic gatekeepers?

As mentioned above, most neuronal populations are not directly involved in beta cell-like glucose sensing. Nevertheless, many of them express functional K-ATP channels. It is likely that these neuronal K-ATP channels act as general energy control elements that adapt the electrical activity and consequently neuronal ATP consumption to the delicate metabolic state of neurons. Increased electrical activity will lead to internal sodium accumulation that stimulates Na^+/K^+ ATPase activity, which is by far the most dominant consumer (40–70%) of neuronal ATP (Erecinska and Dagani 1990). K-ATP channels that have been found on axonal, terminal and somatodendritc membranes, are ideal sensors to detect locally-enhanced activity-dependent changes in ATP/ADP ratio and translate them into an altered channel activity. The activity-dependent activation of K-ATP channels will tend to hyperpolarize the membrane and dampen excitability. Thus, K-ATP channel activation will act as negative feedback in order to match electrical signalling and energy consumption. Supportive of this notion is the evidence that some K-ATP channels are active *in vivo* in the brain under physiological conditions (Fellows *et al.* 1993). In accordance, intracellular sodium loading and increased Na^+/K^+ ATPase activity have been shown to enhance neuronal K-ATP channel activity (Seutin *et al.* 1996). These mechanisms might also be operative at presynaptic membranes that are compartments of high metabolic demands. Here, K-ATP channel activation will reduce neurotransmitter release, which has been demonstrated in particular for GABAergic neurotransmission (Amoroso *et al.* 1990, Mourre *et al.* 1990, Luhmann and Heinemann 1992, Watts *et al.* 1995). Recent evidence suggests that this metabolic tuning of neuronal activity is not simply a homeostatic 'safety' device, but might control higher functions like memory, learning or voluntary movement (Lamensdorf *et al.* 1999, Stefani *et al.* 1999). There is also another possible mechanism to be taken into account. Activity-dependent K-ATP channel activation not only increases membrane conductance, but also leads to enhanced activity-dependent K^+ efflux that rapidly accumulates in the small extracellular space of the CNS (Laming 2000). These activity-dependent changes in external K^+ concentration might serve as coupling between local metabolic demands and cerebrovascular perfusion, as mild increases in external K^+ are potent inducers of local vasodilatation (Quayle *et al.* 1997, Nguyen *et al.* 2000). In this way, K-ATP channel activity might provide two feedback loops: a cellular one that controls the excitability of individual neurons or synapses and a regional one that uses K^+ fluxes as readout to match regional electrical activity with local blood flow. There are

conflicting results in the literature, whether neuronal K-ATP channels are involved in this type of signalling or not (Schaeffer and Lazdunski 1991, Jiang *et al.* 1992, Fellows *et al.* 1993, Zetterstrom *et al.* 1995). In this context, K-ATP channels in dopaminergic neurons might have an additional role, as 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 (Krimer *et al.* 1998). There is increasing evidence that neuronal K-ATP channels are not just pure metabolic sensors, but that they can also serve as effectors for other biochemical pathways and second messenger systems, like G-proteins, PIP cascades, and proteinkinase-mediated phosphorylations. This has already been appreciated for native peripheral and also recombinant heterologously expressed K-ATP channels (Sanchez *et al.* 1998, Baukrowitz and Fakler 2000, Lin *et al.* 2000, Wada *et al.* 2000). Neuronal K-ATP channels can be modulated by coupling to metabotropic receptors, cytoskeletal changes and other mechanisms (Tanaka *et al.* 1996, Hosseinzadeh and Stone 1998, Mironov and Richter 2000). Thus, K-ATP channels act as integrators of multiple signalling pathways that converge on these channels.

Neuronal K-ATP channels and disease

Role of neuronal K-ATP channels in acute ischemia/hypoxia

With respect to acute or chronic perturbations of energy metabolism, the brain is by far the most vulnerable organ. A few minutes of hypoxia or ischemia can lead to irreparable structural and functional loss. Conceptionally, K-ATP channels are attractive targets in the pathophysiological scenario of hypoxia/ischemia, not only in the heart but also in the brain. The initiation and execution of hypoxic/ischemic neuronal death is believed to critically depend on excessive glutamate release and subsequent excitotoxicity (Dirnagl *et al.* 1999). It has been argued that in these acute scenarios, K-ATP channel activation might reduce terminal depolarization and, thus, decrease ischemic glutamate release. In addition, somatodendritic K-ATP channel activation might diminish the glutamate-induced calcium overload and initiation of necrotic or apoptotic cell death (Haddad and Jiang 1994). On the other hand, opening of K-ATP channels by ischemia will accelerate the external K^+ accumulation, which will shift the equilibrium potential of potassium to more positive values, resulting in membrane depolarization and, finally, collapse of ionic gradients. In this context, its is noteworthy that organisms with a high brain-hypoxia tolerance, like deep-diving turtles or new-borns, have a very low density of neuronal K-ATP channels compared to highly vulnerable adult mammalian brains (Jiang *et al.* 1992). Apparently, there is an inevitable trade-off between protecting the integrity of the membrane potential and at the same time accelerating external K^+ accumulation by active K-ATP channels during metabolic challenges (Cameron and Baghdady 1994). However, in rats, K-ATP channel activating drugs have been shown to reduce the infarct volume in a middle cerebral artery occlusion paradigm (Takaba *et al.* 1997). This indicates that activation or overexpression of K-ATP channels can not only be neuroprotective in *in vitro* model systems (Wind *et al.* 1997, Reshef *et al.* 1998, Jovanovic *et al.* 1999), but also appears to be beneficial in *in vivo* focal cerebral ischemia. As K^+ channel openers might also act on vascular K-ATP channels, the specific role of neuronal K-ATP channels in the pathophysiology of acute ischemia needs to be further clarified. It is likely that the best evidence will come from cell-type specific transgenic mouse models. Recent interest focused on the possible contribution of K-ATP channels to a phenomenon called ischemic preconditioning that is studied predominantly in the heart (Cohen *et al.* 2000), but is also present in the brain (Cheung 2000). Here, a short and fully reversible hypoxic or ischemic insult precedes the genuine hypoxia/ischemia test period and induces a set of protective adaptive changes that increases the hypoxic tolerance and speed of recovery for the second challenge. There is increasing evidence that the opening of K-ATP channels could play a central role either as trigger or effector of this protective preconditioning in the brain (Perez-Pinzon and Born 1999, Blondeau *et al.* 2000). Protein kinase C-mediated phosphorylation of K-ATP channels and a subsequent increase in open probability is one of the suggested molecular mechanisms (Wang and Ashraf 1999, Light *et al.* 2000). More work is needed to understand the mechanisms of preconditioning. Deeper insight in the respective roles of plasmamembrane and mitochondrial K-ATP channels in preconditioning could have import clinical implications for neuroprotective therapies.

K-ATP channels in neurodegenerative disease

It has been demonstrated for a variety of brain regions that metabolic inhibition of glycolysis or the mitochondrial respiratory chain act as potent activators of neuronal K-ATP channels (Riepe *et al.* 1992, Roper and Ashcroft 1995, Hyllienmark and Brismar 1996, Ballanyi and Kulik 1998, Koyama *et al.* 1999, Sun *et al.* 2000, Zawar and Neumcke 2000). This is not only relevant for acute metabolic challenges, as discussed above, but also in the more chronic settings of neurodegenerative disorders like Alzheimer's or Parkinson's disease (PD). Chronic impairment of mitochondrial function has been identified in these diseases and is believed to play a crucial role in their pathogenesis (Beal 2000). PD is a common neurodegenerative movement disorder that affects more than 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). Recent work suggests a potential role for K-ATP channel subtypes expressed in dopaminergic neurons of the SNpc for their selective vulnerability in PD. Dopaminergic midbrain neurons play a crucial role in a variety of brain functions like reward, working memory and voluntary movement. The latter is severely affected in PD. However, whilst some subpopulations of dopaminergic neurons are highly vulnerable to the disease process, others remain largely unaffected (Damier *et al.* 1999). This suggests the presence of relevant differences in gene expression that determine the differential vulnerability of single dopaminergic neurons. 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 – NADH ubichinone reductase) of ~40% has been consistently found in PD patients (Beal 2000). However, the pathophysiological downstream events that are induced by CXI inhibition and lead to the selective dopaminergic degeneration are still unclear. In this context, K-ATP channels in DA neurons might act as candidate targets of the chronic metabolic disturbance present in PD. 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 (Roper and Ashcroft 1995, Liss *et al*. 1999a). The activation of K-ATP channels in response to metabolic inhibition leads to an hyperpolarization of the dopaminergic neurons, accompanied by a complete loss of their normal pacemaker activity. 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 the mitochondrial CXI by rotenone activates K-ATP channels only in a subpopulation of ~40% of dopaminergic neurons with a half maximal effective concentration (EC_{50}) of 15 nM. This corresponds to a degree of CXI inhibition similar to that found in brains of PD patients (Beal 1999) and suggests the possibility of K-ATP channel activation in PD. Combined single-cell RT-PCR experiments demonstrated that these highly responsive dopaminergic neurons express the K-ATP channel subunits SUR1 and Kir6.2 (see Figure 2D, upper panel). In contrast, the other population of dopaminergic SNpc neurons possess a more than 100 fold higher EC_{50} (2 μM) for rotenone-induced K-ATP channel activation—indicative of a significantly lower sensitivity to CXI inhibition. Theses neurons, which maintain their pacemaker activity during partial CXI inhibition, express the other SUR isoform, SUR2B, in combination with Kir6.2 mRNA (see Figure 2D) (Liss *et al*. 1999a). By analogy, the activity of SUR2B-expressing dopaminergic neurons might not be perturbed in PD. 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 to SUR2A/Kir6.2 mediated channels (Ashcroft and Gribble 1998). This demonstrates that the alternative expression of SUR subunits is a major determinant of metabolic sensitivity of K-ATP channels.

K-ATP channels in the weaver mouse—a genetic model of dopaminergic degeneration

Studies of dopaminergic mid-brain neurons in the *weaver* mouse, a genetic mouse model of dopaminergic degeneration similar to that in PD (Patil *et al*. 1995), 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 homomers, the mutated GIRK2 channel lost its potassium selectivity and can be constitutively active, resulting in a chronic, depolarizing sodium influx into dopaminergic SNpc neurons (Kofuji *et al*. 1996, Navarro *et al*. 1996, Slesinger *et al*. 1996, Liss *et al*. 1999b, Guatteo *et al*. 2000). The chronic sodium overload is expected to further enhance ATP consumption of the Na^+/K^+ ATPase, which is already a major single consumer of neuronal ATP. It was demonstrated, for dopaminergic neurons in homozygous *weaver*, that SUR1/Kir6.2 containing K-ATP channels are tonically activated in direct consequence to the activity of *weaver* GIRK2 channels. This partially compensates the chronic depolarization of the dopaminergic weaver neurons. Single-cell RT-PCR experiments revealed the complete absence of SUR2B expressing dopaminergic neurons in *weaver* (Liss *et al*. 1999a, b). These results give the first evidence that alternative SUR1 expression and activation of K-ATP channels can affect the process of dopaminergic neurodegeneration. Furthermore, preconditioning might also be a relevant mechanism reducing the vulnerability of dopaminergic neurons towards neurotoxins like MPTP (Duan and Mattson 1999). In summary, there is growing evidence that the differential expression of K-ATP channel subunits in DA neurons not only determines the acute response to metabolic stress, but might also be an important molecular mechanism for their differential vulnerability in chronic neurodegenerative disorders.

To explicitly test the potential roles of molecularly defined K-ATP channels in the brain, as reviewed in this article, one need to combine existing electrophysiological and molecular methods with a new generation of cell-type specific transgenic mouse models of K-ATP channel function.

Acknowledgements

This work was supported by the Medical Research Council and the Deutsche Forschungsgemeinschaft. B.L. was a Blaschko Visiting Research Scholar and is a Todd-Bird Junior Research Fellow at New College. J.R. is the Monsanto Senior Research Fellow at Exeter College.

References

Aguilar-Bryan, L., Clement, J. P., Gonzalez, G., Kunjilwar, K., Babenko, A. and Bryan, J., 1998, Toward understanding the assembly and structure of K-ATP channels. *Physiology Review*, **78**, 227 – 245.

Aguilar-Bryan, L., Nichols, C. G., Wechsler, S. W., Clement, J. P. T., Boyd, A. E., Gonzalez, G., Herrera-Sosa, H., Nguy, K., Bryan, J. and Nelson, D. A., 1995, Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion [see comments]. *Science*, **268**, 423 – 426.

Amoroso, S., Schmid-Antomarchi, H., Fosset, M. and Lazdunski, M., 1990, Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K^+ channels. *Science*, **247**, 852 – 854.

Ashcroft, F. M., 1988, Adenosine 5'-triphosphate-sensitive potassium channels. *Annual Review of Neuroscience*, **11**, 97 – 118.

Ashcroft, F. M. and Gribble, F. M., 1999, ATP-sensitive K^+ channels and insulin secretion: their role in health and disease. *Diabetologia*, **42**, 903 – 919.

Ashcroft, F. M. and Gribble, F. M., 2000, New windows on the mechanism of action of K(ATP) channel openers. [In Process Citation]. *Trends in Pharmacology Science*, **21**, 439 – 445.

Ashford, M. L., Boden, P. R. and Treherne, J. M., 1990, Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K^+ channels. *Pflugers Archives*, **415**, 479 – 483.

Ballanyi, K. and Kulik, A., 1998, Intracellular $Ca2^+$ during metabolic activation of K-ATP channels in spontaneously active dorsal vagal neurons in medullary slices. *European Journal of Neuroscience*, **10**, 2574–2585.

Baukrowitz, T. and Fakler, B., 2000, K(ATP) channels: linker between phospholipid metabolism and excitability. *Biochemical Pharmacology*, **60**,735–740.

Baukrowitz, T., Schulte, U., Oliver, D., Herlitze, S., Krauter, T., Tucker, S. J., Ruppersberg, J. P. and Fakler, B., 1998, PIP2 and PIP as determinants for ATP inhibition of K-ATP channels [see comments]. *Science*, **282**, 1141–1144.

Beal, M. F., 1999, Mitochondria, NO and neurodegeneration. *Biochemical Society Symposium*, **66**, 43–54.

Beal, M. F., 2000, Energetics in the pathogenesis of neurodegenerative diseases. *Trends in Neuroscience*, **23**, 298–304.

Blondeau, N., Plamondon, H., Richelme, C., Heurteaux, C. and Lazdunski, M., 2000, K(ATP) channel openers, adenosine agonists and epileptic preconditioning are stress signals inducing hippocampal neuroprotection [In Process Citation]. *Neuroscience*, **100**, 465–474.

Bryan, J. and Aguilar-Bryan, L., 1999, Sulfonylurea receptors: ABC transporters that regulate ATP-sensitive K(+) channels. *Biochimica et Biophysica Acta*, **1461**, 285–303.

Cameron, J. S. and Baghdady, R., 1994, Role of ATP sensitive potassium channels in long term adaptation to metabolic stress. *Cardiovascular Research*, **28**, 788–796.

Cheung, R. T., 2000, Ischemic preconditioning and tolerance in human brain [letter]. *Stroke*, **31**, 2266–2278.

Chutkow, W. A., Makielski, J. C., Nelson, D. J., Burant, C. F. and Fan, Z., 1999, Alternative splicing of sur2 Exon 17 regulates nucleotide sensitivity of the ATP-sensitive potassium channel. *Journal of Biological Chemistry*, **274**, 13656–13665.

Chutkow, W. A., Simon, M. C., Le Beau, M. M. and Burant, C. F., 1996, Cloning, tissue expression, and chromosomal localization of SUR2, the putative drug-binding subunit of cardiac, skeletal muscle, and vascular K-ATP channels. *Diabetes*, **45**, 1439–1445.

Clement, J. P. T., Kunjilwar, K., Gonzalez, G., Schwanstecher, M., Panten, U., Aguilar-Bryan, L. and Bryan, J., 1997, Association and stoichiometry of K(ATP) channel subunits. *Neuron*, **18**, 827–838.

Cohen, M. V., Baines, C. P. and Downey, J. M., 2000, Ischemic preconditioning: from adenosine receptor of K-ATP channel. *Annual Review of Physiology*, **62**, 79–109.

Dallaporta, M., Perrin, J. and Orsini, J. C., 2000, Involvement of adenosine triphosphate-sensitive K^+ channels in glucose-sensing in the rat solitary tract nucleus. *Neuroscience Letters*, **278**, 77–80.

Damier, P., Hirsch, E. C., Agid, Y. and Graybiel, A M., 1999, The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease [see comments]. *Brain*, **122**, 1437–1448.

Dirnagl, U., Iadecola, C. and Moskowitz, M. A., 1999, Pathobiology of ischaemic stroke: an integrated view. *Trends in Neuroscience*, **22**, 391–397.

Dixon, A. K., Richardson, P. J., Lee, K., Carter, N. P. and Freeman, T. C., 1998, Expression profiling of single cells using 3 prime end amplification (TPEA) PCR. *Nucleic Acids Research*, **26**, 4426–4431.

Duan, W. and Mattson, M. P., 1999, Dietary restriction and 2-deoxyglucose administration improve behavioral outcome and reduce degeneration of dopaminergic neurons in models of Parkinson's disease. *Journal of Neuroscience Research*, **57**, 195–206.

Dunn-Meynell, A. A., Rawson, N. E. and Levin, B. E., 1998, Distribution and phenotype of neurons containing the ATP-sensitive K^+ channel in rat brain. *Brain Research*, **814**, 41–54.

Dunn-Meynell, A. A., Routh, V. H., McArdle, J. J. and Levin, B. E., 1997, Low-affinity sulfonylurea binding sites reside on neuronal cell bodies in the brain. *Brain Research*, **745**, 1–9.

Eberwine, J., Yeh, H., Miyashiro, K., Cao, Y., Nair, S., Finnell, R., Zettel, M. and Coleman, P., 1992, Analysis of gene expression in single live neurons. *Proceedings of the National Academy of Sciences (USA)*, **89**, 3010–3014.

Erecinska, M. and Dagani, F., 1990, Relationships between the neuronal sodium/potassium pump and energy metabolism. Effects of K^+, Na^+, and adenosine triphosphate in isolated brain synaptosomes. *Journal of Genetic Physiology*, **95**, 591–616.

Eto, K., Tsubamoto, Y., Terauchi, Y., Sugiyama, T., Kishimoto, T., Takahashi, N., Yamauchi, N., Kubota, N., Murayama, S., Aizawa, T., Akanuma, Y., Aizawa, S., Kasai, H., Yazaki, Y. and Kadowaki, T., 1999, Role of NADH shuttle system in glucose-induced activation of mitochondrial metabolism and insulin secretion [see comments]. *Science*, **283**, 981–985.

Fellows, L. K., Boutelle, M. G. and Fillenz, M., 1993, ATP-sensitive potassium channels and local energy demands in the rat hippocampus: an in vivo study. *Journal of Neurochemistry*, **61**, 949–954.

Finta, E. P., Harms, L., Sevcik, J., Fischer, H. D. and Illes, P., 1993, Effects of potassium channel openers and their antagonists on rat locus coeruleus neurones. *British Journal of Pharmacology*, **109**, 308–315.

Fujimura, N., Tanaka, E., Yamamoto, S., Shigemori, M. and Higashi, H., 1997, Contribution of ATP-sensitive potassium channels to hypoxic hyperpolarization in rat hippocampal CA1 neurons *in vitro*. *Journal of Neurophysiology*, **77**, 378–385.

Gribble, F. M., Tucker, S. J. and Ashcroft, F. M., 1997, The interaction of nucleotides with the tolbutamide block of cloned ATP-sensitive K^+ channel currents expressed in Xenopus oocytes: a reinterpretation. *Journal of Physiology (London)*, **504**, 35–45.

Guatteo, E., Federici, M., Siniscalchi, A., Knopfel, T., Mercuri, N. B. and Bernardi, G., 1998, Whole cell patch-clamp recordings of rat midbrain dopaminergic neurons isolate a sulphonylurea- and ATP-sensitive component of potassium currents activated by hypoxia. *Journal of Neurophysiology*, **79**, 1239–1245.

Guatteo, E., Fusco, F. R., Giacomini, P., Bernardi, G. and Mercuri, N. B., 2000, The weaver mutation reverses the function of dopamine and GABA in mouse dopaminergic neurons. *Journal of Neuroscience*, **20**, 6013–6020.

Haddad, G. G. and Jiang, C., 1994, Mechanisms of neuronal survival during hypoxia: ATP-sensitive K^+ channels. *Biology Neonate*, **65**, 160–165.

Hernandez-Sanchez, C., Ito, Y., Ferrer, J., Reitman, M. and LeRoith, D., 1999, Characterization of the mouse sulfonylurea receptor 1 promoter and its regulation. *Journal of Biological Chemistry*, **274**, 18261–18270.

Heron, L., Virsolvy, A., Peyrollier, K., Gribble, F. M., Le Cam, A., Ashcroft, F. M. and Bataille, D., 1998, Human alpha-endosulfine, a possible regulator of sulfonylurea-sensitive K-ATP channel: molecular cloning, expression and biological properties. *Proceedings of the National Academy of Sciences (USA)*, **95**, 8387–8391.

Hicks, G. A., Hudson, A. L. and Henderson, G., 1994, Localization of high affinity [3H]glibenclamide binding sites within the substantia nigra zona reticulata of the rat brain. *Neuroscience*, **61**, 285–292.

Higgins, C. F., 1995, The ABC of channel regulation. *Cell*, **82**, 693–696.

Hosseinzadeh, H. and Stone, T. W., 1998, Tolbutamide blocks postsynaptic but not presynaptic effects of adenosine on hippocampal CA1 neurones. *Journal of Neural Transmission*, **105**, 161–172.

Hyllienmark, L. and Brismar, T., 1996, Effect of metabolic inhibition on K^+ channels in pyramidal cells of the hippocampal CA1 region in rat brain slices. *Journal of Physiology (London)*, **496**, 155–164.

Illes, P., Sevcik, J., Finta, E. P., Frohlich, R., Nieber, K. and Norenberg, W., 1994, Modulation of locus coeruleus neurons by extra- and intracellular adenosine 5′-triphosphate. *Brain Research Bulletin*, **35**, 513–519.

Inagaki, N., Gonoi, T., Clement, J. P. T., Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S. and Bryan, J., 1995a, Reconstitution of IK-ATP: an inward rectifier subunit plus the sulfonylurea receptor [see comments]. *Science*, **270**, 1166–1170.

Inagaki, N., Gonoi, T., Clement, J. P., Wang, C. Z., Aguilar-Bryan, L., Bryan, J. and Seino, S., 1996, A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron*, **16**, 1011–1017.

Inagaki, N., Gonoi, T. and Seino, S., 1997, Subunit stoichiometry of the pancreatic beta-cell ATP-sensitive K^+ channel. *FEBS Letters*, **409**, 232–236.

Inagaki, N., Inazawa, J. and Seino, S., 1995b, cDNA sequence, gene structure, and chromosomal localization of the human ATP-sensitive potassium channel, uK-ATP-1, gene (KCNJ8). *Genomics*, **30**, 102–104.

Inagaki, N., Tsuura, Y., Namba, N., Masuda, K., Gonoi, T., Horie, M., Seino, Y., Mizuta, M. and Seino, S., 1995c, Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart. *Journal of Biological Chemistry*, **270**, 5691–5694.

Inoue, I., Nagase, H., Kishi, K. and Higuti, T., 1991, ATP-sensitive K^+ channel in the mitochondrial inner membrane. *Nature*, **352**, 244–247.

Isomoto, S., Kondo, C., Yamada, M., Matsumoto, S., Higashiguchi, O., Horio, Y., Matsuzawa, Y. and Kurachi, Y., 1996, A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. *Journal of Biological Chemistry*, **271**, 24321–24324.

Jiang, C., Xia, Y. and Haddad, G. G., 1992, Role of ATP-sensitive K^+ channels during anoxia: major differences between rat (newborn and adult) and turtle neurons. *Journal of Physiology (London)*, **448**, 599–612.

Jovanovic, N., Jovanovic, S., Jovanovic, A. and Terzic, A., 1999, Gene delivery of Kir6.2/SUR2A in conjunction with pinacidil handles intracellular $Ca2^+$ homeostasis under metabolic stress. *Faseb Journal*, **13**, 923–929.

Karschin, A., Brockhaus, J. and Ballanyi, K., 1998, K-ATP channel formation by the sulphonylurea receptors SUR1 with Kir6.2 subunits in rat dorsal vagal neurons *in situ*. *Journal of Physiology (London)*, **509**, 339–346.

Karschin, C., Ecke, C., Ashcroft, F. M. and Karschin, A., 1997, Overlapping distribution of K(ATP) channel-forming Kir6.2 subunit and the sulfonylurea receptor SUR1 in rodent brain. *FEBS Letters*, **401**, 59–64.

Kofuji, P., Hofer, M., Millen, K. J., Millonig, J. H., Davidson, N., Lester, H. A. and Hatten, M. E., 1996, Functional analysis of the weaver mutant GIRK2 K^+ channel and rescue of weaver granule cells. *Neuron*, **16**, 941–952.

Koster, J. C., Marshall, B. A., Ensor, N., Corbett, J. A. and Nichols, C. G., 2000, Targeted overactivity of beta cell K(ATP) channels induces profound neonatal diabetes. *Cell*, **100**, 645–654.

Koyama, S., Jin, Y. H. and Akaike, N., 1999, ATP-sensitive and $Ca2^+$-activated K^+ channel activities in the rat locus coeruleus neurons during metabolic inhibition. *Brain Research*, **828**, 189–192.

Krimer, L. S., Muly, E. C., Williams, G. V. and Goldman-Rakic, P. S., 1998, Dopaminergic regulation of cerebral cortical microcirculation [see comments]. *National Neuroscience*, **1**, 286–289.

Lambolez, B., Audinat, E., Bochet, P., Crepel, F. and Rossier, J., 1992, AMPA receptor subunits expressed by single Purkinje cells. *Neuron*, **9**, 247–258.

Lamensdorf, I., Meiri, N., Harvey-White, J., Jacobowitz, D. M. and Kopin, I. J., 1999, Kir6.2 oligoantisense administered into the globus pallidus reduces apomorphine-induced turning in 6-OHDA hemiparkinsonian rats. *Brain Research*, **818**, 275–284.

Laming, P. R., 2000, Potassium signalling in the brain: its role in behaviour. *Neurochemistry International*, **36**, 271–290.

Lee, K., Dixon, A. K., Freeman, T. C. and Richardson, P. J., 1998, Identification of an ATP-sensitive potassium channel current in rat striatal cholinergic interneurones. *Journal of Physiology (London)*, **510**, 441–453.

Lee, K., Dixon, A. K., Richardson, P. J. and Pinnock, R. D., 1999, Glucose-receptive neurones in the rat ventromedial hypothalamus express K-ATP channels composed of Kir6.1 and SUR1 subunits. *Journal of Physiology (London)*, **515**, 439–452.

Levin, B. E., Dunn-Meynell, A. A. and Routh, V. H., 1999, Brain glucose sensing and body energy homeostasis: role in obesity and diabetes. *American Journal of Physiology*, **276**, R1223–R1231.

Light, P. E., Bladen, C., Winkfein, R. J., Walsh, M. P. and French, R. J., 2000, Molecular basis of protein kinase C-induced activation of ATP-sensitive potassium channels. *Proceedings of the National Academy of Sciences (USA)*, **97**, 9058–9063.

Lin, Y. F., Jan, Y. N. and Jan, L. Y., 2000, Regulation of ATP-sensitive potassium channel function by protein kinase A-mediated phosphorylation in transfected HEK293 cells. *Embo Journal*, **19**, 942–955.

Liss, B., Bruns, R. and Roeper, J., 1999a, Alternative sulfonylurea receptor expression defines metabolic sensitivity of K-ATP channels in dopaminergic midbrain neurons. *Embo Journal*, **18**, 833–846.

Liss, B., Miki, T., Seino, S. and Roeper, J., 2000, Kir6.2-mediated K-ATP channels are an essential component for glucose sensing in mouse ventromedial hypothalamic neurons. *Journal of Physiology (London)*, **527**, 114P–115P.

Liss, B., Neu, A. and Roeper, J., 1999b, The weaver mouse gain-of-function phenotype of dopaminergic midbrain neurons is determined by coactivation of wvGirk2 and K-ATP channels. *Journal of Neuroscience*, **19**, 8839–8848.

Liu, M., Seino, S. and Kirchgessner, A. L., 1999, Identification and characterization of glucoresponsive neurons in the enteric nervous system. *Journal of Neuroscience*, **19**, 10305–10317.

Luhmann, H. J. and Heinemann, U., 1992, Hypoxia-induced functional alterations in adult rat neocortex. *Journal of Neurophysiology*, **67**, 798–811.

Lynch, R. M., Tompkins, L. S., Brooks, H. L., Dunn-Meynell, A. A. and Levin, B. E., 2000, Localization of glucokinase gene expression in the rat brain. *Diabetes*, **49**, 693–700.

Miki, T., Liss, B., Minami, K., Shiuchi, T., Saraya, A., Horiuchi, M., Ashcroft, F. M., Minokoshi, Y., Kashima, Y., Roeper, J. and Seino, S., 2001, ATP-sensitive potassium channels in ventromedial hypothalamic neurons play an essential role in the maintenance of glucose homeostasis, by controlling glucagon release and food intake. *Nature Neuroscience*, in press.

Miki, T., Nagashima, K., Tashiro, F., Kotake, K., Yoshitomi, H., Tamamoto, A., Gonoi, T., Iwanaga, T., Miyazaki, J. and Seino, S., 1998, Defective insulin secretion and enhanced insulin action in K-ATP channel-deficient mice. *Proceedings of the National Academy of Sciences (USA)*, **95**, 10402–10406.

Miki, T., Tashiro, F., Iwanaga, T., Nagashima, K., Yoshitomi, H., Aihara, H., Nitta, Y., Gonoi, T., Inagaki, N., Miyazaki, J. and Seino, S., 1997, Abnormalities of pancreatic islets by targeted expression of a dominant-negative K-ATP channel. *Proceedings of the National Academy of Sciences (USA)*, **94**, 11969–11973.

Minami, T., Oomura, Y. and Sugimori, M., 1986, Electrophysiological properties and glucose responsiveness of guinea-pig ventromedial hypothalamic neurones in vitro. *Journal of Physiology (London)*, **380**, 127–143.

Mironov, S. L. and Richter, D. W., 2000, Intracellular signalling pathways modulate K(ATP) channels in inspiratory brainstem neurones and their hypoxic activation: involvement of metabotropic receptors, G-proteins and cytoskeleton. *Brain Research*, **853**, 60–67.

Mironova, G. D., Skarga, Y. Y., Grigoriev, S. M., Negoda, A. E., Kolomytkin, O. V. and Marinov, B. S., 1999, Reconstitution of the mitochondrial ATP-dependent potassium channel into bilayer lipid membrane. *Journal of Bioenergy and Biomembranes*, **31**, 159–163.

Monyer, H. and Lambolez, B., 1995, Molecular biology and physiology at the single-cell level. *Current Opinions in Neurobiology*, **5**, 382–387.

Moritz, W., Leech, C. A., Ferrer, J. and Habener, J. F., 2001, Regulated expression of adenosine triphosphate-sensitive potassium channel subunits in pancreatic ss-cells. *Endocrinology*, **142**, 129–138.

Mourre, C., Smith, M. L., Siesjo, B. K. and Lazdunski, M., 1990, Brain ischemia alters the density of binding sites for glibenclamide, a specific blocker of ATP-sensitive K^+ channels. *Brain Research*, **526**, 147–152.

Nasonkin, I., Alikasifoglu, A., Ambrose, C., Cahill, P., Cheng, M., Sarniak, A., Egan, M. and Thomas, P. M., 1999, A novel sulfonylurea receptor family member expressed in the embryonic Drosophila dorsal vessel and tracheal system. *Journal of Biological Chemistry*, **274**, 29420–29425.

Navarro, B., Kennedy, M. E., Velimirovic, B., Bhat, D., Peterson, A. S. and Clapham, D. E., 1996, Nonselective and G betagamma-insensitive weaver K^+ channels. *Science*, **272**, 1950–1953.

Nguyen, T. S., Winn, H. R. and Janigro, D., 2000, ATP-sensitive potassium channels may participate in the coupling of neuronal activity and cerebrovascular tone. *American Journal of Physiology and Heart Circulatory Physiology*, **278**, H878–H885.

Noma, A., 1983, ATP-regulated K^+ channels in cardiac muscle. *Nature*, **305**, 147–148.

Nualart, F., Godoy, A. and Reinicke, K., 1999, Expression of the hexose transporters GLUT1 and GLUT2 during the early development of the human brain. *Brain Research*, **824**, 97–104.

Ohno-Shosaku, T. and Yamamoto, C., 1992, Identification of an ATP-sensitive K^+ channel in rat cultured cortical neurons. *Pflugers Archives*, **422**, 260–266.

Okuyama, Y., Yamada, M., Kondo, C., Satoh, E., Isomoto, S., Shindo, T., Horio, Y., Kitakaze, M., Hori, M. and Kurachi, Y., 1998, The effects of nucleotides and potassium channel openers on the SUR2A/Kir6.2 complex K^+ channel expressed in a mammalian cell line, HEK293T cells. *Pflugers Archives*, **435**, 595–603.

Patil, N., Cox, D. R., Bhat, D., Faham, M., Myers, R. M. and Peterson, A. S., 1995, A potassium channel mutation in weaver mice implicates membrane excitability in granule cell differentiation [see comments]. *National Genetics*, **11**, 126–129.

Pelletier, M. R., Pahapill, P. A., Pennefather, P. S. and Carlen, P. L., 2000, Analysis of single K(ATP) channels in mammalian dentate gyrus granule cells. *Journal of Neurophysiology*, **84**, 2291–2301.

Perez-Pinzon, M. A. and Born, J. G., 1999, Rapid preconditioning neuroprotection following anoxia in hippocampal slices: role of the K^+ ATP channel and protein kinase C. *Neuroscience*, **89**, 453–459.

Quayle, J. M., Nelson, M. T. and Standen, N. B., 1997, ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiology Review*, **77**, 1165–1232.

Rayner, D. V., Thomas, M. E. and Trayhurn, P., 1994, Glucose transporters (GLUTs 1-4) and their mRNAs in regions of the rat brain: insulin-sensitive transporter expression in the cerebellum [published erratum appears in *Canadian Journal of Physiology and Pharmacology*, 1994, **72**, 1098]. *Canadian Journal of Physiology and Pharmacology*, **72**, 476–479.

Reimann, F. and Ashcroft, F. M., 1999, Inwardly rectifying potassium channels. *Current Opinions in Cell Biology*, **11**, 503–508.

Reshef, A., Sperling, O. and Zoref-Shani, E., 1998, Opening of ATP-sensitive potassium channels by cromakalim confers tolerance against chemical ischemia in rat neuronal cultures. *Neuroscience Letters*, **250**, 111–114.

Riepe, M., Hori, N., Ludolph, A. C., Carpenter, D. O., Spencer, P. S. and Allen, C. N., 1992, Inhibition of energy metabolism by 3-nitropropionic acid activates ATP-sensitive potassium channels. *Brain Research*, **586**, 61–66.

Roeper, J., Hainsworth, A. H. and Ashcroft, F. M., 1990, Activation of ATP-sensitive K channels by hypoglycaemia or metabolic inhibition in guinea-pig isolated substantia nigra neurones. *Journal of Physiology (London)*, **430**, P130.

Roper, J. and Ashcroft, F. M., 1995, Metabolic inhibition and low internal ATP activate K-ATP channels in rat dopaminergic substantia nigra neurones. *Pflugers Archives*, **430**, 44–54.

Sakura, H., Ammala, C., Smith, P. A., Gribble, F. M. and Ashcroft, F. M., 1995, Cloning and functional expression of the cDNA encoding a novel ATP-sensitive potassium channel subunit expressed in pancreatic beta-cells, brain, heart and skeletal muscle. *FEBS Letters*, **377**, 338–344.

Sanchez, J. A., Gonoi, T., Inagaki, N., Katada, T. and Seino, S., 1998, Modulation of reconstituted ATP-sensitive $K(^+)$-channels by GTP-binding proteins in a mammalian cell line. *Journal of Physiology (London)*, **507**, 315–324.

Schaeffer, P. and Lazdunski, M., 1991, K^+ efflux pathways and neurotransmitter release associated to hippocampal ischemia: effects of glucose and of K^+ channel blockers. *Brain Research*, **539**, 155–158.

Schuit, F. C., Huypens, P., Heimberg, H. and Pipeleers, D. G., 2001, Glucose sensing in pancreatic beta-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. [In Process Citation]. *Diabetes*, **50**, 1–11.

Schwanstecher, C. and Bassen, D., 1997, K-ATP-channel on the somata of spiny neurones in rat caudate nucleus: regulation by drugs and nucleotides. *British Journal of Pharmacology*, **121**, 193–198.

Schwanstecher, C. and Panten, U., 1993, Tolbutamide- and diazoxide-sensitive K^+ channel in neurons of substantia nigra pars reticulata. *Naunyn Schmiedebergs Archives of Pharmacology*, **348**, 113–117.

Schwanstecher, C. and Panten, U., 1994, Identification of an ATP-sensitive K^+ channel in spiny neurons of rat caudate nucleus. *Pflugers Archives*, **427**, 187–189.

Seghers, V., Nakazaki, M., DeMayo, F., Aguilar-Bryan, L. and Bryan, J., 2000, Sur1 knockout mice. A model for K(ATP) channel-independent regulation of insulin secretion. *Journal of Biological Chemistry*, **275**, 9270–9277.

Seino, S., 1999, ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. *Annual Review of 61*, 337–362.

Seino, S., Iwanaga, T., Nagashima, K. and Miki, T., 2000, Diverse roles of K(ATP) channels learned from Kir6.2 genetically engineered mice. *Diabetes*, **49**, 311–318.

Seutin, V., Shen, K. Z., North, R. A. and Johnson, S. W., 1996, Sulfonylurea-sensitive potassium current evoked by sodium-loading in rat midbrain dopamine neurons. *Neuroscience*, **71**, 709–719.

Shiraishi, T., Oomura, Y., Sasaki, K. and Wayner, M. J., 2000, Effects of leptin and orexin-A on food intake and feeding related hypothalamic neurons. *Physiology of Behaviour*, **71**, 251–261.

Shyng, S. and Nichols, C. G., 1997, Octameric stoichiometry of the K-ATP channel complex. *Journal of General Physiology*, **110**, 655–664.

Shyng, S. L. and Nichols, C. G., 1998, Membrane phospholipid control of nucleotide sensitivity of K-ATP channels [see comments]. *Science*, **282**, 1138–1141.

Silver, I. A. and Erecinska, M., 1998, Glucose-induced intracellular ion changes in sugar-sensitive hypothalamic neurons. *Journal of Neurophysiology*, **79**,1733–1745.

Slesinger, P. A., Patil, N., Liao, Y. J., Jan, Y. N., Jan, L. Y. and Cox, D. R., 1996, Functional effects of the mouse weaver mutation on G protein-gated inwardly rectifying K^+ channels. *Neuron*, **16**, 321–331.

Spanswick, D., Smith, M. A., Groppi, V. E., Logan, S. D. and Ashford, M. L., 1997, Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature*, **390**, 521–525.

Spanswick, D., Smith, M. A., Mirshamsi, S., Routh, V. H. and Ashford, M. L., 2000, Insulin activates ATP-sensitive K^+ channels in hypothalamic neurons of lean, but not obese rats. *National Neuroscience*, **3**, 757–758.

Stanford, I. M. and Lacey, M. G., 1995, Regulation of a potassium conductance in rat midbrain dopamine neurons by intracellular adenosine triphosphate (ATP) and the sulfonylureas tolbutamide and glibenclamide. *Journal of Neuroscience*, **15**, 4651–4657.

Stanford, I. M. and Lacey, M. G., 1996, Electrophysiological investigation of adenosine trisphosphate-sensitive potassium channels in the rat substantia nigra pars reticulata. *Neuroscience*, **74**, 499–509.

Stefani, M. R., Nicholson, G. M. and Gold, P. E., 1999, ATP-sensitive potassium channel blockade enhances spontaneous alternation performance in the rat: a potential mechanism for glucose-mediated memory enhancement. *Neuroscience*, **93**, 557–563.

Steffens, A. B., Strubbe, J. H., Balkan, B. and Scheurink, J. W., 1990, Neuroendocrine mechanisms involved in regulation of body weight, food intake and metabolism. *Neuroscience Biobehaviour Review*, **14**, 305–313.

Pflugers Archives, **440**, 530–547.

Suzuki, M., Kotake, K., Fujikura, K., Inagaki, N., Suzuki, T., Gonoi, T., Seino, S. and Takata, K., 1997, Kir6.1: a possible subunit of ATP-sensitive K^+ channels in mitochondria. *Biochemistry and Biophysics Research Communications*, **241**, 693–697.

Takaba, H., Nagao, T., Yao, H., Kitazono, T., Ibayashi, S. and Fujishima, M., 1997, An ATP-sensitive potassium channel activator reduces infarct volume in focal cerebral ischemia in rats. *American Journal of Physiology*, **273**, R583–R586.

Tanaka, T., Yoshida, M., Yokoo, H., Mizoguchi, K. and Tanaka, M., 1996, ATP-sensitive K^+ channel openers block sulpiride-induced dopamine release in the rat striatum. *European Journal of Pharmacology*, **297**, 35–41.

Trapp, S. and Ballanyi, K., 1995, K-ATP channel mediation of anoxia-induced outward current in rat dorsal vagal neurons in vitro. *Journal of Physiology (London)*, **487**, 37–50.

Treherne, J. M. and Ashford, M. L., 1991, The regional distribution of sulphonylurea binding sites in rat brain. *Neuroscience*, **40**, 523–531.

Tucker, S. J., Gribble, F. M., Proks, P., Trapp, S., Ryder, T. J., Haug, T., Reimann, F. and Ashcroft, F. M., 1998, Molecular determinants of K-ATP channel inhibition by ATP. *Embo Journal*, **17**, 3290–3296.

Tucker, S. J., Gribble, F. M., Zhao, C., Trapp, S. and Ashcroft, F. M., 1997, Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor. *Nature*, **387**, 179–183.

Wada, Y., Yamashita, T., Imai, K., Miura, R., Takao, K., Nishi, M., Takeshima, H., Asano, T., Morishita, R., Nishizawa, K., Kokubun, S. and Nukada, T., 2000, A region of the sulfonylurea receptor critical for a modulation of ATP-sensitive K(+) channels by G-protein betagamma-subunits [In Process Citation]. *Embo Journal*, **19**, 4915–4925.

Wang, Y. and Ashraf, M., 1999, Role of protein kinase C in mitochondrial K-ATP channel-mediated protection against $Ca2^+$ overload injury in rat myocardium. *Circulation Research*, **84**, 1156–1165.

Watts, A. E., Hicks, G. A. and Henderson, G., 1995, Putative pre- and postsynaptic ATP-sensitive potassium channels in the rat substantia nigra in vitro. *Journal of Neuroscience*, **15**, 3065–3074.

Wind, T., Prehn, J. H., Peruche, B. and Krieglstein, J., 1997, Activation of ATP-sensitive potassium channels decreases neuronal injury caused by chemical hypoxia. *Brain Research*, **751**, 295–299.

Yamada, M., Isomoto, S., Matsumoto, S., Kondo, C., Shindo, T., Horio, Y. and Kurachi, Y., 1997, Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K^+ channel. *Journal of Physiology (London)*, **499**, 715–720.

Yang, X. J., Kow, L. M., Funabashi, T. and Mobbs, C. V., 1999, Hypothalamic glucose sensor: similarities to and differences from pancreatic beta-cell mechanisms. *Diabetes*, **48**, 1763–1772.

Ye, G. L., Leung, C. K. and Yung, W. H., 1997, Pre-synaptic effect of the ATP-sensitive potassium channel opener diazoxide on rat substantia nigra pars reticulata neurons. *Brain Research*, **753**, 1–7.

Yokoshiki, H., Sunagawa, M., Seki, T. and Sperelakis, N., 1998, ATP-sensitive K^+ channels in pancreatic, cardiac, and vascular smooth muscle cells. *American Journal of Physiology*, **274**, C25–C37.

Zawar, C. and Neumcke, B., 2000, Differential activation of ATP-sensitive potassium channels during energy depletion in CA1 pyramidal cells and interneurones of rat hippocampus. *Pflugers Archives*, **439**, 256–262.

Zawar, C., Plant, T. D., Schirra, C., Konnerth, A. and Neumcke, B., 1999, Cell-type specific expression of ATP-sensitive potassium channels in the rat hippocampus. *Journal of Physiology (London)*, **514**, 327–341.

Zerangue, N., Schwappach, B., Jan, Y. N. and Jan, L. Y., 1999, A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. *Neuron*, **22**, 537–548.

Zetterstrom, T. S., Vaughan-Jones, R. D. and Grahame-Smith, D. G., 1995, A short period of hypoxia produces a rapid and transient rise in $[K^+]e$ in rat hippocampus in vivo which is inhibited by certain K(+)-channel blocking agents. *Neuroscience*, **67**, 815–821.

Zhou, M., Tanaka, O., Sekiguchi, M., Sakabe, K., Anzai, M., Izumida, I., Inoue, T., Kawahara, K. and Abe, H., 1999, Localization of the ATP-sensitive potassium channel subunit (Kir6. 1/uK(ATP)-1) in rat brain. *Brain Research and Molecular Brain Research*, **74**, 15–25.

Zini, S., Tremblay, E., Roisin, M. and Ben-Ari, Y., 1991, Two binding sites for H3 glibenclamide in the rat brain. *Brain Research*, **542**, 151–154.

Received 16 January 2001, and in revised form 19 March 2001.