Acta Biochimica Polonica

Vol. 56 No. 3/2009, 385–392

on-line at: www.actabp.pl

Review

Potassium channels in brain mitochondria

Piotr Bednarczyk✉

Department of Biophysics, Warsaw University of Life Sciences — SGGW, Warszawa, Poland; Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warszawa, Poland

Received: 20 June, 2009; revised: 03 August, 2009; accepted: 15 September, 2009
available on-line: 17 September, 2009

Potassium channels are the most widely distributed class of ion channels. These channels are transmembrane proteins known to play important roles in both normal and pathophysiological functions in all cell types. Various potassium channels are recognised as potential therapeutic targets in the treatment of Parkinson's disease, Alzheimer's disease, brain/spinal cord ischaemia and sepsis. In addition to their importance as therapeutic targets, certain potassium channels are known for their beneficial roles in anaesthesia, cardioprotection and neuroprotection. Some types of potassium channels present in the plasma membrane of various cells have been found in the inner mitochondrial membrane as well. Potassium channels have been proposed to regulate mitochondrial membrane potential, respiration, matrix volume and Ca^{2+} ion homeostasis. It has been proposed that mitochondrial potassium channels mediate ischaemic preconditioning in various tissues. However, the specificity of a pharmacological agents and the mechanisms underlying their effects on ischaemic preconditioning remain controversial. The following potassium channels from various tissues have been identified in the inner mitochondrial membrane: ATP-regulated (mitoK_{ATP}) channel, large conductance Ca^{2+}-regulated (mitoBK_{Ca}) channel, intermediate conductance Ca^{2+}-regulated (mitoIK_{Ca}) channel, voltage-gated (mitoKv1.3 type) channel, and twin-pore domain (mitoTASK-3) channel. It has been shown that increased potassium flux into brain mitochondria induced by either the mitoK_{ATP} channel or mitoBK_{Ca} channel affects the beneficial effects on neuronal cell survival under pathological conditions. Recently, differential distribution of mitoBK_{Ca} channels has been observed in neuronal mitochondria. These findings may suggest a neuroprotective role for the mitoBK_{Ca} channel in specific brain structures. This minireview summarises current data on brain mitochondrial potassium channels and the efforts to identify their molecular correlates.

Keywords: brain, mitochondria, potassium channel

INTRODUCTION

The progress in the field of ion channel biology has been phenomenal during the last 60 years. Ion channel research has become highly interdisciplinary, combining the approaches of electrophysiology, pharmacology, molecular biology and genetics. Julius Bernstein (1902) was the first to postulate the existence of selective potassium permeability in excitable cell membranes, and he also proposed that membrane permeability to other ions increases during excitation. Since then, genetics studies have

✉Corresponding author: Piotr Bednarczyk, Department of Biophysics, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, 02-776 Warszawa, Poland; Tel.: (48) 22 589 2239; Fax: (48) 22 822 5342; e-mail: piotr_bednarczyk@sggw.pl

Abbreviations: 5-HD, 5-hydroxydecanoic acid; ANT, adenine nucleotide translocator; $BK_{Ca,}$ plasma membrane large conductance Ca^{2+}-regulated potassium channel; ChTx, charybdotoxin; IbTx, iberiotoxin; IPC, ischaemic precondition; $K_{ATP,}$ plasma membrane ATP-regulated potassium channel; $K_{Ca}3.1$, plasma membrane Ca^{2+}-regulated potassium channel; Kv, plasma membrane voltage-gated potassium channel; mABC1, ATP-binding cassette protein-1; mito$BK_{Ca,}$ mitochondrial large conductance Ca^{2+}-regulated potassium channel; mito$IK_{Ca'}$ mitochondrial intermediate conductance Ca^{2+}-regulated potassium channel; mitoK_{ATP}, mitochondrial ATP-regulated potassium channel; mitoKv1.3, mitochondrial voltage-gated potassium channel; mitoTASK-3, mitochondrial twin-pore domain potassium channel; MgTx, margatoxin; PIC, inorganic phosphate carrier; PLB, lanar lipid bilayer technique; SDH, succinate dehydrogenase; TASK-3, plasma membrane twin-pore domain potassium channel.

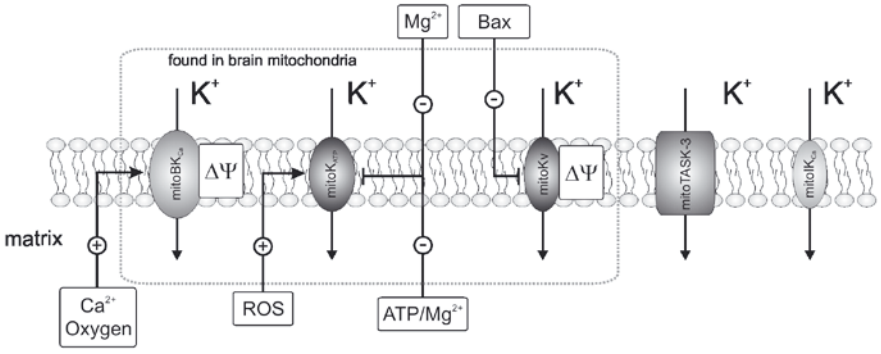

Figure 1. Mitochondrial potassium channels.
Shown are: ATP-regulated potassium channel (mitoK_{ATP}), large conductance Ca^{2+}-regulated potassium channel (mitoB-K_{Ca}), intermediate conductance Ca^{2+}-regulated potassium channel (mitoIK_{Ca}), voltage-dependent potassium channel (mitoKv1.3), and twin-pore potassium channel (mitoTASK-3).

revealed over 80 mammalian genes that encode subunits of plasma membrane potassium channels. Today it is well known that ion channels specific for potassium are present in the plasma membranes of most cell types and control a wide variety of cellular functions (Hille, 2001). An increasing body of literature also suggests that mitochondrial channelopathies can play crucial roles in neurodegenerative disorders such as Parkinson's, Huntington's and Alzheimer's diseases.

Potassium channels similar to those present in the plasma membrane of various cells have been found in the inner mitochondrial membrane (Fig. 1) (Szewczyk *et al.,* 2009). ATP-regulated potassium channels (Inoue *et al.,* 1991), large conductance Ca^{2+}-regulated potassium channels (Siemen *et al.,* 1999), intermediate conductance Ca^{2+}-regulated potassium channels (De Marchi *et al.,* 2009), voltage-gated potassium channels (Szabo *et al.,* 2005) and twin-pore potassium channels (Rusznak *et al.,* 2008) have all been found in the inner mitochondrial membrane, where they are involved in the regulation of mitochondrial volume (Halestrap, 1994; Bednarczyk *et al.,* 2008a), membrane potential (Debska *et al.,* 2001) or acidification (Bednarczyk *et al.,* 2008) and apoptosis (Szabo *et al.,* 2008). Mitochondrial potassium channels have been suggested to be triggers and end effectors during neuroprotection and cardioprotection, but the mechanisms of these processes are still unknown. This minireview summarises the current knowledge in the field of brain mitochondrial potassium channels and the efforts to understand their molecular similarity to plasma membrane potassium channels.

MITOCHONDRIAL ATP-REGULATED POTASSIUM CHANNEL

Biochemical and electrophysiological studies have provided evidence for the presence of ATP-regulated potassium channels (mitoK_{ATP} channels) in the inner mitochondrial membrane of various cell types. The channel has been described in liver (Inoue *et al.,* 1991), heart (Paucek *et al.,* 1992), brain (Bajgar *et al.,* 2001; Debska *et al.,* 2001; Kulawiak & Bednarczyk, 2005), renal (Cancherini *et al.,* 2003), skeletal muscle (Debska *et al.,* 2002), human T-lymphocytes (Dahlem *et al.,* 2004) and amoeba mitochondria (Kicinska *et al.,* 2007). The mitoK_{ATP} channel has also been functionally described in plant mitochondria but channel activity was not detected (Pastore *et al.,* 1999).

Despite efforts by several groups, the molecular identity of mitoK_{ATP} channel is still unknown. Several observations regarding its pharmacological profile suggest that the mitoK_{ATP} channel belongs to the inward rectifier K^+ channel family, Kir6.x (Suzuki *et al.,* 1997; Zhou *et al.,* 1999). Specific antibodies against Kir6.x or SUR have been reported to give an immunoreactive band in purified mitochondrial proteins (Szewczyk *et al.,* 1997; 1999; Bajgar *et al.,* 2001; Singh *et al.,* 2003; Lacza *et al.,* 2003). Recently, it was proposed that a complex of five proteins in the mitochondrial inner membrane is capable of transporting K^+ with characteristics similar to those of the mitoK_{ATP} channel. This complex contains: ATP-binding cassette protein-1 (mABC1), succinate dehydrogenase (SDH), ATP synthase (ATPase), inorganic phosphate carrier (PIC) and adenine nucleotide translocator (ANT). The pore-forming unit of the channel has not been characterized yet (Ardehali *et al.,* 2004).

The proposal that the mitoK_{ATP} channel may play a role in preconditioning is still an open question, in particular because the gene coding for the channel is not known. A minority of researchers does not believe in the existence of the mitoK_{ATP} channel or suggest that it is considerably different from the plasma membrane K_{ATP} channel (Hanley & Daut, 2005).

The planar lipid bilayer and patch-clamp techniques have successfully been applied to study

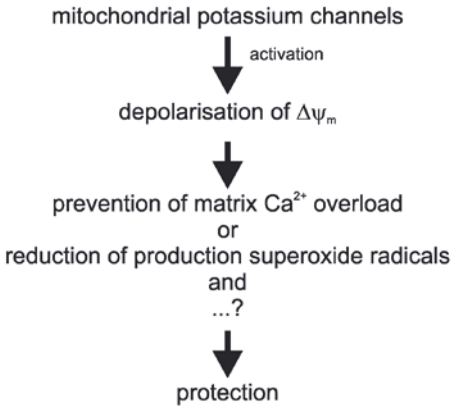

Figure 2. Schematic representation of mechanisms protection mediated by mitochondrial potassium channels. Opening mitoK_{ATP} or mitoBK_{Ca} channels depolarizes $\Delta\psi_m$, which reduces the driving force for Ca^{2+} influx or reduces production of superoxide radicals and …? Scheme shows only main mitochondrial events during IPC.

single channel properties of the mitoK_{ATP} channel (Zhang *et al.,* 2001; Nakae *et al.,* 2003; Bednarczyk *et al.,* 2004). The patch-clamp studies showed that the human mitoK_{ATP} channel is modulated by calcium and nitric oxide (Dahlem *et al.,* 2004). The planar lipid studies showed that oxidative stress results in the activation of the mitoK_{ATP} channel, inhibited by 5-hydroxydecanoic acid (5-HD) or a sulfhydryl alkylating compound, N-ethylmaleimide (Zhang *et al.,* 2001). Additionally, the anaesthetic isoflurane was found to directly activate the mitoK_{ATP} channel (Nakae *et al.,* 2003). Recently, regulation of the cardiac mitoK_{ATP} channel by quinine, magnesium ions, protons and adenine nucleotides was described (Table 1) (Bednarczyk *et al.,* 2004; 2005; 2008). Moreover, the mitoK_{ATP} channel is likely regulated by multiple phosphorylation events (Bednarczyk *et al.,* 2008).

It has been reported that ischaemic preconditioning (brief episodes of ischaemia protect against subsequent lethal ischaemia) involves an endogenous cellular protective mechanisms, as observed in various animal models of brain ischaemia (Watanabe *et al.,* 2008) and in human stroke patients (Wegener *et al.,* 2004). There is data suggesting that the opening of mitochondrial ATP-regulated potassium channels during an ischaemic episode has protective effects on neuronal functions (Busija *et al.,* 2008; Watanabe *et al.,* 2008; Gaspar *et al.,* 2008). To investigate the role of this type of channel on neuroprotection, effects of selective mitoK_{ATP} channel openers, diazoxide (Watanabe *et al.,* 2008) and BMS-191095 (Gaspar *et al.,* 2008), were assessed in various ischaemic preconditioning (IPC) models (Fig. 2). Diazoxide significantly enhanced the protective effects of IPC in gerbil and rat models. The mitochondrial membranes that were depolarized with IPC and 5-HD treatment significantly reduced this effect (Watanabe *et al.,* 2008). It was also shown that the selective mitoK_{ATP} channel opener BMS-191095 induces neuronal IPC; however, the direct mechanism of the BMS-induced neuroprotection remains unclear.

MITOCHONDRIAL LARGE CONDUCTANCE Ca^{2+}-REGULATED POTASSIUM CHANNEL

One of the first reports about Ca^{2+}-regulated potassium channels came from microelectrode studies of molluscan neurons (Meech, 1974; Meech & Standen, 1974). When the patch-clamp technique was introduced, it became possible to easily record the channel unitary currents that were dependent on calcium level with different properties (Wei *et al.,* 2005). The investigators were surprised to find prominent large conductance Ca^{2+}-regulated potassium (BK_{Ca}, also called Maxi-K or slo1) channels, intermediate conductance Ca^{2+}-regulated potassium (IK_{Ca}) channels, and small conductance Ca^{2+}-regulated potassium (SK_{Ca}) channels. The three different types of channels were analysed based on their voltage dependence, Ca^{2+} sensitivity, conductance and pharmacology. The peptide toxin apamin from honeybee venom and iberiotoxin (IbTx) and charybdotoxin (ChTx) from scorpion venom have been useful for discovering the properties of these channels.

The large conductance Ca^{2+}-regulated potassium (BK_{Ca}) channels are widely distributed in the plasma membrane of both excitable and non-excitable cells. These channels are located in smooth muscle (Giangiacomo *et al.,* 1995), brain (Knaus *et al.,* 1996) and chromaffin cells (Lingle *et al.,* 1996). The functional BK_{Ca} channel is a tetramer of the pore-forming α-subunit (Butler *et al.,* 1993). However, depending on the tissue, the α-subunits may be associated with distinct β-subunits phenotypes, which determine the electrophysiological and pharmacological properties of the channel. Currently, four putative β-subunit types (β1–β4) have been cloned (Ghatta *et al.,* 2006). Of the four subtypes, β2 and β4 are localised in neurons (Wallner *et al.,* 1999).

The large conductance Ca^{2+}-regulated potassium channel was also found in brain mitochondria (Kulawiak & Bednarczyk, 2005) and the human glioma cell line LN229 (Siemen *et al.,* 1999). The channel was stimulated by Ca^{2+} and blocked by charybdotoxin. A channel with properties similar to the plasma membrane BK_{Ca} channel was observed in mitoplasts of guinea pig ventricular cells (Xu *et al.,* 2002). The channel was stimulated by the potassium channel opener NS1619 and blocked by ChTx, IbTx and paxilline, which inhibit mitoBK_{Ca} channels. Immunoblots of cardiac mitochondria with antibodies against the C-terminal region of the BK_{Ca} channel identified a 55 kDa protein as a putative channel

Table 1. Potassium channel types identified in mitochondria.

See text for details and abbreviations.

Type	Tissue or cell localisation	Experimental setup Conductance (in ~150 mM KCl)	Modulators	Putative role	References
mitoK$_{ATP}$	liver, heart, brain, renal, skeletal muscle, human T-lymphocytes, amoeba, plant mitochondria	PLB, patch-clamp, isolated mitochondria, proteoliposome flux, mitochondrial flux, molecular biology ~ 10 to 110 pS	ATP, GTP, palmitoyl CoA, Mg^{2+}, pH, glibenclamide, 5-HD, quinine, *N*-ethylamide, diazoxide, cromakalim, pinacidil, BMS-191095, RP66471, nicorandil, P1075, isofurane	volume regulation, protection, apoptosis	Inoue *et al.*, 1991 Paucek *et al.*, 1992 Pastore *et al.*, 1999 Bajgar *et al.*, 2001 Debska *et al.*, 2001 Debska *et al.*, 2002 Cancherini *et al.*, 2003 Dahlem *et al.*, 2004 Bednarczyk *et al.*, 2004 Bednarczyk *et al.*, 2005 Kicinska *et al.*, 2007
mitoBK$_{Ca}$*	heart, brain, skeletal muscle mitochondria	PLB, patch-clamp, mitochondrial flux, isolated mitochondria, molecular biology ~ 265 to 310 pS	Ca^{2+}, voltage, iberiotoxin, paxilline, charybdotoxin, NS1619	volume regulation	Siemen *et al.*, 1999 Xu *et al.*, 2002 Sato *et al.*, 2005 Cheng *et al.*, 2008 Piwonska *et al.*, 2008 Skalska *et al.*, 2009
mitoIK$_{Ca}$	human colon tumour cells mitochondria	Patch-clamp, molecular biology ~ 10 to 90 pS	Ca^{2+},TRAM-34, clotrimazole	regulation of volume, pH and metabolism	De Marchi *et al.*, 2009
mitoKv**	T lymphocyte mitochondria	Patch-clamp, molecular biology ~ 25 pS	voltage, MgTx, TEACl, Bax	apoptosis	Szabo *et al.*, 2005 Szabo *et al.*, 2008
mitoTASK-3	melanoma, keratinocyte, HaCaT cells mitochondria	molecular biology no data about conductance	no data	proliferation and growth of tumour cells	Rusznak *et al.*, 2008

*possible candidate for mitoBK$_{Ca}$ channel is the BKα splice variant known as BK-DEC (Kathiresan *et al.*, 2009); **pJK/mito-EYFP-Kv1.3 construct specifically targeted the expression of Kv1.3 to mitochondria (Szabo *et al.*, 2008).

that may contribute to the cardioprotective effect of potassium influx into mitochondria. Additionally, it has been reported that the activation of cardiac mitoBK$_{Ca}$ by NS1619 (as measured by flavoprotein oxidation) is amplified by 8-bromoadenosine-3′,5′-cyclic monophosphate and forskolin but not by PKC-activating phorbol ester — PMA (Sato *et al.*, 2005). These results suggest that the opening of mitoBK$_{Ca}$ is modulated by a cAMP-dependent protein kinase.

Recently, it was found that mitochondrial ion channels are sensitive to low levels of oxygen. Using patch-clamp techniques, it was reported that hypoxia inhibits the permeability transition pore but substantially increases the mitoBK$_{Ca}$ channel activity of rat liver and astrocyte mitochondria (Cheng *et al.*, 2008). The Siemen's group observations indicate a possible role for the mitoBK$_{Ca}$ channel during hypoxia that could be interpreted as an anti-apoptotic mechanism.

A combined approach using Western blot analysis, high-resolution immunofluorescence and immunoelectron microscopy with the use of antibodies directed against four distinct β-subunits demonstrated the presence of the BK$_{Ca}$ channel β4 subunit (KCNMB4) in the inner membrane of neuronal mitochondria (Piwonska *et al.*, 2008). Within the cell, the expression of the β4 subunit was restricted to a subpopulation of mitochondria. The analysis of β4 subunit distribution throughout the brain has revealed that the highest expression levels occur in the thalamus and the brain stem (Piwonska *et al.*, 2008).

The mitochondrial response to changes in the cytosolic calcium concentration has a strong impact on neuronal cell metabolism and viability. It was observed that the addition of Ca^{2+} to isolated rat brain mitochondria induced dissipation of the mitochondrial membrane potential and in consequence increased mitochondrial respiration (Skalska *et al.*, 2009). The Ca^{2+} effects were blocked by IbTx or ChTx, inhibitors of the BK$_{Ca}$ channel. Furthermore, NS1619, a BK$_{Ca}$ channel opener, induced potassium flux similar to that induced by Ca^{2+}. These findings suggest the presence of a large conductance Ca^{2+}-regulated potassium channel (mitoBK$_{Ca}$ channel) in rat brain mitochondria. The presence of the mitoBK$_{Ca}$ channel was also confirmed by reconstitution of submitochondrial particles into planar lipid bilayers. The conductance of the reconstituted channel was

265 pS under gradient (50/450 mM KCl) conditions. The reversal potential was equal to 50 mV, which indicated that the examined channel was cation-selective (Table 1). Additionally, immunohistochemical analysis confirmed the predominant occurrence of β4 subunit in neuronal mitochondria (Skalska *et al.*, 2009).

The cumulative research up to this point suggests that the mitoBK$_{Ca}$ channel may represent a novel link between cellular/mitochondrial calcium signalling and mitochondrial membrane potential-dependent reactions. In addition, the data supports a neuroprotective role for the mitochondrial large conductance Ca-regulated potassium channel in specific brain structures.

MITOCHONDRIAL VOLTAGE-GATED POTASSIUM CHANNEL

Voltage-gated potassium channels (Kv channels) belong to an ion channel family, which is regulated by changes in membrane potential (Gutman *et al.*, 2005). They play a crucial role during action potentials by returning the depolarized cell to a resting state. The Kv1.3 type potassium channel is primarily expressed in T lymphocytes, but it is also present in the kidney (Yao *et al.*, 1996), epithelia (Grunnet *et al.*, 2003) and central nervous system (Mourre *et al.*, 1999). In T lymphocytes, it has been shown that Kv1.3 channels play a crucial role in proliferation and volume regulation (Chandy *et al.*, 2004). Accordingly, dysfunction of Kv channels causes various neuronal, immune and cardiac disorders (Shieh *et al.*, 2000).

In 2005, a margatoxin-sensitive voltage-gated Kv1.3 channel was identified in T lymphocyte mitochondria (Table 1) (Szabo *et al.*, 2005). Biophysical, biochemical, pharmacological and genetic data have confirmed the functional expression of the Kv1.3 type channel in lymphocyte mitochondria. First, electron microscopy studies on peripheral blood lymphocytes showed that the channel is present in the inner mitochondrial membrane. Second, a patch-clamp study showed the localization of the mitoKv1.3 channel in the inner mitochondrial membrane with properties similar to this type of channel from the plasma membrane. Third, analysis of mitochondria from Kv1.3-deficient and Kv1.3-reconstituted T lymphocyte cell line CTLL-2 confirmed the identity of the mitoKv1.3 channel. Interestingly, it was shown that the Kv1.3 channel is present both in the plasma and mitochondrial membranes, despite lacking the N-terminal mitochondrial targeting sequence. It may be possible that the mitochondrial Kv1.3 channel represents an important factor in apoptotic signal transduction. It has also been shown that Bax mediates cytochrome *c* release and mitochondrial depolarization, at least in part, *via* its interaction with the mitoKv1.3 channel (Szabo *et al.*, 2008). Recently, we have observed mitoKv1.3 channel activity in gerbil hippocampal mitochondria (Bednarczyk *et al.*, unpublished).

MITOCHONDRIAL TWIN-PORE DOMAIN POTASSIUM CHANNEL

The TASK-3 channels are members of the twin-pore domain potassium channel family. They have four transmembrane segments and are active as tandem pore-domain channels. These channels are acid-sensitive potassium channels and are weak inward rectifier potassium channels-related (Table 1) (Meadows & Randall, 2001). Their tissue distribution is wide, and they are found in both excitable and non-excitable cells (Lesage & Lazdunski, 2000). They are involved in a number of physiological functions, including the adjustment of neuronal excitability (Kim *et al.*, 2000; Meuth *et al.*, 2003) or shaping the duration of postsynaptic events (Chemin *et al.*, 2003). Recently, TASK-3 was described in mitochondria (Rusznak *et al.*, 2008). Immunochemical and molecular biological methods were used to establish the mitochondrial localisation of the TASK-3 channel in melanoma and keratinocyte cells. Until now, the functional properties of the TASK-3 channels in the inner mitochondrial membrane are unknown. Expression of the twin-pore domain potassium channels in brain mitochondria has yet to be confirmed.

MITOCHONDRIAL INTERMEDIATE CONDUCTANCE Ca^{2+}-REGULATED POTASSIUM CHANNEL

Data published in 2009 indicate that the intermediate conductance Ca^{2+}-regulated potassium (mitoIK$_{Ca}$) channels are present in the inner membranes of mitochondria isolated from the human colon tumour cell line, HCT116 (De Marchi *et al.*, 2009). The channel properties were assessed using the patch-clamp technique on mitoplasts. The biophysical characteristics of the detected channel are similar to those of the plasma membrane Ca^{2+}-regulated potassium channel (K$_{Ca}$3.1 channel). The channel is potassium-selective with a conductance ranging from 10 to 90 pS without changing the probability of opening at positive or negative voltages (Table 1). The pharmacological properties of the mitoIK$_{Ca}$ channel were also similar to those of the plasma membrane ion channel. Activity was completely abolished at low calcium levels, and K$_{Ca}$3.1 channel inhibitors such as clotrimazole and TRAM-34 inhibited its activity at nanomolar concentrations (De Marchi *et al.*,

2009). Additionally, localisation of that channel was confirmed by Western blotting and fluorescent tools. Until now, a channel similar to the mitoIK$_{Ca}$ channel has not been detected in brain tissue.

FINAL REMARKS

In recent years, five different types of potassium channels have been detected in the inner mitochondrial membrane. These findings include the ATP-regulated, large conductance Ca^{2+}-regulated, twin-pore, voltage-gated and intermediate conductance Ca^{2+}-regulated potassium channels. However, there is still insufficient information about the molecular identity and single-channel properties of the mitochondrial potassium channels. Recent observations indicate that both the mitoK$_{ATP}$ and mitoBK$_{Ca}$ channels regulate mitochondrial K^+ flux and mediate neuronal ischaemic preconditioning (IPC). The mechanism of this process, including the activation of the mitoBK$_{Ca}$ channel, can be partially attributed to the reduced driving force for calcium influx or the production of superoxide radicals (Fig. 2). Using different experimental models, it has been shown that the activation of mitoK$_{ATP}$ channels results in substantial acute and delayed protective effects in the brain and in cultured neurons. However, the specificity of the pharmacological agents used in those studies and the mechanisms underlying their effects on neuronal preconditioning remain unclear and controversial.

Acknowledgements

The author wishes to thank Adam Szewczyk and Krzysztof Dołowy for stimulating discussions, and the Polish Mitochondrial Network (MitoNet.pl) and the Ministry of Science and Higher Education grant No. P-N301/2006 and No. 301-053-31/1676 (to A.S.) for financial support.

I would like to apologize to all authors whose work could not be cited in this minireview due to restricted space.

REFERENCES

Ardehali H, Chen Z, Ko Y, Mejia-Alvarez R, Marban E (2004) Multiprotein complex containing succinate dehydrogenase confers mitchondrial ATP-sensitive K^+ channel activity. *Proc Natl Acad Sci USA* **101**: 11880–11885.

Bajgar R, Seetharaman S, Kowaltowski AJ, Garlid KD, Paucek P (2001) Identification and properties of a novel intracellular (mitochondrial) ATP-sensitive potassium channel in brain. *J Biol Chem* **276**: 33369–33374.

Bednarczyk P, Kicinska A, Kominkova V, Ondrias K, Dolowy K, Szewczyk A (2004) Quinine inhibits mitochondrial ATP-regulated potassium channel from bovine heart. *J Membr Biol* **199**: 63–72.

Bednarczyk P, Dolowy K, Szewczyk A (2005) Matrix Mg^{2+} regulates mitochondrial ATP-dependent potassium channel from heart. *FEBS Lett* **579**: 1625–1632.

Bednarczyk P, Barker GD, Halestrap AP (2008a) Determination of the rate of K^+ movement through potassium channels in isolated rat heart and liver mitochondria. *Biochim Biophys Acta* **1777**: 540–548.

Bednarczyk P, Dołowy K, Szewczyk A (2008b) New properties of mitochondrial ATP-regulated potassium channels. *J Bioenerg Biomembr* **40**: 325–335.

Busija DW, Gaspar T, Domoki F, Katakam PV, Bari F (2008) Mitochondrial-mediated suppression of ROS production upon exposure of neurons to lethal stress: mitochondrial targeted preconditioning. *Adv Drug Deliv Rev* **60**: 1471–1417.

Butler A, Tsunoda S, McCobb DP, Wei A, Salkoff L (1993) mSlo, a complex mouse gene encoding "maxi" calcium-activated potassium channels. *Science* **261**: 221–224.

Cancherini DV, Trabuco LG, Reboucas NA, Kowaltowski AJ (2003) ATP-sensitive K^+ channels in renal mitochondria. *Am J Physiol Renal Physiol* **285**: F1291–F1296.

Chandy KG, Wulff H, Beeton C, Pennington M, Gutman GA, Cahalan MD (2004) K^+ channels as targets for specific immunomodulation. *Trends Pharmacol Sci* **25**: 280–289.

Chemin J, Girard C, Duprat F, Lesage F, Romey G, Lazdunski M (2003) Mechanisms underlying excitatory effects of group I metabotropic glutamate receptors via inhibition of 2P domain K^+ channels. *EMBO J* **22**: 5403–5411.

Cheng Y, Gu XQ, Bednarczyk P, Wiedemann FR, Haddad GG, Siemen D (2008) Hypoxia increases activity of the BK-channel in the inner mitochondrial membrane and reduces activity of the permeability transition pore. *Cell Physiol Biochem* **22**: 127–136.

Dahlem YA, Horn TF, Buntinas L, Gonoi T, Wolf G, Siemen D (2004) The human mitochondrial K$_{ATP}$ channel is modulated by calcium and nitric oxide: a patch-clamp approach. *Biochim Biophys Acta* **1656**: 46–56.

Debska G, Kicinska A, Skalska J, Szewczyk A, May R, Elger CE, Kunz WS (2002) Opening of potassium channels modulates mitochondrial function in rat skeletal muscle. *Biochim Biophys Acta* **1556**: 97–105.

Debska G, May R, Kicinska A, Szewczyk A, Elger CE, Kunz WS (2001) Potassium channel openers depolarize hippocampal mitochondria. *Brain Res* **892**: 42–50.

De Marchi U, Sassi N, Fioretti B, Catacuzzeno L, Cereghetti GM, Szabo I, Zoratti M (2009) Intermediate conductance Ca^{2+}-activated potassium channel (KCa3.1) in the inner mitochondrial membrane of human colon cancer cells. *Cell Calcium* **45**: 509–516.

Gaspar T, Snipes JA, Busija AR, Kis B, Domoki F, Bari F, Busija DW (2008) ROS-independent preconditioning in neurons via activation of mitoK$_{ATP}$ channels by BMS-191095. *J Cereb Blood Flow Metab* **28**: 1090–1103.

Ghatta S, Nimmagadda D, Xu X, O'Rourke ST (2006) Large-conductance, calcium-activated potassium channels: structural and functional implications. *Pharmacol Ther* **110**: 103–116.

Giangiacomo KM, Garcia-Calvo M, Knaus HG, Mullmann TJ, Garcia ML, McManus O (1995) Functional reconstitution of the large-conductance, calcium-activated potassium channel purified from bovine aortic smooth muscle. *Biochemistry* **34**: 15849–15862.

Grunnet M, Rasmussen HB, Hay-Schmidt A, Klaerke DA (2003) The voltage-gated potassium channel subunit, Kv1.3, is expressed in epithelia. *Biochim Biophys Acta* **1616**: 85–94.

Gutman GA, Chandy KG, Grissmer S, Lazdunski M, McKinnon D, Pardo LA, Robertson GA, Rudy B, Sanguinetti MC, Stühmer W, Wang X (2005) International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol Rev* **57**: 473–508.

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

Halestrap AP (1994) Regulation of mitochondrial metabolism through changes in matrix volume. *Biochem Soc Trans* **22**: 522–529.

Hanley PJ, Daut JK (2005) K_{ATP} channels and preconditioning: a re-examination of the role of mitochondrial K_{ATP} channels and an overview of alternative mechanisms. *J Mol Cell Cardiol* **39**: 17–50.

Hille B (2001) Potassium channels and chloride channels. In *Ion channels of excitable membranes*. Chapter 5, pp 131–167. Sunderland, Mass: Sinauer.

Kathiresan T, Harvey M, Orchard S, Sakai Y, Sokolowski B (2009) A protein interaction network for the large conductance Ca^{2+}-activated K^+ channel in the mouse cochlea. *Mol Cell Proteomics* **8**: 1972–1987.

Kicinska A, Swida A, Bednarczyk P, Koszela-Piotrowska I, Choma K, Dolowy K, Szewczyk A, Jarmuszkiewicz W (2007) ATP-sensitive potassium channel in mitochondria of the eukaryotic microorganism, *Acanthamoeba castellanii*. *J Biol Chem* **282**: 17433–17441.

Kim Y, Bang H, Kim D (2000) TASK-3, a new member of the tandem pore K^+ channel family. *J Biol Chem* **275**: 9340–9347.

Knaus HG, Schwarzer C, Koch RO, Eberhart A, Kaczorowski GJ, Glossmann H, Wunder F, Pongs O, Garcia ML, Sperk G (1996) Distribution of high-conductance Ca^{2+}-activated K^+ channels in rat brain: targeting to axons and nerve terminals. *J Neurosci* **16**: 955–963.

Kulawiak B, Bednarczyk P (2005) Reconstitution of brain mitochondria inner membrane into planar lipid bilayer. *Acta Neurobiol Exp* **65**: 271–276.

Lacza Z, Snipes JA, Kis B, Szabo C, Grover G, Busija DW (2003) Investigation of the subunit composition and the pharmacology of the mitochondrial ATP-dependent K^+ channel in the brain. *Brain Res* **994**: 27–36.

Lesage F, Lazdunski M (2000) Molecular and functional properties of two-pore-domain potassium channels. *Am J Physiol* **279**: F793–F801.

Lingle CJ, Solaro CR, Prakriya M, Ding JP (1996) Calcium-activated potassium channels in adrenal chromaffin cells. *Ion Channels* **4**: 261–301.

Meadows HJ, Randall AD (2001) Functional characterisation of human TASK-3, an acid-sensitive two-pore domain potassium channel. *Neuropharmacology* **40**: 551–559.

Meech RW (1974) The sensitivity of *Helix aspersa* neurones to injected calcium ions. *J Physiol* **237**: 259–277.

Meech RW, Standen NB (1974) Calcium-mediated potassium activation in *Helix* neurones. *J Physiol* **237**: 43P-44P.

Meuth SG, Budde T, Kanyshkova T, Broicher T, Munsch T, Pape H-C (2003) Contribution of TWIK-related acid-sensitive K^+ channel 1 (TASK1) and TASK3 channels to the control of activity modes in thalamocortical neurons. *J Neurosci* **23**: 6460–6469.

Mourre C, Chernova MN, Martin-Eauclaire MF, Bessone R, Jacquet G, Gola M, Alper SL, Crest M (1999) Distribution in rat brain of binding sites of kaliotoxin, a blocker of Kv1.1 and Kv1.3 alpha-subunits. *J Pharmacol Exp Ther* **291**: 943–952.

Nakae Y, Kwok WM, Bosnjak ZJ, Jiang MT (2003) Isoflurane activates rat mitochondrial ATP-sensitive K^+ channels reconstituted in lipid bilayers. *Am J Physiol* **284**: H1865–H1871.

Pastore D, Stoppelli MC, Di Fonzo N, Passarella S (1999) The existence of the K^+ channel in plant mitochondria. *J Biol Chem* **274**: 26683–26690.

Paucek P, Mironova G, Mahdi F, Beavis AD, Woldegiorgis G, Garlid KD (1992) Reconstitution and partial purification of the glibenclamide-sensitive, ATP-dependent K^+ channel from rat liver and beef heart mitochondria. *J Biol Chem* **267**: 26062–26069.

Piwonska M, Wilczek E, Szewczyk A, Wilczynski GM (2008) Differential distribution of Ca^{2+}-activated potassium channel β4 subunit in rat brain: immunolocalization in neuronal mitochondria. *Neuroscience* **153**: 446–460.

Rusznak Z, Bakondi G, Kosztka L, Pocsai K, Diens B, Fodor J, Telek A, Gomczi M, Szucs G, Csernoch L (2008) Mitochondrial expression of the two-pore domain TASK-3 channels in malignantly transformed and non-malignant human cells. *Virchows Arch* **452**: 415–426.

Sato T, Saito T, Saegusa N, Nakaya H (2005) Mitochondrial Ca^{2+}-activated K^+ channels in cardiac myocytes: A mechanism of the cardioprotective effect and modulation by protein kinase A. *Circulation* **111**: 198–203.

Shieh CC, Coghlan M, Sullivan JP, Gopalakrishnan M (2000) Potassium channels: molecular defects, diseases, and therapeutic opportunities. *Pharmacol Rev* **52**: 557–594.

Siemen D, Loupatatzis C, Borecky J, Gulbins E, Lang F (1999) Ca^{2+}-activated K channel of the BK-type in the inner mitochondrial membrane of a human glioma cell line. *Biochem Biophys Res Commun* **257**: 549–554.

Singh H, Hudman D, Lawrence CL, Rainbow RD, Lodwick D, Norm RI (2003) Distribution of Kir6.0 and SUR2 ATP-sensitive potassium channel subunits in isolated ventricular myocytes. *J Mol Cell Cardiol* **35**: 433–435.

Skalska J, Bednarczyk P, Piwońska M, Kulawiak B, Wilczynski G, Dołowy K, Kudin AP, Kunz WS, Szewczyk A (2009) Calcium ions regulate K uptake into brain mitochondria: The evidence for a novel potassium channel. *Int J Mol Sci* **10**: 1104–1120.

Szabo I, Bock J, Grassmé H, Soddemann M, Wilker B, Lang F, Zoratti M, Gulbins E (2008) Mitochondrial potassium channel Kv1.3 mediates Bax-induced apoptosis in lymphocytes. *Proc Natl Acad Sci USA* **105**: 14861–14866.

Szabo I, Bock J, Jekle A, Soddemann M, Adams C, Lang F, Zoratti M, Gulbins E (2005) A novel potassium channel in lymphocyte mitochondria. *J Biol Chem* **280**: 12790–12798.

Szewczyk A, Jarmuszkiewicz W, Kunz WS (2009) Mitochondrial potassium channels. *IUBMB Life* **61**: 134–143.

Szewczyk A, Wojcik G, Lobanov NA, Nalecz MJ (1999) Modification of the mitochondrial sulfonylurea receptor by thiol reagents. *Biochem Biophys Res Commun* **262**: 255–258.

Szewczyk A, Wojcik G, Lobanov NA, Nalecz MJ (1997) The mitochondrial sulfonylurea receptor: identification and characterization. *Biochem Biophys Res Commun* **230**: 611–615.

Suzuki M, Kotake K, Fujikura K, Inagaji N, Suzuki T, Gonoi T, Seino S, Takata K (1997) Kir6.1: a possible subunit of ATP-sensitive K^+ channels in mitochondria. *Biochem Biophys Res Commun* **241**: 693–697.

Wallner M, Meera P, Toro L (1999) Molecular basis of fast inactivation in voltage and Ca^{2+}-activated K^+ channels: a transmembrane beta-subunit homolog. *Proc Natl Acad Sci USA* **96**: 4137–4142.

Watanabe M, Katsura K, Ohsawa I, Mizukoshi G, Takahashi K, Asoh S, Ohta S, Katayama Y (2008) Involve-

ment of mitoK_{ATP} channel in protective mechanisms of cerebral ischemic tolerance. *Brain Res* **1238**: 199–207.

Wegener S, Gottschalk B, Jovanovic V, Knab R, Fiebach JB, Schellinger PD, Kucinski T, Jungehülsing GJ, Brunecker P, Müller B, Banasik A, Amberger N, Wernecke KD, Siebler M, Röther J, Villringer A, Weih M; MRI in Acute Stroke Study Group of the German Competence Network Stroke (2004) Transient ischemic attacks before ischemic stroke: preconditioning the human brain? A multicenter magnetic resonance imaging study. *Stroke* **35**: 616–621.

Wei AD, Gutman GA, Aldrich R, Chandy KG, Grissmer S, Wulff H (2005) International Union of Pharmacology. LII. Nomenclature and molecular relationships of calcium-activated potassium channels. *Pharmacol Rev* **57**: 463–472.

Xu W, Liu Y, Wang S, McDonald T, Van Eyk JE, Sidor A, O'Rourke B (2002) Cytoprotective role of Ca^{2+}-activated K^+ channels in the cardiac inner mitochondrial membrane. *Science* **298**: 1029–1033.

Yao X, Chang AY, Boulpaep EL, Segal AS, Desir GV (1996) Molecular cloning of a glibenclamide-sensitive, voltage-gated potassium channel expressed in rabbit kidney. *J Clin Invest* **97**: 2525–2533.

Zhang DX, Chen YF, Campbell WB, Zou AP, Gross GJ, Li PL (2001) Characteristics and superoxide-induced activation of reconstituted myocardial mitochondrial ATP-sensitive potassium channels. *Circ Res* **89**: 1177–1183.

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