Biochemical and Biophysical Research Communications 376 (2008) 625–628

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The *C. elegans* mitochondrial K^+_{ATP} channel: A potential target for preconditioning

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ARTICLE INFO

Article history:
Received 9 September 2008
Available online 20 September 2008

Keywords:
ATP sensitive potassium channel
mK_{ATP}
Preconditioning
Ischemia
Mitochondria
C. elegans

ABSTRACT

Ischemic preconditioning (IPC) is an evolutionarily conserved endogenous mechanism whereby short periods of non-lethal exposure to hypoxia alleviate damage caused by subsequent ischemia reperfusion (IR). Pharmacologic targeting has suggested that the mitochondrial ATP-sensitive potassium channel (mK_{ATP}) is central to IPC signaling, despite its lack of molecular identity. Here, we report that isolated *Caenorhabditis elegans* mitochondria have a K_{ATP} channel with the same physiologic and pharmacologic characteristics as the vertebrate channel. Since *C. elegans* also exhibit IPC, our observations provide a framework to study the role of mK_{ATP} in IR injury in a genetic model organism.

Mitochondria are essential for cellular function and are a sensitive target for damage induced by ischemia reperfusion (IR) injury, such as the overproduction of reactive oxygen species (ROS) and overload of Ca^{2+}, both of which can trigger the formation of the mitochondrial permeability transition (PT) pore at reperfusion [1–3]. Mitochondrial dysfunction has significant implications for post-ischemic recovery [4,5]. Despite being the site of such pathological events, mitochondria are also involved in the protective mechanism of ischemic preconditioning (IPC) whereby short non-lethal periods of ischemia protect the heart from subsequent prolonged ischemic insults [6]. IPC triggers an array of cell signaling pathways, many of which converge at the mitochondrion. Recent work has suggested that a primary target of IPC signaling is the mitochondrial ATP sensitive K^+ channel (mK_{ATP}) [7,8], and that this channel is essential for the mechanism of cardioprotection recruited by IPC.

The mK_{ATP} was first described electro-physiologically [9], then subsequently via its pharmacological sensitivity, both of which demonstrate similarities to plasma membrane and sarcoplasmic reticulum ATP sensitive K^+ channels or "surface" K_{ATP} channels. For example, the sulfonylurea diazoxide's cardioprotective activity was originally thought to be due to triggering of surface K_{ATP} channels, but its protective effects were later shown to relate specifically to mK_{ATP} channel opening [10]. Moreover, the protective effects of IPC can be mimicked by mK_{ATP} channel activators [11–13] and prevented by channel inhibitors [14,15]. The protection mediated through the opening of the mK_{ATP} is thought to involve membrane depolarization through K^+ influx [16], which may translate to decreased Ca^{2+} uptake and ROS generation [17]. Additionally, K^+ entry obligates the influx of anions and water, thereby augmenting matrix volume [18] and potentially improving energy metabolism and interfering with PT pore assembly [16,19]. Despite the central role of the mK_{ATP} in IPC and the well-documented molecular characterization of the surface K_{ATP} channels, none of the sequences or splice variants of the inward rectifying K^+ channel (Kir) or sulfonylurea receptor (SUR) genes that comprise surface K_{ATP} channels [20] contain mitochondrial targeting sequences, and it is unclear whether the mK_{ATP} channel is derived from surface K_{ATP} channels [21–23] or is a unique protein entity [24].

Efforts centered on mammalian model systems have yielded no definitive answer regarding the molecular identity of the mK_{ATP} channels. One example of the problems faced by this field is the recent discovery that many of the antibodies used to identify SUR and Kir subunits in purified mitochondria can recognize off-target proteins unrelated to K^+ channel function [25]. Furthermore, knockout mice for Kir and SUR genes have been generated and are viable, but the ability to test these mice in IPC experiments is confounded by the obligatory effects of knocking out surface K_{ATP} channel function on various cardiovascular functions. A key exam-

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0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.09.043

ple is the SUR2$^{-/-}$ mouse which exhibits elevated blood glucose, a known risk factor for increased IR injury [26]. These limitations may be further compounded by potential nonspecific effects of pharmacological agents used in assaying the mK_{ATP} channel [27–29].

One potential solution to these limitations is to employ a model organism. In this regard, IPC in the nematode worm *C. elegans* results in protection from ischemic death similar to "late" preconditioning seen in mammals [30,31]. To begin testing the hypothesis that IPC in worms occurs through an evolutionarily conserved mechanism involving mK_{ATP}, we assayed mK_{ATP} activity in purified mitochondria using a K^+ swelling assay [32–35]. Our results demonstrate mK_{ATP} channel activity that both pharmacologically and physiologically mimics its mammalian counterpart. The presence of the channel in this model organism provides several advantages in determining its molecular identity. The reduced cellular complexity of worms and absence of vasculature may reduce confounding systemic effects, while the use of genetics to eliminate candidate channel expression should eliminate off-target pharmacologic effects. Future determination of the identity of the mK_{ATP} channel in worms will represent a significant advance in the field of IR injury and protection by IPC.

Materials and methods

All chemicals and reagents were from Sigma (St. Louis MO) unless otherwise stated. Culture and growth of *C. elegans* N2 strain was performed essentially as described [36]. 2×10^5 embryos or starved L1 worms were plated onto 2×150 mm plates, each seeded with 6 ml of HB101 bacteria (~50% slurry), condensed from a 500 ml culture of Terrific Broth. Plates were incubated in a humidified atmosphere at 20 °C for 3–4 days, resulting in staged adult worms ready for mitochondrial isolation.

Mitochondria were isolated from adult *C. elegans* by a method based on that described in [37]. Worms from 2 plates were transferred at 25 °C into 50 ml of M9 media (22 mM KH_2PO_4, 42 mM Na_2HPO_4, 86 mM NaCl, 1 mM $MgSO_4$, pH 7) in a conical tube, then incubated for 20 min on ice resulting in precipitation/settling. Supernatant (45 ml) was removed, and the worm pellet rinsed twice with 45 ml ice-cold M9 and once with 45 ml ice-cold mitochondrial isolation medium (IM: 220 mM mannitol, 70 mM sucrose, 5 mM MOPS, 2 mM EGTA, pH 7.4, filtered) containing 0.4% BSA. Worms were settled by gravity for 10 min. between rinses, and finally by centrifugation for 1 min at 1000 *g*, and suspension of the final pellet in ~1.5 ml of IM. Worms were transferred using a cut-off pipet tip to a mortar on-ice containing 2 g of washed sand per ml of worms, followed by grinding with a pestle for 1 min. Ground worms were extracted from the sand using 3×3 ml aliquots of IM, transferring to a 10 ml conical tube. Following three additional settling/wash steps in IM+BSA to remove excess sand, the worm suspension was then homogenized with a glass Dounce homogenizer in IM + BSA. The homogenate was diluted and centrifuged at 600 *g* for 5 min. The supernatant was then filtered through 300 μm mesh and centrifuged at 7000 *g* for 10 min. The pellet was resuspended in 1.5 ml IM, and centrifuged at 7000 *g* for 5 min. Another wash/centrifugation step was performed, and the final mitochondrial pellet suspended in 150 μl IM and stored on ice until use (within 2 h). Protein was determined by the Folin–Phenol method [38], against a standard curve constructed with BSA. Two plates of worms typically yielded ~6 mg mitochondrial protein.

Immediately following isolation, mitochondrial viability was determined by the measuring respiratory function (O_2 consumption) using a Clark-type O_2 electrode in a stirred chamber at 25 °C. Mitochondria (0.25 mg protein) were suspended in 0.25 ml respiration buffer (RB: 195 mM mannitol, 25 mM sucrose, 40 mM HEPES, 0.25% w/v fat-free BSA, pH 7.35 at 25 °C). Addition of substrates (10 mM pyruvate plus 5 mM malate, or 5 mM succinate) was used to initiate state 2, followed by the addition of ADP (200 μM) to initiate state 3 respiration. Oligomycin (1 μg/ml) was then added to enforce state 4 respiration.

mK_{ATP} channel activity was measured by the commonly used swelling/light-scatter assay [32–35] at 520 nm using a Beckman DU800 spectrophotometer. Mitochondria (0.25 mg/ml) were rapidly added to a stirred cuvette containing mK_{ATP} buffer (100 mM KCl, 10 mM HEPES, 2 mM succinate, 2 mM $MgCl_2$, 2 mM KH_2PO_4, and 1 μg/ml oligomycin, pH 7.2 at 37 °C). In some experiments, as indicated by figure legends, K^+ in the buffer was replaced with Na^+. All differences between experimental groups were determined using ANOVA; $p < 0.05$ was considered significant.

Results

We and others have previously noted that the mK_{ATP} channel activity degrades rapidly in mammalian mitochondria [32,33], thus contraindicating the use of proteases to break the *C. elegans* cuticle. Instead, a 2-step homogenization protocol was developed in which whole worms were first ground with sand in a pestle and mortar, followed by several passes in a glass Dounce homogenizer. This was followed by several centrifugation steps, resulting in a mitochondrial fraction. The respiratory properties of these isolated mitochondria are shown in Fig. 1, indicating that the mitochondria were moderately well coupled. Respiratory control ratio (RCR) values were not as high as those typically seen in mammalian mitochondria, however, worms are poikilotherms and prolonged incubation at 37 °C is lethal. Correspondingly, a lower incubation temperature was used in the respiration reactions (25 °C vs. 37 °C for mammalian mitochondria [33]), which slows down state 3 respiration considerably, and this may partly account for the lower RCR.

The rapid osmotic swelling of mitochondria in K^+ based media, which results in a decrease in light scattering that is assayed spectrophotometrically, has been widely used to assay mK_{ATP} channel function [32–35]. As shown in Fig. 2a, we were able to recapitulate mK_{ATP} mediated osmotic swelling of *C. elegans* mitochondria, and this activity persisted for up to 3 h following mitochondrial isolation. In Fig. 2b, we demonstrated that the channel exhibits the clas-

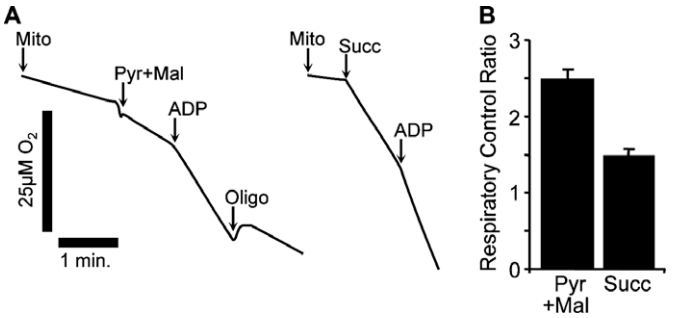

Fig. 1. O_2 consumption by isolated *C. elegans* mitochondria. Mitochondria were isolated as described and suspended at a concentration of 1 mg protein/ml in respiration buffer containing 2.5 mg/ml of BSA. O_2 consumption was measured using a Clark-type O_2 electrode in a thermostatic chamber at 25 °C. (A) Representative respiration traces: following the addition of mitochondria, left trace: 10 mM Pyruvate plus 5 mM Malate (Pyr+Mal) were added to induce state 2 respiration, 200 μM ADP to induce state 3, and 1 μg/ml oligomycin (Oligo) to induce state 4. Right trace: 10 mM Succinate (Succ) was added followed by 200 μM ADP. (B) Quantitation of the respiratory control ratio (state 3 divided by state 4) from several experiments as shown in A. Data are means ± SEM, $N > 8$.

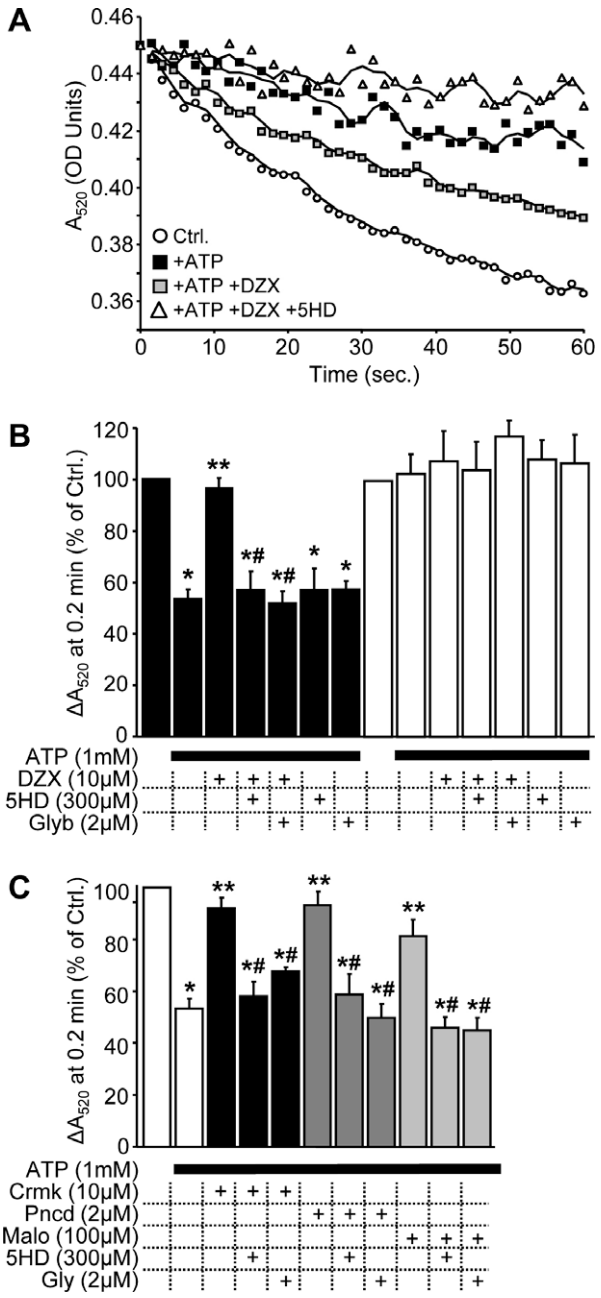

Fig. 2. The *C. elegans* mK_{ATP} channel. (A) Osmotic swelling assay for determination of the mK_{ATP} channel activity. Representative swelling trace of mitochondria (~0.25 mg/ml) in K^+ media. Where indicated, 1 mM ATP, 10 μM diazoxide (DZX, a K_{ATP} opener), or 300 μM of 5-hydroxydecanoate (5-HD, a K_{ATP} antagonist) were present in the media. (B) Magnitude of swelling, relative to the control, as determined by a decrease in A_{520} after 0.2 min incubation in K^+ media (filled bars) or in Na^+ media (open bars). (C) The pharmacological profile of the *C. elegans* mK_{ATP} channel demonstrated in response to activators and inhibitors in K^+ media. Chromakalim (Crmk), pinacidil (Pncd) and malonate (Malo) are all K_{ATP} openers, while 5-HD and glyburide (Gly) are K_{ATP} antagonists. The 3 conditions for each opener (i.e. opener alone and with 2 antagonists) are shaded the same. Control swelling after 0.2 min resulted in a decrease in A_{520} of 0.0233 ± 0.0013 OD units in K^+ media and 0.0167 ± 0.004 OD units in Na^+ media. Experimental conditions are listed below the *x*-axis. Data are means ± SEM $^{*}p < 0.05$ vs. control, $^{**}p < 0.05$ vs. ATP, $^{***}p < 0.05$ vs. ATP^+ channel activator; $N \geqslant 4$.

sical mK_{ATP} pharmacologic profile as previously reported in the literature and in this laboratory [10,15,17,33,39], i.e. swelling under baseline conditions in K^+ based media (open channel), inhibition by ATP, re-activation by diazoxide superseding the effect of ATP, and inhibition again by 5-HD over-riding the effect of diazoxide plus ATP. No effect of these agents on swelling was observed in Na^+ based media [33], and mitochondria isolated from adult worms staged either as embryos or as starved L1 larvae behaved similarly (data not shown).

Fig. 2C shows that the *C. elegans* mK_{ATP} channel also responds to a variety of other reagents in exactly the same manner as previously observed for the mammalian mitochondrial channel [10,15,17,33,39]. The mK_{ATP} channel openers cromakalim, pinacidil and malonate all reversed the inhibition of swelling by ATP, and the mK_{ATP} channel antagonists 5-HD and glyburide both reversed the effects of cromakalim, pinacidil and malonate.

Discussion

Our results demonstrate that viable mitochondria isolated from *C. elegans* contain a K_{ATP} channel identical to the mammalian mK_{ATP} channel in every aspect so far examined. This fundamental observation is the first report on the existence of an mK_{ATP} channel in multicellular invertebrates, suggesting that channel activity is evolutionarily conserved. Given the ability of *C. elegans* to undergo IPC [30], we further suggest that the mK_{ATP} channel is likely a fundamental component of all evolutionarily conserved preconditioning signal pathways.

Another advantage to the *C. elegans* model system in regard to mK_{ATP} channel function is the discovery herein that the channel activity was stable in isolated mitochondria for up to 3 h. The mK_{ATP} channel swelling assay has been criticized recently due to its poor reproducibility by some laboratories [40], and as discussed above a key factor governing the ability to assay the channel by this method is the freshness of isolated mitochondria [32,33]. The issue of channel stability may account for several related discrepancies in the mK_{ATP} channel literature, and thus the greater stability of the *C. elegans* mK_{ATP} channel activity may permit greater consensus in the future studies on this channel.

Numerous studies have centered on unraveling the molecular mechanisms of IPC over the past two decades. Despite significant advances, the molecular identity of mK_{ATP} is unknown, and this impairs progress in developing therapeutics to protect the heart and other organs from IR injury, a major cause of morbidity and mortality worldwide [41]. In addition it is known that IPC is less effective in certain patient cohorts (e.g. diabetics) [42], but the mechanisms underlying this are unclear. It is possible that differences in mitochondrial K^+ channel activity may underlie such effects, and thus identification of these channels will permit enhanced diagnosis and treatment of individuals refractory to IPC.

Despite surface K_{ATP} channels being well characterized at the molecular level (i.e. with well defined genes and protein structures, see [20] for review), none of the known Kir or SUR genes or their splice variants have mitochondrial targeting sequences. In addition, several antibodies used to identify SUR and Kir subunits in purified mitochondria are now known to recognize off-target unrelated proteins [25]. Thus, a competing hypothesis has emerged, that the mK_{ATP} channel may not be comprised of classical Kir/SUR proteins [21–23], and instead is comprised of other mitochondrial proteins [24]. Candidates for mK_{ATP} that have been proposed include: succinate dehydrogenase (complex II), the phosphate carrier, mitochondrial ATP-binding cassette protein-1 (mABC-1), adenine nucleotide translocator, and ATP synthase [24]. The conservation of function and direct genetic orthologies between mammalian and worm counterparts makes *C. elegans* an attractive model to test whether these molecules are components of or perhaps regulate mK_{ATP}. Our demonstration that worms possess an

mK_{ATP} channel activity is fundamental to this process, and the future analysis of candidate mutants should allow unambiguous insight into the identity of the mK_{ATP} channel.

Furthermore, it has recently been discovered that, analogous to mammals, *C. elegans* can also be protected from ischemic/hypoxic injury by preconditioning with volatile anesthetics (so-called "anesthetic preconditioning", APC) [31]. In addition, emerging work suggests that Ca^{2+} activated K^+ channels in the mitochondrion (mK_{Ca}) may participate in APC signaling, possibly playing a similar mechanistic role to mK_{ATP} channels. Thus, it is possible that the application of mitochondrial isolation methods and channel activity assays outlined herein, could also be applied to the molecular identification of the mK_{Ca} channel and its regulators.

Acknowledgments

We thank Phil. Morgan and Marge Sedensky (Seattle Children's Hospital) for an introduction to preparing *C. elegans* mitochondria. This work was supported in part by PHS Grants R01 HL080810 (to K.N.) and RO1 HL071158 (to P.S.B.). A.P.W. is the recipient of an American Heart Association Founders Affiliate pre-doctoral fellowship award.

References

[1] P.S. Brookes, Y. Yoon, J.L. Robotham, M.W. Anders, S.S. Sheu, Calcium, ATP, and ROS: a mitochondrial love-hate triangle, Am. J. Physiol. Cell Physiol. 287 (2004) C817–C833.
[2] J.S. Kim, Y. Jin, J.J. Lemasters, Reactive oxygen species, but not Ca^{2+} overloading, trigger pH- and mitochondrial permeability transition-dependent death of adult rat myocytes after ischemia-reperfusion, Am. J. Physiol. Heart Circ. Physiol. 290 (2006) H2024–H2034.
[3] E.J. Griffiths, A.P. Halestrap, Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion, Biochem. J. 307 (Pt 1) (1995) 93–98.
[4] F.G. Akar, M.A. Aon, G.F. Tomaselli, B. O'Rourke, The mitochondrial origin of postischemic arrhythmias, J. Clin. Invest. 115 (2005) 3527–3535.
[5] E.J. Lesnefsky, S. Moghaddas, B. Tandler, J. Kerner, C.L. Hoppel, Mitochondrial dysfunction in cardiac disease: ischemia–reperfusion, aging, and heart failure, J. Mol. Cell Cardiol. 33 (2001) 1065–1089.
[6] C.E. Murry, R.B. Jennings, K.A. Reimer, Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium, Circulation 74 (1986) 1124–1136.
[7] H.T. Facundo, M. Fornazari, A.J. Kowaltowski, Tissue protection mediated by mitochondrial K+ channels, Biochim. Biophys. Acta 1762 (2006) 202–212.
[8] K.D. Garlid, S.P. Dos, Z.J. Xie, A.D. Costa, P. Paucek, Mitochondrial potassium transport: the role of the mitochondrial ATP-sensitive K(+) channel in cardiac function and cardioprotection, Biochim. Biophys. Acta 1606 (2003) 1–21.
[9] I. Inoue, H. Nagase, K. Kishi, T. Higuti, ATP-sensitive K+ channel in the mitochondrial inner membrane, Nature 352 (1991) 244–247.
[10] K.D. Garlid, P. Paucek, V. Yarov-Yarovoy, H.N. Murray, R.B. Darbenzio, A.J. D'Alonzo, N.J. Lodge, M.A. Smith, G.J. Grover, Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K+ channels. Possible mechanism of cardioprotection, Circ. Res. 81 (1997) 1072–1082.
[11] S.C. Armstrong, G.S. Liu, J.M. Downey, C.E. Ganote, Potassium channels and preconditioning of isolated rabbit cardiomyocytes: effects of glyburide and pinacidil, J. Mol. Cell Cardiol. 27 (1995) 1765–1774.
[12] G.J. Gross, J.A. Auchampach, Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs, Circ. Res. 70 (1992) 223–233.
[13] T. Mizumura, K. Nithipatikom, G.J. Gross, Bimakalim, an ATP-sensitive potassium channel opener, mimics the effects of ischemic preconditioning to reduce infarct size, adenosine release, and neutrophil function in dogs, Circulation 92 (1995) 1236–1245.
[14] J.A. Auchampach, G.J. Grover, G.J. Gross, Blockade of ischaemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate, Cardiovasc. Res. 26 (1992) 1054–1062.
[15] M. Jaburek, V. Yarov-Yarovoy, P. Paucek, K.D. Garlid, State-dependent inhibition of the mitochondrial KATP channel by glyburide and 5-hydroxydecanoate, J. Biol. Chem. 273 (1998) 13578–13582.
[16] M.M. da Silva, A. Sartori, E. Belisle, A.J. Kowaltowski, Ischemic preconditioning inhibits mitochondrial respiration, increases H2O2 release, and enhances K+ transport, Am. J. Physiol Heart Circ. Physiol. 285 (2003) H154–H162.
[17] A.J. Kowaltowski, S. Seetharaman, P. Paucek, K.D. Garlid, Bioenergetic consequences of opening the ATP-sensitive K(+) channel of heart mitochondria, Am. J. Physiol. Heart Circ. Physiol. 280 (2001) H649–H657.
[18] R.A. Eliseev, B. Vanwinkle, R.N. Rosier, T.E. Gunter, Diazoxide-mediated preconditioning against apoptosis involves activation of cAMP-response element-binding protein (CREB) and NFkappaB, J. Biol. Chem. 279 (2004) 46748–46754.
[19] A.D. Costa, C.L. Quinlan, A. Andrukhiv, I.C. West, M. Jaburek, K.D. Garlid, The direct physiological effects of mitoK(ATP) opening on heart mitochondria, Am. J. Physiol. Heart Circ. Physiol. 290 (2006) H406–H415.
[20] L.V. Zingman, A.E. Alekseev, D.M. Hodgson-Zingman, A. Terzic, ATP-sensitive potassium channels: metabolic sensing and cardioprotection, J. Appl. Physiol. 103 (2007) 1888–1893.
[21] G.D. Mironova, A.E. Negoda, B.S. Marinov, P. Paucek, A.D. Costa, S.M. Grigoriev, Y.Y. Skarga, K.D. Garlid, Functional distinctions between the mitochondrial ATP-dependent K+ channel (mitoKATP) and its inward rectifier subunit (mitoKIR), J. Biol. Chem. 279 (2004) 32562–32568.
[22] P. Paucek, G. Mironova, F. Mahdi, A.D. Beavis, G. Woldegiorgis, K.D. Garlid, Reconstitution and partial purification of the glibenclamide-sensitive, ATP-dependent K+ channel from rat liver and beef heart mitochondria, J. Biol. Chem. 267 (1992) 26062–26069.
[23] R. Bajgar, S. Seetharaman, A.J. Kowaltowski, K.D. Garlid, P. Paucek, Identification and properties of a novel intracellular (mitochondrial) ATP-sensitive potassium channel in brain, J. Biol. Chem. 276 (2001) 33369–33374.
[24] H. Ardehali, Z. Chen, Y. Ko, R. Mejia-Alvarez, E. Marban, Multiprotein complex containing succinate dehydrogenase confers mitochondrial ATP-sensitive K+ channel activity, Proc. Natl. Acad. Sci. USA 101 (2004) 11880–11885.
[25] F.D. Brian, J.J. Rucker, E. Marban, Is Kir6.1 a subunit of mitoK(ATP)?, Biochem Biophys. Res. Commun. 366 (2008) 649–656.
[26] W.A. Chutkow, V. Samuel, P.A. Hansen, J. Pu, C.R. Valdivia, J.C. Makielski, C.F. Burant, Disruption of Sur2-containing K(ATP) channels enhances insulin-stimulated glucose uptake in skeletal muscle, Proc. Natl. Acad. Sci. USA 98 (2001) 11760–11764.
[27] P.P. Dzeja, P. Bast, C. Ozcan, A. Valverde, E.L. Holmuhamedov, D.G. Van Wylen, A. Terzic, Targeting nucleotide-requiring enzymes: implications for diazoxide-induced cardioprotection, Am. J. Physiol. Heart Circ. Physiol. 284 (2003) H1048–H1056.
[28] G. Schafer, C. Wegener, R. Portenhauser, D. Bojanovski, Diazoxide, an inhibitor of succinate oxidation, Biochem. Pharmacol. 18 (1969) 2678–2681.
[29] P.J. Hanley, K.V. Gopalan, R.A. Lareau, D.K. Srivastava, M.M. von, J. Daut, Beta-oxidation of 5-hydroxydecanoate, a putative blocker of mitochondrial ATP-sensitive potassium channels, J. Physiol. 547 (2003) 387–393.
[30] N. Dasgupta, A.M. Patel, B.A. Scott, C.M. Crowder, Hypoxic preconditioning requires the apoptosis protein CED-4 in *C. elegans*, Curr. Biol. 17 (2007) 1954–1959.
[31] B. Jia, C.M. Crowder, Volatile anesthetic preconditioning present in the invertebrate *Caenorhabditis elegans*, Anesthesiology 108 (2008) 426–433.
[32] H.T. Facundo, J.G. de Paula, A.J. Kowaltowski, Mitochondrial ATP-sensitive K+ channels are redox-sensitive pathways that control reactive oxygen species production, Free Radic. Biol. Med. 42 (2007) 1039–1048.
[33] A.P. Wojtovich, P.S. Brookes, The endogenous mitochondrial complex II inhibitor malonate regulates mitochondrial ATP-sensitive potassium channels: implications for ischemic preconditioning, Biochim. Biophys. Acta 1777 (2008) 882–889.
[34] A.D. Beavis, R.D. Brannan, K.D. Garlid, Swelling and contraction of the mitochondrial matrix. I. A structural interpretation of the relationship between light scattering and matrix volume, J. Biol. Chem. 260 (1985) 13424–13433.
[35] K.D. Garlid, A.D. Beavis, Swelling and contraction of the mitochondrial matrix. II. Quantitative application of the light scattering technique to solute transport across the inner membrane, J. Biol. Chem. 260 (1985) 13434–13441.
[36] S. Brenner, The genetics of Caenorhabditis elegans, Genetics 77 (1974) 71–94.
[37] E.B. Kayser, P.G. Morgan, C.L. Hoppel, M.M. Sedensky, Mitochondrial expression and function of GAS-1 in *Caenorhabditis elegans*, J. Biol. Chem. 276 (2001) 20551–20558.
[38] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[39] K.D. Garlid, P. Paucek, Mitochondrial potassium transport: the K(+) cycle, Biochim. Biophys. Acta 1606 (2003) 23–41.
[40] M. Das, J.E. Parker, A.P. Halestrap, Matrix volume measurements challenge the existence of diazoxide/glibencamide-sensitive KATP channels in rat mitochondria, J. Physiol. 547 (2003) 893–902.
[41] T. Thom, N. Haase, W. Rosamond, V.J. Howard, J. Rumsfeld, T. Manolio, Z.J. Zheng, K. Flegal, C. O'Donnell, S. Kittner, D. Lloyd-Jones, D.C. Goff Jr., Y. Hong, R. Adams, G. Friday, K. Furie, P. Gorelick, B. Kissela, J. Marler, J. Meigs, V. Roger, S. Sidney, P. Sorlie, J. Steinberger, S. Wasserthiel-Smoller, M. Wilson, P. Wolf, Heart disease and stroke statistics—2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee, Circulation 113 (2006) e85–e151.
[42] J.R. Kersten, W.G. Toller, E.R. Gross, P.S. Pagel, D.C. Warltier, Diabetes abolishes ischemic preconditioning: role of glucose, insulin, and osmolality, Am. J. Physiol. Heart Circ. Physiol. 278 (2000) H1218–H1224.