Mitochondrial ATP-sensitive K⁺ channels modulate cardiac mitochondrial function

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Mitochondrial ATP-sensitive K⁺ channels modulate cardiac mitochondrial function. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1567–H1576, 1998.—Discovered in the cardiac sarcolemma, ATP-sensitive K⁺ (KATP) channels have more recently also been identified within the inner mitochondrial membrane. Yet the consequences of mitochondrial KATP channel activation on mitochondrial function remain partially documented. Therefore, we isolated mitochondria from rat hearts and used K⁺ channel openers to examine the effect of mitochondrial KATP channel opening on mitochondrial membrane potential, respiration, ATP generation, Ca²⁺ transport, and matrix volume. From a mitochondrial membrane potential of −180 ± 15 mV, K⁺ channel openers, pinacidil (100 µM), cromakalim (25 µM), and levcromakalim (20 µM), induced membrane depolarization by 10 ± 7, 25 ± 9, and 24 ± 10 mV, respectively. This effect was abolished by removal of extramitochondrial K⁺ or application of a KATP channel blocker. K⁺ channel opener-induced membrane depolarization was associated with an increase in the rate of mitochondrial respiration and a decrease in the rate of mitochondrial ATP synthesis. Furthermore, treatment with a K⁺ channel opener released Ca²⁺ from mitochondria preloaded with Ca²⁺, an effect also dependent on extramitochondrial K⁺ concentration and sensitive to KATP channel blockade. In addition, K⁺ channel openers, cromakalim and pinacidil, increased matrix volume and released mitochondrial proteins, cytochrome c and adenylate kinase. Thus, isolated cardiac mitochondria, KATP channel openers depolarized the membrane, accelerated respiration, slowed ATP production, released accumulated Ca²⁺, produced swelling, and stimulated efflux of intermembrane proteins. These observations provide direct evidence for a role of mitochondrial KATP channels in regulating functions vital for the cardiac mitochondria.

heart; mitochondria; potassium channel openers; calcium

THE POTASSIUM ION is the major cytoplasmic and mitochondrial cation, and net flux of K⁺ across the inner mitochondrial membrane critically regulates mitochondrial activity (16). This includes regulation of energy production and maintenance of cellular Ca²⁺ homeostasis, two mitochondrial functions essential for cellular survival (12, 15, 23, 24). To allow for bidirectional K⁺ cycling across an otherwise K⁺-impermeable inner membrane, mitochondria express specialized K⁺ conduits (16). These include the well-characterized electro-neutral K⁺/H⁺ antiporter responsible for K⁺ efflux, along with less known pathways for K⁺ influx (8, 15).

A candidate mechanism for K⁺ entry is an ATP-sensitive K⁺ (KATP) channel (15, 16). This channel, known as the mitochondrial KATP channel, has been recently identified within the inner mitochondrial membrane (29), and the molecular identity of channel subunits has been partially characterized (16, 59, 60). Purified channel proteins, when incorporated into artificial bilayers or liposomes, reconstitute an ATP-sensitive K⁺ conductance with properties similar to those previously described for other members of the KATP Channel family (52). This channel family regroups nucleotide-gated inwardly rectifying K⁺ channels, which sense the metabolic state of a cell and regulate K⁺ flux and membrane excitability (1, 2, 31, 42, 46, 59, 67).

KATP Channels have been originally described in the cardiac sarcolemma (50), and opening of sarcolemmal KATP channels has been associated with shortening of cardiac action potential, decrease in intracellular Ca²⁺ loading, and cardioprotection during ischemia (5, 14, 20, 21, 32, 33, 44). However, the consequences of activation of mitochondrial KATP channels on mitochondrial functions, including oxidative phosphorylation and Ca²⁺ transport, have not been determined.

K⁺ channel-opening drugs are the established tool used to activate KATP channels (21, 54, 66) and also target the mitochondrial KATP channels (17, 18, 61, 62). Therefore, in this study we used K⁺ channel openers to examine the effect of mitochondrial KATP channel opening on mitochondrial functions, including membrane potential, respiration, ATP generation, Ca²⁺ transport, and membrane integrity. We present evidence that activation of mitochondrial KATP channels modulates the function of isolated cardiac mitochondria.

MATERIALS AND METHODS

Isolated cardiac mitochondria. Cardiac mitochondria were isolated from ether- or pentobarbital-anesthetized rats by differential centrifugation (25, 43). After thoracotomy, hearts were rapidly excised into an ice-cold isolation buffer (50 mM sucrose, 200 mM mannitol, 5 mM KH₂PO₄, 1 mM EGTA, 5 mM MOPS, and 0.1% BSA, pH 7.15 adjusted with KOH), atria were removed, and 1-mm³ pieces of ventricular myocardium were homogenized (30 ml of isolation buffer per heart) using a PT10/35 Polytron (Brinkman). Three 20-s homogenization cycles were performed on ice, and then the samples were centrifuged for 10 min (at 750 g) using a Sorvall II centrifuge equipped with a GSA rotor. The supernatant containing the mitochondrial fraction, was further centrifuged at 7,000 g for 20 min, and the pellet was resuspended in 30 ml of isolation buffer (with no EGTA) and spun at 7,000 g for 20 min. Finally, mitochondria were resuspended in the...
isolation buffer (with no EGTA), and protein concentration was determined using a protein kit (Bio-Rad). Mitochondrial suspension (~30–40 mg protein/ml) was kept on ice before experiments.

Mitochondrial membrane potential. Measurements were made at 30°C under continuous stirring of the mitochondrial suspension (1 mg protein/ml incubation medium) placed into a multichannel chamber of an ESON-6ch computerized analyzer (25–28). The standard incubation medium contained (in mM) 110 KCl, 5 K₂HPO₄, 5 succinate, 5 pyruvate, and 10 MOPS (pH 7.15). Mitochondrial membrane potential was measured with tetraphenylphosphonium (TPP⁺)-sensitive minielectrodes manufactured and calibrated as described elsewhere (38). TPP⁺ (200 nM) was added to the incubation medium in the chamber before addition of mitochondria, and mitochondrial membrane potential was calculated according to the following equation:

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\Delta \psi = 59 \times \log (V/V) - 59 \times \log [10^{(E_0 - E_0)} - 1],
$$

where $\Delta \psi$ is mitochondrial membrane potential (mV), $V$ is mitochondrial matrix volume (1.6 µl/mg mitochondrial protein) (53), $V$ is volume of incubation medium (1 ml), and $E_0$ and $E$ are electrode potentials before and after addition of mitochondria, respectively (49). Membrane potential was measured in the presence of K⁺ channel openers [pinacidil (RBI), cromakalim (Sigma Chemical), and levocromakalim (a gift from SmithKline Beecham)], K⁺ openers [pinacidil (RBI), cromakalim (Sigma Chemical), and levocromakalim (a gift from SmithKline Beecham)], K⁺ ionophore (valinomycin), and/or K⁺ channel blockers [glyburide and 5-hydroxydecanoic acid (5-HD), both from RBI]. In addition, mitochondrial membrane potential was also measured after addition of ADP, in the absence and presence of a K⁺ channel opener.

Mitochondrial respiration. O₂ consumption of mitochondria was measured in the multichannel chamber using a calibrated Clark-type O₂ minielectrode (11, 25). For calibration purposes, depletion of O₂ content within the mitochondrial suspension was achieved by sodium hydrosulfite. Data acquisition and processing were the same as with the TPP⁺ electrode. The effects of drugs were assessed on “state 2” mitochondrial respiration (7). In addition, in certain experiments, ADP-stimulated mitochondrial respiration (“state 3”) was measured in the absence and presence of a K⁺ channel opener.

Mitochondrial Ca²⁺ uptake. Mitochondrial Ca²⁺ uptake was measured as a change in the free Ca²⁺ concentration within the suspension using calibrated Ca²⁺-selective minielectrodes (Microelectrodes) (11, 26, 28). Data acquisition and processing were the same as with the TPP⁺ electrode. The effect of K⁺ channel openers on mitochondrial Ca²⁺ retention and release was studied in mitochondria preloaded with Ca²⁺ (3 consecutive 40-nmol Ca²⁺ pulses/mg mitochondrial protein).

Mitochondrial ATP synthesis. Mitochondrial ATP content was determined in mitochondrial extracts (9). Briefly, 200 µl of mitochondrial suspension were treated with 20 µl of 3.3 M HClO₄, and precipitated proteins were removed by centrifugation (60 s, 14,000 rpm, 4°C). After neutralization of the supernatant with 80 µl of a mixture containing 2.5 M K₂CO₃ in 1 M HEPES, the precipitate was separated by centrifugation (60 s, 14,000 rpm, 4°C), and the concentration of ATP within the extract was determined by coupled enzymatic analysis based on spectrophotometric detection of NADH (40) in the absence and presence of a K⁺ channel opener.

Mitochondrial volume and integrity. Light scattering of mitochondrial suspensions, a measure of matrix volume (18, 24, 25), was determined within a 10-mm cuvette (maintained at 22°C) in the presence of K⁺ channel openers or a K⁺ ionophore. The absorbance of the mitochondrial suspension was measured at 540 nm using a spectrophotometer (model DU-7400, Beckman).

The integrity of the mitochondrial membrane, in the absence and presence of K⁺ channel openers, was assessed by measuring release of cytochrome c and adenylate kinase, two intermembrane proteins (47, 56). Supernatants, obtained after sedimentation (at 14,000 g, 15 min, 4°C) of the mitochondrial suspension, were concentrated by ultrafiltration (Centriflo, Amicon), and an aliquot (30 µl) was immobilized on nitrocellulose membranes (Optitran BA-NC) using a Minifold I dot-blot system. Membrane dot blots were probed with a mouse anti-cytochrome c monoclonal antibody (clone 6H2.2B4, Pharmingen) and detected with a rabbit anti-mouse IgG conjugated with horseradish peroxidase (Amersham), which served as a secondary antibody. The blot was placed on the SuperSignal CL-HRP substrate (Pierce) and exposed to an X-Omat film (Eastman Kodak) for 60 s. Adenylate kinase activity was determined within the supernatant spectrophotometrically (9, 10, 40).
RESULTS

Effect on mitochondrial membrane potential. Isolated cardiac mitochondria had a membrane potential of $-180 \pm 15$ mV ($n = 12$), as demonstrated using a potential-sensitive probe, TPP$^+$ (Fig. 1A). Mitochondria maintained a steady membrane potential throughout the duration of recordings (up to 40 min). Addition of the K$^+$ channel opener pinacidil (100 µM) induced rapid depolarization (Fig. 1A). On average, mitochondrial membrane potential decreased by $10 \pm 7$ mV ($n = 5$) in the presence of 100 µM pinacidil. The effect of pinacidil was concentration dependent (Fig. 1B). The K$^+$ ionophore valinomycin (12.5 ng/mg mitochondrial protein) also reduced mitochondrial membrane potential, indicating that depolarization can be triggered by promoting K$^+$ flux across the mitochondrial membrane (Fig. 1A).

Cromakalim (Fig. 2A) and levocromakalim (Fig. 3A), K$^+$ channel openers that are structurally unrelated to pinacidil, also induced mitochondrial membrane depolarization when applied at concentrations known to activate plasmalemmal K$_{ATP}$ channels (21, 65). On average, cromakalim (25 µM) and levocromakalim (20 µM) decreased the mitochondrial membrane potential by 25 ± 9 and 24 ± 10 mV, respectively ($n = 4$).

In nominally K$^+$-free medium, cromakalim could no longer induce mitochondrial depolarization (Fig. 2B). Introduction of KCl into the medium bathing mitochondria restored the depolarizing effect of cromakalim (Fig. 2B). Conversely, in mitochondria in which the potential was dissipated by antimycin A (an inhibitor of the mitochondrial respiratory chain) and oligomycin (an inhibitor of mitochondrial ATPase), cromakalim, in the absence of extramitochondrial K$^+$, induced hyperpolarization of the mitochondrial membrane (Fig. 2C). This suggests that the effect of the K$^+$ channel opener on mitochondrial membrane potential is dependent on the electrochemical gradient for K$^+$.

The depolarizing effect of K$^+$ channel openers was sensitive to 5-HD, a selective antagonist of K$_{ATP}$ channels (51). By itself, 5-HD (20 µM) had no effect on the steady-state mitochondrial membrane potential but prevented mitochondrial depolarization by levocromakalim (20 µM; Fig. 3; $n = 7$).

ADP, a mitochondrial substrate used in the production of ATP, is known to induce transient mitochondrial depolarization, the duration of which depends on the time required for conversion of ADP to ATP (43). In the absence of a K$^+$ channel opener, ADP (500 µM) induced depolarization of cardiac mitochondria that lasted 105 ± 15 s (Fig. 4A; $n = 6$). In the presence of pinacidil (100 µM - 1 mM), the ADP-induced mitochondrial membrane depolarization was significantly prolonged (Fig. 4).

Effect on mitochondrial respiration. The rate of state 2 respiration of isolated cardiac mitochondria was $14.0 \pm 6.1.3$ nmol O$_2 \cdot$min$^{-1} \cdot$mg protein$^{-1}$ ($n = 20$). Pinacidil (100 µM - 1 mM), in a concentration-dependent manner, induced an increase in the rate of mitochondrial respiration (Fig. 5A). Pretreatment (3-5 min) of mitochondria with the K$_{ATP}$ channel blocker glyburide (1 µM) inhibited the effect of pinacidil on mitochondrial respiration (Fig. 5B; $n = 3$).

Fig. 2. Cromakalim (CROM)-induced mitochondrial membrane depolarization and extramitochondrial concentration of K$^+$. A: cromakalim (20 µM)-induced membrane depolarization. Valinomycin (12.5 ng/mg mitochondrial protein) was added as a positive control. B: in nominally K$^+$-free solution (i.e., KCl replaced with 110 mM choline chloride, K$_2$HPO$_4$ with 5 mM Na$_2$HPO$_4$, and pH adjusted with Trizma base), cromakalim (20 µM) was without effect. Addition of KCl (in 4 mM steps) restored the depolarizing effect of cromakalim. C: in deenergized mitochondria treated with antimycin A (AntIA, 1 µg/mg mitochondrial protein) and oligomycin (Oligo, 1 µg/mg mitochondrial protein), cromakalim (in 25 µM steps) induced mitochondrial membrane hyperpolarization in nominally K$^+$-free solution. Arrowheads, addition of isolated cardiac mitochondria to a solution containing TPP$^+$. Mitochondrial membrane potential was measured as described in Fig. 1 legend.
ADP (500 µM) induced transition of mitochondria from state 2 to state 3 respiration (Fig. 6; n = 6). Pinacidil (100 µM) slowed ADP-induced state 3 respiration (from 165 ± 7 to 55 ± 5 natsoms O₂·min⁻¹·mg protein⁻¹ in the absence and presence of pinacidil, respectively) without changing the total amount of the ADP-stimulated O₂ consumption, which was 340 ± 10 and 353 ± 8 natsoms in the absence and presence of the K⁺ channel opener (n = 3; Fig. 6).

Effect on mitochondrial ATP synthesis. Isolated cardiac mitochondria efficiently phosphorylated ADP (500 µM) so that ~90% of added ADP was converted to ATP within 100 ± 15 s (n = 3; Fig. 7). The initial rate of ATP synthesis was 401 ± 35 nmol ATP·min⁻¹·mg protein⁻¹, and the half-time required for complete conversion of added ADP to ATP was 27 ± 5 s (n = 3; Fig. 7). Pinacidil (100 µM) decreased the rate of initial ATP synthesis to 187 ± 23 nmol ATP·min⁻¹·mg protein⁻¹ and increased the half-time required for conversion of ADP to ATP to 87 ± 7 s (n = 3; Fig. 7). Although the rate of mitochondrial ATP synthesis was slowed, pinacidil (100 µM) did not prevent conversion of ADP to ATP (Fig. 7).

Effect on mitochondrial Ca²⁺ loading. Mitochondria are known to accumulate and to retain Ca²⁺ within the matrix (22). Isolated cardiac mitochondria efficiently took up and retained added Ca²⁺ when repeatedly challenged with extramitochondrial Ca²⁺ pulses (in total 120 nmol Ca²⁺/mg mitochondrial protein; Fig. 8). Addition of pinacidil (250 µM) induced rapid release of Ca²⁺ from preloaded mitochondria (Fig. 8, A–C). The efficacy of pinacidil to induce release of mitochondrial Ca²⁺ was dependent on the extramitochondrial concentration of K⁺ (Fig. 8, A–C). At 1, 10, and 110 mM extramitochondrial KCl, the magnitude of pinacidil-induced Ca²⁺ release was 1, 3, and 5 nmol Ca²⁺/mg mitochondrial protein, respectively (Fig. 8, A–C). The Ca²⁺-releasing effect of pinacidil (250 µM) was suppressed by pretreatment of mitochondria with the K⁺ channel blocker glyburide (1 µM; Fig. 8D).
Effect on mitochondrial matrix volume and integrity. The volume of mitochondria depends on the permeability of the inner membrane to osmotically active particles, such as K\(^+\) (6). The K\(^+\) channel opener cromakalim (100 µM) induced swelling of mitochondria, as revealed by a decrease in the absorbance of the mitochondrial suspension in K\(^+\)-containing media by 0.1 ± 0.02 optical density unit (n = 3; Fig. 9A). Under this condition, the K\(^+\) ionophore valinomycin also induced significant mitochondrial swelling (not shown). In contrast, in K\(^+\)-free medium, cromakalim had no significant effect on mitochondrial swelling (Fig. 9; n = 3). K\(^+\)-dependent changes in mitochondrial volume were also observed with pinacidil (250 µM; Fig. 9B).

Cytochrome c and adenylate kinase are located within the intermembrane space of mitochondria (58). Under control conditions, isolated cardiac mitochondria show no significant release of cytochrome c (Fig. 10) or adenylate kinase (Fig. 11). K\(_{\text{ATP}}\) channel openers pinacidil (250 µM) and cromakalim (100 µM) induced release of cytochrome c (Fig. 10) and adenylate kinase (Fig. 11). The K\(^+\) ionophore valinomycin (12.5 ng/mg mitochondrial protein), used as a positive control, also induced release of cytochrome c (Fig. 10) and adenylate kinase (Fig. 11).

DISCUSSION

In the present study we report that, in mitochondria isolated from cardiac muscle, K\(^+\) channel openers in-
duced membrane depolarization, accelerated respiration, slowed ATP production, triggered release of Ca$^{2+}$, and produced swelling associated with efflux of intermembrane proteins. In accord with an activation of mitochondrial K$\text{ATP}$ channels, these effects were dependent on the extramitochondrial concentration of K$^+$, were inhibited by blockers of K$\text{ATP}$ channels, and could be induced by structurally distinct K$^+$ channel openers. These observations provide direct evidence for a role of mitochondrial K$\text{ATP}$ channels in the regulation of mitochondrial functions.

That K$\text{ATP}$ channels are present within the inner membrane of mitochondria was initially demonstrated on the basis of electrophysiological measurements of a K$^+$-selective, ATP-gated ion conductance in fused giant mitoplasts (29). The presence of functional K$\text{ATP}$ channels in mitochondria was confirmed after isolation of an inner membrane protein fraction from rat liver and beef heart mitochondria, which, when reconstituted in liposomes or planar lipid bilayers, catalyzed electrophoretic ATP-sensitive K$^+$ flux (52). This ATP-inhibited K$^+$ flux was activated by K$^+$ channel openers such as cromakalim (18) and suppressed by K$^+$ channel blockers such as glyburide and 5-HD (3, 17, 51, 63). K$^+$ influx through this channel was hypothesized to serve as a mechanism for osmotic regulation of mitochondrial volume (15, 23, 29, 62). In mitochondria from noncardiac tissue, such as liver and pancreatic $\beta$-cells, openers of K$\text{ATP}$ channels were found to affect mitochondrial energy metabolism (16, 19, 63). Thus the present findings that opening of mitochondrial K$\text{ATP}$ channels can modulate multiple functions in isolated cardiac mitochondria provide direct evidence for a K$\text{ATP}$ channel-mediated regulation of cardiac mitochondrial behavior.

Pinacidil, cromakalim, and levcromakalim share the property to activate K$\text{ATP}$ channels (21, 54, 66) and were found here to depolarize the membrane potential of cardiac mitochondria. However, it has remained controversial whether opening of mitochondrial K$\text{ATP}$ channels can be associated with changes in mitochondrial membrane potential (19, 61). In pancreatic $\beta$-cells, glyburide, a K$\text{ATP}$ channel blocker, failed to antagonize the depolarizing effect of diazoxide, a drug with multiple sites of action, including K$^+$ channel activation, suggesting that diazoxide-induced membrane depolarization is not associated with activation of K$\text{ATP}$ channels, at least in this cell type (19). Moreover, in isolated liver mitochondria, other K$^+$ channel openers, including aprikalim and nicorandil, were without effect on membrane potential, although in the same preparation, RP-66471, a more recently developed K$\text{ATP}$ channel opener, induced mitochondrial depolarization (61).

Here, in cardiac mitochondria, a K$\text{ATP}$ channel-mediated regulation of mitochondrial membrane potential is supported by three lines of evidence. First, structurally distinct K$^+$ channel openers were effective in depolarizing mitochondria. Second, the effect of K$^+$ channel openers on mitochondrial membrane potential
was dependent on the electrochemical gradient for K⁺. Third, 5-HD, a selective K⁺ATP channel blocker (51), did not affect membrane potential by itself but prevented the depolarizing action of a K⁺ channel opener. Our findings are in accord with the property of cromakalim and other K⁺ channel openers to induce K⁺ influx through mitochondrial K⁺ATP channels (18). Under the present experimental condition (110 mM extramitochondrial K⁺ and ΔΨ = −180 mV), opening of K⁺ATP channels is expected to lead to K⁺ influx and depolarization of mitochondria. In turn, a decrease in membrane potential would dissipate the driving force for ATP synthesis, triggering a compensatory activation of the mitochondrial respiratory chain. It has previously been shown that increase in the K⁺ permeability by valinomycin of the inner membrane decreases the rate of ATP synthesis and promotes respiration (53). The present study extends this concept and shows that activation of K⁺ATP channels not only induces membrane depolarization but can also reduce the rate of ATP synthesis and increase O₂ consumption in cardiac mitochondria.

In addition to energy production, mitochondria have the capacity to store Ca²⁺, a function critical in the maintenance of cellular Ca²⁺ homeostasis (13). In the present study, isolated cardiac mitochondria, when challenged with repeated Ca²⁺ pulses, displayed the property to accumulate and store Ca²⁺. Opening of mitochondrial K⁺ATP channels rapidly released Ca²⁺ from preloaded mitochondria, an effect also seen with K⁺ ionophores. Although the precise mechanism for such an effect on Ca²⁺ release is unknown, the sensitivity toward K⁺ATP channel blockade and the dependence on extramitochondrial K⁺ concentration are in line...
with a $K_{\text{ATP}}$ channel-mediated membrane depolarization and promotion of $Ca^{2+}$ release. Such interpretation also concurs with the notion that setting the mitochondrial membrane potential is critical for cyclic accumulation and release of $Ca^{2+}$ (30).

In the present study we further demonstrated that opening of mitochondrial $K_{\text{ATP}}$ channels increased matrix volume, which is manifested through mitochondrial swelling. This finding supports previous reports that mitochondrial $K_{\text{ATP}}$ channels can regulate the mitochondrial swelling. This finding supports previous reports that mitochondrial $K_{\text{ATP}}$ channels promote cellular survival (17, 20, 41).

In summary, the present study provides direct evidence that opening of mitochondrial $K_{\text{ATP}}$ channels modulates essential functions of isolated cardiac mitochondria. The cellular consequences of mitochondrial $K_{\text{ATP}}$ channel opening are not known. It has been proposed that this ion conductance may participate in the protection of cardiac muscle from ischemia-reperfusion injury (17). Because mitochondrial $Ca^{2+}$ overload is implicated in cellular injury (13), our findings may suggest a possible beneficial effect of mitochondrial $K_{\text{ATP}}$ channel opening through regulation of mitochondrial $Ca^{2+}$ levels. The present study also identifies a $K_{\text{ATP}}$ channel-mediated decrease in the rate of ATP synthesis and a release of mitochondrial proteins. Because a decrease in cellular ATP content coupled with the presence of cytochrome $c$ within the cytosol has been associated with cellular apoptosis (48, 56), this further suggests that opening of mitochondrial $K_{\text{ATP}}$ channels may participate in the regulation of cellular viability. The net effect of opening of mitochondrial $K_{\text{ATP}}$ channels is, however, difficult to predict in view of the diversity in cellular protective mechanisms (20, 21, 34, 35, 44, 57) and the complex nature of $K_{\text{ATP}}$ channel gating (2, 4, 16, 36, 65). Indeed, it is still controversial whether in excitable tissues opening of $K_{\text{ATP}}$ channels leads to programmed cell death (68) or promotes cellular survival (17, 20, 41).

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