

Influence of K_{ATP} channel openers on the permeability of cardiac mitochondrial membranes for potassium ions, protons and ADP during mild acidosis

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The inhibition of ATP-ase or the impairment of ADP/ATP transport is suggested to be responsible for the cardioprotective effects of K_{ATP} channel openers. In this study, we investigated the ability of K_{ATP} channel openers to affect K^+ and H^+ flux to the matrix of rat heart mitochondria as well as permeability of mitochondrial membranes for ADP under mildly acidic (pH 6.8) conditions. K^+ and H^+ flux to the mitochondrial matrix was registered spectrophotometrically as the swelling of non-respiring mitochondria at 540 nm in the KNO_3 and NH_4NO_3 medium. The apparent K_M for ADP of mitochondria was estimated from the least-squares fit to the Michaelis–Menten equation. The results showed that the K_{ATP} channel openers diazoxide and pinacidil could activate potassium ion and proton flux to the mitochondrial matrix both under normal (pH 7.4) and mildly acidic (pH 6.8) conditions. Mild acidosis increased the apparent K_M for ADP of mitochondria two times (up to $60.6 \pm 3.4 \mu M$) as compared to control ($33.0 \pm 3.2 \mu M$). The K_{ATP} channel opener diazoxide (100 μM) increased the apparent K_M for ADP by 40% under control and by 30% under mildly acidic conditions. Our results suggest that K_{ATP} channel openers could suppress the ADP/ATP exchange during ischemia and thus promote preservation of ATP in cardiomyocytes.

Key words: rat heart mitochondria, K_{ATP} channel, ADP/ATP exchange, diazoxide, pinacidil, acidosis

INTRODUCTION

Extensive investigations of the mechanism of cardioprotection by K_{ATP} channel openers have raised several hypotheses which all contribute to preservation of mitochondrial functions. K_{ATP} channel openers increase mitochondrial matrix swelling [1, 2], modulate ROS production [3–6], induce moderate uncoupling of mitochondrial oxidative phosphorylation [7, 8]. Since K_{ATP} channel openers preserve ATP in cardiomyocytes during ischemia, one of the currently proposed mechanisms of cardioprotection by K_{ATP} channel openers is inhibition of ATP hydrolysis [9, 10] due to direct action on ATP-ase [11, 12] or due to alteration of mitochondrial inner membrane permeability for ADP and ATP [13].

Our previous investigations have shown that K_{ATP} channel openers partially inhibit ADP/ATP exchange under normal conditions [14]. Since cytosolic acidification is one of the markers of ischemia [15, 16], in this study we investigated the ability of K_{ATP} channel openers to affect K^+ and H^+ flux to the mitochondrial matrix as

well as permeability for ADP under mildly acidic (pH 6.8) conditions.

MATERIALS AND METHODS

Isolation of rat heart mitochondria. The experiments were carried out on mitochondria isolated from male Wistar rat hearts by differential centrifugation procedure. After rat decapitation, hearts were excised and rinsed in ice-cold isolation medium, containing 220 mM manitol, 70 mM sucrose, 5 mM N-tris[Hydroxymethyl]methyl-2-aminoethane-sulfonic acid (TES) and 0.5 mM EGTA (pH 7.4, adjusted with Trizma base; 2 °C). Mitochondria were isolated in the same medium supplemented with 2 mg/ml bovine serum albumin (BSA; fraction V, A4503, Sigma). The homogenate was centrifugated for 5 min at 750×g, then the supernatant was centrifugated for 10 min at 6740×g and the pellet was washed once in the isolation medium without BSA, suspended in it and kept on ice. Mitochondrial protein concentration was determined by the biuret method [17] using BSA as a standard. The final mitochondrial protein concentration in all experiments was 0.5 mg/ml.

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Measurement of mitochondrial swelling

Swelling of non-respiring mitochondria was spectrophotometrically recorded as a decreased light scattering at 540 nm in KNO_3 medium (120 mM KNO_3 , 10 mM HEPES, 3 mM rotenone, 1 μg oligomycin/mg mitochondrial protein; 200 μM ATP; pH 7.4 or 6.8, adjusted with Trizma base, 25 °C) or NH_4NO_3 medium, where KNO_3 was replaced by NH_4NO_3 .

Measurement of oxygen consumption rate

The rates of oxygen uptake were recorded at 37 °C using the Clark-type electrode system in KCl medium (120 mM KCl, 5 mM KH_2PO_4 , 5 mM TES and 1 mM MgCl_2 ; pH 7.4 or 6.8, adjusted with Trizma base, 37 °C). Pyruvate and malate (6+6 mM) were used as substrates. The solubility of oxygen was taken to be 422 ngatoms/ml [18]. Respiration rates were expressed as $\text{nmol O} \times \text{min}^{-1} \times \text{mg mitochondrial protein}^{-1}$.

Estimation of the apparent K_M for ADP of mitochondria

1.2 IU/ml yeast hexokinase (Type V; EC 2.7.1.1, Sigma) and 24 mM glucose were used as an ADP regeneration system. The apparent K_M for ADP of mitochondria was estimated from the least-squares fit to the Michaelis–Menten equation by GraphPad Prism v.3.0.

Statistical analysis

The results are expressed as means \pm S.E. of three independent experiments. A comparison between groups was performed using ANOVA followed by the Tukey test. A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The ability of K_{ATP} channel openers to increase K^+ and H^+ flux to the mitochondrial matrix was evaluated by swelling experiments in the KNO_3 and NH_4NO_3 medium. Since NO_3^- freely permeates membranes, the swelling rate of non-respiring mitochondria (respiration is blocked by rotenone and phosphorylation or ATP hydrolysis by oligomycin) is limited respectively by the potassium and proton permeability of the mitochondrial inner membrane under these conditions. Our results show that the K_{ATP} channel openers diazoxide and pinacidil increased mitochondrial swelling both in KNO_3 (Fig. 1A) and NH_4NO_3 (Fig. 1B) media. K_{ATP} channel opener-induced swelling was negligibly less under mildly acidic (Fig. 1; dashed line) than in normal (Fig. 1; solid line) conditions. Thus, our results indicate that diazoxide and pinacidil could activate K^+ and H^+ flux through the inner membrane of cardiac mitochondria both under normal and mildly acidic conditions.

K^+ and H^+ transport to the mitochondrial matrix was suggested to occur via mitochondrial adenine nucleotide translocase (ANT) [19, 20] which catalyses the transmembrane exchange between ATP generated inside mi-

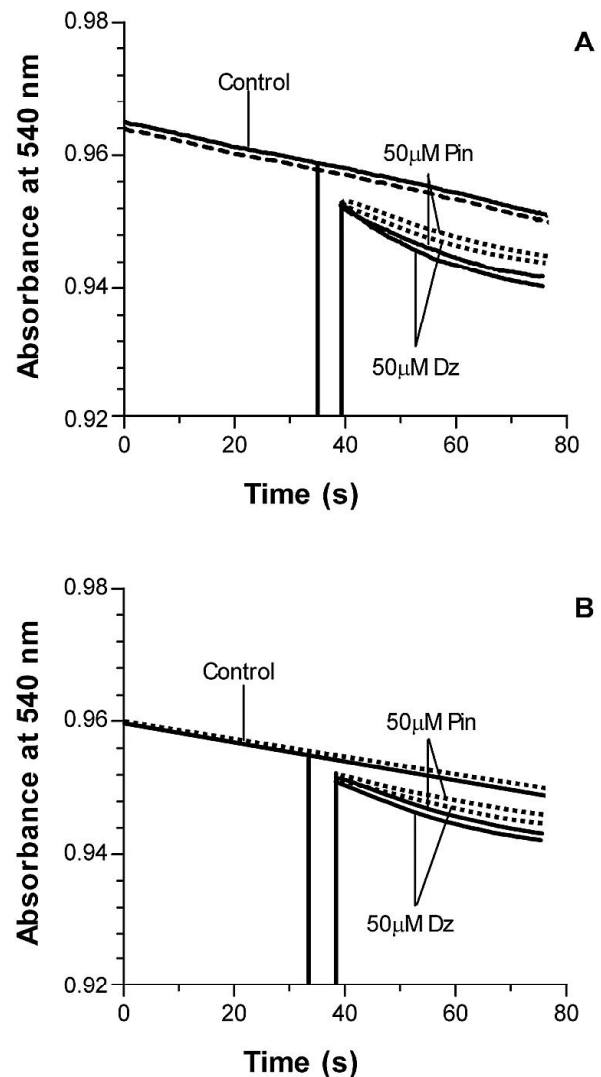


Fig. 1. Diazoxide (Dz)- and pinacidil (Pin)-induced swelling of rat heart mitochondria in KNO_3 (A) and NH_4NO_3 (B) medium under normal (solid line; pH 7.4) and mildly acidic (dashed line; pH 6.8) conditions. Experiments were performed at 25 °C. Similar curves were obtained in three independent experiments

tochondria through oxidative phosphorylation and cytosolic ADP [21–23]. Recently, the involvement of the ANT in the K^+ transport in brain mitochondria was shown by Brustovetsky's group [24]. Our previous results revealed the sensitivity of K_{ATP} channel opener-induced K^+ and H^+ flux through the mitochondrial inner membrane to inhibitors of ANT – carboxyatractyloside and bongkrekcic acid as well as to adenine nucleotides [14]. Therefore, we tested the effects of K_{ATP} channel openers on the permeability of mitochondrial membranes to ADP. Our results (Fig. 2) show that mild acidosis (pH 6.8) increased the apparent K_M for ADP of heart mitochondria two times (up to $60.6 \pm 3.4 \mu\text{M}$) compared to control ($33.0 \pm 3.2 \mu\text{M}$). This finding is consistent with earlier observations that a decreased extramitochondrial pH causes inhibition of State 3 respiration and suppressed ATP production in mitochondria

[25–27]. K_{ATP} channel opener diazoxide (100 μ M) increased the apparent K_M for ADP by 40% under control and by 30% under mildly acidic conditions (Fig. 2). Thus, diazoxide similarly decreases the permeability of mitochondrial membranes to ADP at pH 7.4 and pH 6.8. These results suggest that the K_{ATP} channel openers could also suppress the ADP/ATP exchange during ischemia.

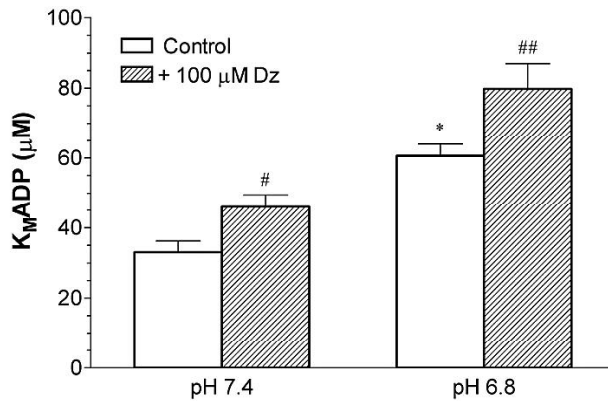


Fig. 2. Influence of mild acidosis (pH 6.8) and K_{ATP} channel opener diazoxide (Dz) on the apparent K_M for ADP of rat heart mitochondria

Experiments were performed in KCl medium at 37 °C, substrate – pyruvate and malate (6 + 6 mM), $n = 3$. # $P < 0.05$; ## $P < 0.05$ – statistically significant effect of diazoxide compared to control under normal and acidic conditions; * $P < 0.05$ – statistically significant effect of acidosis compared to control (pH 7.4). The results were analyzed with ANOVA followed by Tukey test.

Mitochondrial adenine nucleotide translocase is one of the key regulators of oxidative phosphorylation during development of ischemic injury [28]. During ischemia, instead of providing ADP for phosphorylation, this protein can reverse and allow the entry of ATP into the mitochondria for hydrolysis by ATP-ase [29]. Since the K_{ATP} channel openers not only increased K^+ and H^+ flux to mitochondrial matrix, but simultaneously increased the apparent K_M for ADP, our results suggest that K_{ATP} channel openers could diminish the selectivity of adenine nucleotide translocase to adenine nucleotides, thus suppressing the ADP/ATP exchange and promoting preservation of ATP in cardiomyocytes during ischemia.

ACKNOWLEDGEMENTS

This work was supported by the Lithuanian State Science and Studies Foundation.

Received 10 March 2006
Accepted 18 August 2006

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**K_{ATP} KANALŲ AKTYVATORIŲ POVEIKIS ŠIRDIES
MITOCHONDRIJŲ MEMBRANŲ LAIDUMUI KALIO
JONAMS, PROTONAMS IR ADP ACIDOZĖS METU**

S a n t r a u k a

Šiame darbe tyrėme K_{ATP} kanalų aktyvatorių poveikį K^+ ir H^+ srautui į žiurkės širdies mitochondrijų užpildą bei mitochondrijų membranų laidumui ADP švelnios acidozės metu. Gauti rezul-

tatai rodo, kad K_{ATP} kanalų aktyvatoriai diazoksidas ir pinacidilas normaliomis (pH 7,4) ir rūgštinėmis sąlygomis (pH 6,8) panašiai didino K^+ ir H^+ srautą į mitochondrijų matiksą. Rūgštinėmis sąlygomis, lyginant su kontrole ($33,0 \pm 3,2 \mu M$), du kartus (iki $60,6 \pm 3,4 \mu M$) padidėjo mitochondrijų tariamoji K_M ADP. Diazoksidas ($100 \mu M$) padidino tariamąją K_M ADP 40% normaliomis ir 30% rūgštinėmis sąlygomis. Taigi mūsų duomenys leidžia daryti prielaidą, kad K_{ATP} kanalų aktyvatoriai galėtų sulėtinti ADP/ATP mainus ischemijos metu ir taip padėtų išsaugoti kardiomiocituose ATP.