PINACIDIL MODULATES MITOCHONDRIAL FUNCTION UNDER EXPERIMENTAL MYOCARDIUM DYSTROPHY IN RATS

HALYNA TKACHENKO AND NATALIA KURHALYUK

Department of Hygiene and Toxicology,
Danylo Halutsky Lviv National Medical University, 79010 Lviv, Ukraine

1Department of Zoology and Animal Physiology, Institute of Biology and Environment Protection,
Pomeranian Pedagogical University, 76-200 Słupsk, Poland

kurhalyuk@pap.edu.pl

Received for publication April 24, 2006.

Abstract

The effects of KATP channels opener pinacidil (0.06 mg/kg) and blocker glibenclamide (1 mg/kg), on the function of mitochondria and lipid peroxidation processes in the heart of rats with low (LR), and high resistance (HR) to hypoxia under adrenaline myocardium dystrophy were studied. Succinate (0.35 mM) and α-ketoglutarate (1 mM) were used as oxidative substrates. Pinacidil caused an increase in the efficiency of oxygen consumption in heart mitochondria of rats with HR and economical utilization in rats with LR to hypoxia under succinate oxidation. The effects of pinacidil on mitochondrial oxygen consumption were more significant under succinic oxidation in rats with HR, than α-ketoglutarate oxidation in rats with LR to hypoxia. These changes caused the increase in α-ketoglutarate oxidation efficiency, with a temporary decrease in lipid peroxidation processes in rats with LR to hypoxia. Glibenclamide, when administered before the adrenaline myocardium dystrophy, abolished the protection induced by pinacidil.

Key words: rats, KATP-channels, pinacidil, glibenclamide, myocardium dystrophy, mitochondria, resistance, hypoxia.

Adrenaline induces heart injury, and is an important medical problem. Several studies have shown that KATP channel openers such as pinacidil, cromakalim, and diazoxide, are protective against ischaemia-reperfusion in experimental model (4, 6-11). The KATP channel is the end effector protein, mediating the cardioprotective effect. The KATP channel has been demonstrated to be an important component of acute ischaemic preconditioning, in perfused heart and cellular models (6-11, 14, 18). Under certain experimental conditions, KATP channel openers have been shown to render cells and tissues resistant to oxidative stress (1). The interaction of KATP channel and mitochondrial respiratory chain may be important in mediating the adaptive effects of KATP channel openers. Regulation of mitochondrial respiration by KATP channel openers to mediate a number of dysfunctions, which may include hypoxia, shock, ischaemia, and other organs failure (10, 22, 23).

A number of KATP channel openers have been shown to produce a beneficial effect on the myocardium in numerous models of ischaemia, and the KATP channel has been demonstrated to be a key component of the phenomenon termed ischaemic preconditioning, in which single or multiple brief periods of ischaemia have been shown to produce a marked cardioprotection against injury, produced by a subsequent prolonged ischaemic insult (22). The protective action of the KATP channel openings is not limited to the heart, but also observed in the brain (2). Therefore, modulations of the KATP channel functions have important implications in the physiology and pathophysiology of the heart, as well as other organs. Early studies suggested that the cardioprotective effect of KATP channel agonists were mediated through the sarcolemmal channel (24). However, the following findings all support a mitochondrial site of action (6-8, 14, 18, 22). Nevertheless, a role for the sarcolemmal KATP channel has not been totally dismissed.

Tkachenko et al. (26) recently showed that pharmacological treatment with KATP channel openers induced protection under stress in rat liver (26), and experimental myocardium dystrophy in guinea pig liver (17). Our studies have shown that protection induced by KATP channel openers was mediated by the oxidation of α-ketoglutarate in the rat liver under stress conditions (26). We hypothesised the relation between protection induced by KATP channel openers, and oxidation of NAD-generating substrates (α-ketoglutarate) under experimental myocardium dystrophy. Accordingly, the goal of the present study was to show that the pharmacological treatment with pinacidil, an opener of the KATP channel, induces protection under myocardium dystrophy in rats with different resistance to hypoxia.
Material and Methods

Animals. The experiments were carried out on adult male Wistar rats weighing 200–220 g. The study was conducted in conformity with the policies and procedures, detailed in the Guide for the Care and Use of Laboratory Animals. Animals were divided in two groups: rats with low resistance (LR) and with high resistance (HR) to hypoxia (3). Resistance of rats to hypoxia was evaluated as survival time (min) in the altitude chamber 11000 m (170 mm Hg) above sea level. Survival time was measured after achieving the altitude. Cessation of breathing served as the criterion for resistance to hypoxia.

Influence of K$_{ATP}$ channel opener pinacidil and blocker glibenclamide on the mitochondrial function under adrenaline myocardium dystrophy, was evaluated in 4 groups of rats with LR and HR to hypoxia: 1) a control group that was injected intraperitoneally with a isotonic solution; 2) a group that was induced adrenaline myocardium dystrophy, the results were analysed up to 24 h after the injection of adrenaline; 3) a group that was treated with K$_{ATP}$ channel opener pinacidil before adrenaline myocardium dystrophy induction; 4) a group that was induced myocardium dystrophy and injected with glibenclamide before adrenaline myocardium dystrophy induction.

Drugs. Pinacidil and glibenclamide were purchased from Sigma Chemical. Glibenclamide was dissolved in saline. Pinacidil was dissolved in 0.1 M NaOH and saline. Pinacidil and glibenclamide were administered intraperitoneally at a dose of 0.06 mg/kg, and 1 mg/kg, respectively, 30 min before myocardium dystrophy was induced.

Model of experimental myocardium dystrophy. The adrenaline myocardium dystrophy was evoked with injection of adrenaline hydrochloride solution at the dose of 1.5 mg of adrenaline/kg body wt. The dose of adrenaline was chosen based on the results of the preliminary experiments (21). It is known that the most noticeable morphological and biochemical changes are during the first 24 h, under treatment with high doses of adrenaline; therefore to reveal the maximal breach of energetic metabolism we studied the processes of ADP-stimulated respiration of rat heart mitochondria at the 24th h from the beginning of the experiment (21). Before the experiment the group of control animals was injected with 1 ml of isotonic solution.

Mitochondrial isolation and measurement of mitochondrial respiration. Mitochondria were isolated by differential centrifugation from the heart according to Kondrashova (15). Briefly, isolated rat hearts were quickly excised, chilled, weighed, and homogenised in a glass Potter-Elvehejm with a motor-driven Teflon pestle. The homogenisation medium contained 180 mM KCl, 10 mM HEPES, 10 mM EGTA, and 0.5% bovine serum albumin; pH 7.2 was adjusted with KOH. The nuclear fraction was isolated from a 10% suspension of homogenate centrifuged at 150 g for 3 min, and at 350 g for 4 min. The mitochondrial pellet was obtained by centrifugation of supernatant for 20 min at 10 000 g. Finally, mitochondria were re-suspended in the isolation buffer. Mitochondrial suspension (~2-4 mg protein/ml) was kept on ice before experiments.

Mitochondria were added to the respiration chamber containing a total volume of 1.0 ml of respiration medium. The medium contained 30 mM tris-HCl, 125 mM KCl, 10 mM NaCl, 5 mM KH$_2$PO$_4$, 1.5 mM MgCl$_2$, and 3 mM EGTA. Potassium hydroxide (1.0 N) was used to adjust the pH of the medium to 7.20 at 26°C. Succinate (0.35 mM final concentration) and α-ketoglutarate (1 mM) were used as oxidative substrates. ADP (phosphate acceptor) was administered in 0.2 mM concentration.

Oxygen consumption of mitochondrial respiration was measured in the multichannel chamber using a Clark-type electrode (5). The effects of drugs were assessed on “state 2”, “state 3” and “state 4” mitochondrial respiration, respiratory control ratio by Chance (V$_3$/V$_4$), and efficiency of phosphorylation (ADP-to-oxygen-ratio, ADP/O). Oxygen consumption was determined with succinate or α-ketoglutarate as substrates in the presence (state 3) or the absence (state 4) of phosphate acceptor, and recorded as nanogram of oxygen atoms per minute, per milligram of mitochondrial protein. The respiratory control ratio by Chance was calculated as the ratio of state 3 to state 4 respiration rate. The ADP-to-oxygen-ratio (ADP/O) was calculated as the ratio of nmoles of added ADP per nanogram of oxygen atoms utilized during state 3.

Protein concentration was determined by the Lowry assay, using bovine serum albumin as the standard (20). Lipid peroxidation processes (TBA-active products content) were determined spectrophotometrically (25).

Statistical analyses. Results were statistically presented as means ± SEM and treated with Students t-test. Statistical differences were considered significant if the P value was <0.05.

Results

Tables 1 and 2 show the respiratory parameters of mitochondria in the heart of rats with low and high resistance to hypoxia, using FAD-generating (succinic acid), and NADH-generating substrates (α-ketoglutarate).

The rates of state 3 respirations of isolated mitochondria were 59.14 ± 4.18 and 63.06 ± 5.31 ng at O/min mg protein in rats with LR and HR to hypoxia, respectively, and succinate oxidation (Table 1). The model of experimental myocardium dystrophy induced the changes in the rate of mitochondrial respiration. Therefore, the state 3 of respiration (V$_3$) was increased by 29.9% (P<0.05), and decreased by 48.7% (P<0.01) in rats with LR and HR to hypoxia, respectively, under succinate oxidation. Changes of the rate in state 3 were correlated with reduction of respiratory control ratio by 32.2% (P<0.01), and efficiency of phosphorylation (ADP/O) by 35.2% (P<0.01) in heart mitochondria of rats with HR to hypoxia. Oxidation of NAD-dependent substrate (α-ketoglutarate) in mitochondria under myocardium dystrophy was correlated with decreasing
Table 1
Mitochondrial respiration data of heart mitochondria of rats with different resistance to hypoxia under influence of pinacidil or glibenclamide and adrenaline myocardium dystrophy (substrate of oxidation – 0.35 mM succinate)

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>$V_{3}$, ng at O/min · mg mitochondrial protein</th>
<th>Respiratory control ratio, $V_{3}/V_{4}$</th>
<th>ADP/O, µM ADP/ng at O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rats with low resistance</td>
<td>59.14±4.18</td>
<td>2.25±0.18</td>
<td>1.30±0.14</td>
</tr>
<tr>
<td>rats with high resistance</td>
<td>63.06±5.31</td>
<td>2.30±0.14</td>
<td>1.96±0.17</td>
</tr>
<tr>
<td>Myocardium dystrophy:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rats with low resistance</td>
<td>76.85±6.68*</td>
<td>2.14±0.19</td>
<td>1.98±0.10*</td>
</tr>
<tr>
<td>rats with high resistance</td>
<td>32.34±4.71*</td>
<td>1.56±0.13*</td>
<td>1.27±0.18*</td>
</tr>
<tr>
<td>Pinacidil and myocardium dystrophy:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rats with low resistance</td>
<td>53.81±7.34**</td>
<td>2.30±0.09</td>
<td>1.99±0.14</td>
</tr>
<tr>
<td>rats with high resistance</td>
<td>76.96±9.16**</td>
<td>3.81±0.14**</td>
<td>1.79±0.11**</td>
</tr>
<tr>
<td>Glibenclamide and myocardium dystrophy:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rats with low resistance</td>
<td>30.11±4.74**</td>
<td>1.61±0.06**</td>
<td>1.05±0.12**</td>
</tr>
<tr>
<td>rats with high resistance</td>
<td>36.86±6.31</td>
<td>1.91±0.07**</td>
<td>1.22±0.06</td>
</tr>
</tbody>
</table>

*P<0.05 between control and myocardium dystrophy group, ** P<0.05 between myocardium dystrophy group and pinacidil and glibenclamide groups.

Table 2
Mitochondrial respiration data of heart mitochondria of rats with different resistance to hypoxia under influence of pinacidil or glibenclamide and adrenaline myocardium dystrophy (substrate of oxidation – 1 mM α-ketoglutarate)

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>$V_{3}$, ng at O/min · mg mitochondrial protein</th>
<th>Respiratory control ratio, $V_{3}/V_{4}$</th>
<th>ADP/O, µM ADP/ng at O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rats with low resistance</td>
<td>48.26±5.62</td>
<td>2.36±0.11</td>
<td>1.38±0.18</td>
</tr>
<tr>
<td>rats with high resistance</td>
<td>67.13±6.22</td>
<td>2.50±0.16</td>
<td>1.91±0.10</td>
</tr>
<tr>
<td>Myocardium dystrophy:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rats with low resistance</td>
<td>45.21±7.12</td>
<td>1.64±0.17*</td>
<td>0.69±0.04*</td>
</tr>
<tr>
<td>rats with high resistance</td>
<td>55.44±6.14</td>
<td>1.71±0.11*</td>
<td>1.73±0.11</td>
</tr>
<tr>
<td>Pinacidil and myocardium dystrophy:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rats with low resistance</td>
<td>71.42±6.73**</td>
<td>3.63±0.12**</td>
<td>2.66±0.19**</td>
</tr>
<tr>
<td>rats with high resistance</td>
<td>64.38±5.68</td>
<td>3.71±0.34**</td>
<td>2.27±0.12**</td>
</tr>
<tr>
<td>Glibenclamide and myocardium dystrophy:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rats with low resistance</td>
<td>35.40±7.11</td>
<td>1.60±0.16</td>
<td>0.96±0.05**</td>
</tr>
<tr>
<td>rats with high resistance</td>
<td>52.16±5.33</td>
<td>1.63±0.14</td>
<td>1.04±0.09**</td>
</tr>
</tbody>
</table>

For legend see Table 1.

respiratory control ratio by 30.5% (P<0.01), and 31.6% (P<0.01) in heart mitochondria of rats with LR and HR to hypoxia (Table 2).

Treatment with pinacidil under myocardium dystrophy resulted in an increase in state 3 of respiration by 138% (P<0.001), respiratory control ratio – by 144.2% (P<0.001), and efficiency of phosphorylation by 41% (P<0.01) in heart mitochondria of rats with HR to hypoxia under succinate oxidation. However, the effects of pinacidil on mitochondrial oxygen consumption data were more significant under α-ketoglutarate oxidation, especially in heart mitochondria of rats with LR to hypoxia. The increase in rate ADP-induced mitochondrial respiration of state 3 deals with increasing the efficiency of phosphorylation by 286% (P<0.001), and the respiratory control ratio by 121% (p<0.001) in mitochondria of rats with LR to hypoxia. The use of glibenclamide under myocardium dystrophy, proved to reduce the reliability of respiratory control ratio, correlating with the decrease of efficiency of phosphorylation (ADP/O).

The lipid peroxidation processes in myocardium were also determined (Fig.1). It is shown, that model of myocardium dystrophy was accompanied by a considerable increase in TBA-active products content by 94.4% (P<0.001), in the heart of rats with LR.

The same test for the group of animals with HR caused intensification of lipid peroxidation processes only by 73.6% (P<0.001). Treatment with pinacidil induced the considerable decrease in lipid peroxidation marker (TBA-active products content) in the heart by 37% (P<0.01), and by 37.3% (P<0.01) for the group of animals with HR and LR, respectively.
**Discussion**

The main goal of this investigation was to demonstrate whether pinacidil, an opener of the K\textsubscript{ATP} channel, induces cardioprotective effects under adrenaline myocardium dystrophy in the rat heart. Our results show that, pharmacological treating with pinacidil induces cardioprotective effects, as indicated by a significant increase in the efficiency of oxidative phosphorylation and decrease in TBA-active products content, compared to that in non-treated controls. Glibenclamide, the blocker of K\textsubscript{ATP} channels, when administered before the myocardium dystrophy, abolished the cardioprotection induced by pinacidil.

Our data show that the adrenaline myocardium dystrophy caused the succinate oxidation in heart mitochondria with decrease in the NAD-dependent oxidation. However, the efficiency of oxidation processes considerably depends on resistance to hypoxia. The activation of succinate-dependent way of oxidation was examined in the group of animals with LR to hypoxia. Consequently, the myocardium dystrophy in animals with an initial high physiological reactivity is related to metabolic preserving the NAD-dependent oxidation. It may preserve the mitochondrial oxygen consumption capacity.

Loukyanova (19) showed the reason of functioning breach during hypoxia. It consists not so much in the inhibition of cytochrome c oxidase activity, as in a gradual inactivation of electron transport function, which begins on NAD-dependent part of mitochondrial respiratory chain; and afterwards spreads on the region of cytochromes b-c\textsubscript{1}. The indemnification of energy support processes is related to activating the oxidizing ability of two basic metabolic ways, NADH- and FAD-dependent, in mitochondrial respiratory chain during hypoxia. The NADH-dependent way prevails in the region of 75-50% oxygen concentration; the FAD-dependent way turns out at a lower oxygen level (50-20%). NADH- and FAD-dependent ways of electron transport in mitochondrial respiratory chain, determines individual sensitiveness to hypoxia. Predominance of NAD-dependent oxidation is explored in the brain of animals with HR and more intensive succinate oxidation in animals with LR to hypoxia (19).

Animals with LR to hypoxia are characterised by tension of regulatory mechanisms, and a decrease in antioxidant system ability, which results in activating lipid peroxidation processes under stress conditions (16, 19). Therefore, the intensification of regulatory role of K\textsubscript{ATP} channels, caused effects of stress damage on mitochondrial membranes under K\textsubscript{ATP} channel opener treatment under myocardium dystrophy. The specific effect of adrenaline consists in the considerable oxygen consumption and substrates utilisation. The increasing of the macroergics level through strengthening the transport systems activity is unreal. Muscles, liver, and myocardium, are able to overstrain and function in autonomy using only its own resources. The ineffective anaerobic processes with the increasing of lactate concentration are prevailed. However, these processes are uniquely reliable under these conditions. The effective functioning of mitochondrial respiratory chain induces the decrease in lipid peroxidation processes intensity and cellular acidosis. These processes are mediated by the K\textsubscript{ATP} channels and can be a deciding factor in adaptation.

Holmuhamedov et al. (13) demonstrated that pinacidil induced mitochondrial swelling, a decrease in the mitochondrial membrane potential, and accelerated respiration in isolated cardiomyocyte mitochondria. These effects can be reversed by the potassium channel blocker glibenclamide. Recently, Hanley et al. (12) showed that pinacidil inhibited NADH oxidation...
without any effects on succinate oxidation. They showed that these effects produced by pinacidil occurred in the absence of any changes in mitochondrial membrane potential.

Our results demonstrate that pinacidil caused an increase in the efficiency of oxygen consumption for macroergic synthesis (FAD-dependent substrate oxidation); in mitochondria of rats with HR to hypoxia and economical utilization under succinate oxidation, and increase in oxygen consumption data under α-ketoglutarate oxidation for rats with LR to hypoxia. Glibenclamide, when administered before the myocardium dystrophy protocol, abolished the protection induced by pinacidil, and induced the decrease in the rate of mitochondrial respiration with the lowest value of efficiency of phosphorylation in heart mitochondria. Consequently, the economisation of oxygen consumption processes under succinate oxidation with the reduction of lipid peroxidation processes; is one of the indications of forming the oxidation with the reduction of lipid peroxidation oxygen consumption processes under succinate mitochondria. Consequently, the economisation of energy support processes under this condition. Pinacidil has a protective effect on heart mitochondria energy support under this condition.

In conclusion, our studies established that pharmacological treatment with K<sub>ATP</sub> channel opener pinacidil; induces the elevation of efficiency of NAD-dependent substrate oxidation, in particular α-ketoglutarate of rats with LR, and economical utilization under succinate oxidation in rats with HR and succinate oxidation with temporary decrease in lipid peroxidation processes, under myocardium dystrophy. Pinacidil has a protective effect on heart mitochondria energy support under this condition.

References


