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K-ATP channel independent effects of pinacidil on ATP production in isolated cardiomyocyte or pancreatic β-cell mitochondria

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Abstract

Evidence has been presented that mitochondria contain ATP sensitive potassium channels (mK-ATP channels), which may confer tissue protection upon activation. It is, however, not known whether activation of mK-ATP channels has a direct effect on mitochondrial ATP production. This study was performed to define the effect of pinacidil (PIN) on ATP production by oxidative phosphorylation in isolated cardiomyocyte or pancreatic β-cell mitochondria. Cardiomyocyte mitochondria produced seven times more ATP than β-cell mitochondria in the presence of pyruvate/malate. PIN inhibited pyruvate/malate-induced mitochondrial ATP production with half maximal effect at 360 μM in both cell types. The inclusion of 5-hydroxydecanoate (5-HD) did not prevent this inhibition. Succinate induced a similar ATP production in cardiomyocyte or β-cell mitochondria. In β-cell mitochondria succinate-induced ATP production was inhibited by PIN with half maximal effects at 500 μM PIN. However, in cardiomyocyte mitochondria PIN stimulated succinate-induced ATP production 3-fold with half maximal effect at 100 μM and maximal effect at 200 μM. This PIN-dependent stimulation was mimicked by rotenone. The inclusion of 5-HD could not prevent these PIN effects. In conclusion, PIN may inhibit complex 1 of the respiratory chain without indications of opening mK-ATP channels. In cardiomyocytes with metabolically inhibited succinate dehydrogenase this results in a stimulation of ATP production conferring tissue protection. In β-cells without a metabolically inhibited succinate dehydrogenase, there is no stimulation by PIN and tissue protection by PIN is not to be expected.

Keywords: Pinacidil; 5-Hydroxydecanoate; β-Cells; Cardiomyocytes; Mitochondrial ATP production; Mitochondrial K-ATP channels

1. Introduction

Brief ischemic periods prior to prolonged ischemia may protect hearts from reperfusion injury [1]. During IPC the matrix volume increases and the respiratory chain is activated providing more ATP to the recovering heart [2,3]. Electrophysiological and pharmacological evidence has led to the proposal that opening of mK-ATP channels is implicated in this process [4–6]. The potassium channel opener diazoxide is usually used to mimic preconditioning allegedly by opening mK-ATP channels. However, the precise mode of action of diazoxide is in doubt since diazoxide has additional mitochondrial effects such as inhibition of the respiratory chain [7–9], which leads to a reduction, rather than an activation of respiratory activity [2].

Pinacidil, which is chemically unrelated to diazoxide, is another K-ATP channel opener providing cardioprotection [10]. In isolated cardiomyocyte mitochondria PIN induces mitochondrial swelling, a decrease of the mitochondrial membrane potential, and increased respiration [11]. These effects can be reversed by the potassium channel blocker glyburide, which indicates that mK-ATP channels are involved [11].

However, in the case of PIN there may be a mK-ATP channel independent target in mitochondria. Thus, matrix swelling, a decrease of the mitochondrial membrane potential and an increase of mitochondrial respiration can be observed even when extramitochondrial potassium is replaced by lithium although this ion is not transported through mK-ATP channels [12]. One site of action may be the adenine nucleotide translocase since increased respiration is reversed in the presence of carboxyatractyloside

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Abbreviations: mK-ATP channel, mitochondrial K-ATP channel; 5-HD, 5-hydroxydecanoate; PIN, pinacidil; DAPP, diadenosine pentaphosphate; IPC, ischemic preconditioning.

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[13]. Another site may be the respiratory chain since PIN caused an inhibition of NADH oxidation in disrupted mitoplasts [7].

There is at present no information about the effect of PIN on ATP production in intact mitochondria. We addressed this issue by measuring the effect of PIN on mitochondrial ATP production in two different tissues. Heart mitochondria were chosen since most of the previous studies have been performed in that tissue. β-Cell mitochondria were selected since the potassium channel blockers and activators, which were routinely used in β-cell studies, are regarded as plasma membrane selective but may have intracellular side effects [8], which might be exploited for tissue protection. For ATP production the bioluminometric technique specifically developed for monitoring ATP production from pancreatic β-cell mitochondria during incubation at 37° was applied [14–16].

2. Materials and methods

2.1. Chemicals

Microbial collagenase P (EC 3.4.24.3), ADP (potassium salt), ATP (potassium salt), DAPP, (trilithium salt), and electrophoretically homogenous, lyophilized bovine serum albumin were purchased from La Roche Biochemicals whereas bovine serum albumin (fraction V) was from Miles Laboratories. Firefly luciferase (EC 1.13.12.7) and D-luciferin were purchased from Biothema AB. Succinate, malate, PIN, and antimycin A were from Sigma. Pyruvate was from Aldrich. Hepes was obtained from Calbiochem. NaCl, KCl, KH_2PO_4, $MgSO_4$ and KOH (Suprapur) were from Merck. ATP was from Roche and $MgCl_2$ was from BDH Chemicals. Quartz bidistilled water was used throughout.

2.2. Methods

2.2.1. Assay of mitochondrial ATP production

For the isolation of heart mitochondria adult lean female mice, starved overnight, were used. After decapitation part of the cardiac ventricular tissue was immediately excised. For the isolation of β-cell mitochondria adult female ob/ob mice, starved overnight, were used. After decapitation, large islets with a high content of β-cells (>90% [17]) were isolated and the mitochondria obtained may be regarded to represent pure β-cells. Previous studies from this group demonstrated that there is no difference in ATP production between mitochondria isolated from ob/ob mice or their lean litter mates thus ob/ob mice mitochondria can be regarded as normal β-cell mitochondria [14–16].

Mitochondria were prepared and incubated as described [14–16]. In brief, 5 μL cold mitochondrial suspension was added to 995 μL incubation medium prewarmed to 37°. The incubation medium consisted of (mM) 20 Hepes, 3 KH_2PO_4, 1 EGTA, 20 NaCl, 80 KCl, 0.3 $MgCl_2$ and electrophoretically homogeneous albumin (0.5 mg/mL). The pH was adjusted with KOH to 7.10 at 37°. The incubation medium for determination of mitochondrial ATP production by adenylate kinase contained ADP (50 μM) as sole substrate.

The incubation medium for determination of mitochondrial ATP production by oxidative phosphorylation contained the appropriate substrates, ADP and DAPP (1 μM). DAPP is a specific inhibitor of adenylate kinase activity and is routinely used in assays of mitochondrial ATP production in order to prevent extramitochondrial ATP production from ADP. The incubation was stopped after 10 min by the addition of a mixture of antimycin A (0.5 μM), D-luciferin (0.4 μM) and luciferase (0.5 nM). The samples were then rapidly cooled to room temperature and the ATP produced was determined by integrating the light emission over a period of 6 s in a Packard Tricarb scintillation spectrometer, operated out of coincidence.

2.2.2. Normalization of mitochondrial ATP production in isolated mitochondria from cardiomyocytes and β-cells

Although mitochondria from the heart can be isolated in large amounts and ATP production may be calibrated against protein content, the amount of β-cell mitochondria is far less and therefore an appropriate normalization procedure was described recently [16]. It is based on the ATP production by mitochondrial adenylate kinase as a measure of intact mitochondria after purification. A measure of the oxidative phosphorylation activity is obtained as the ratio of ATP production (nmol ATP) by oxidative phosphorylation and ATP production (nmol ATP) from adenylate kinase determined in parallel incubations [16]. This quotient is denoted as normalized ATP production and it is dimensionless.

2.2.3. Data processing and statistics

Duplicate samples were carried through the entire assay procedure. From the difference between duplicate samples the random error (SD) of a single determination was calculated to be 3% of the duplicate mean throughout the range of ATP concentrations measured in the present study. The mean value of duplicates was entered as one observation when calculating the means ± SEM of separate experiments.

3. Results

3.1. Effect of 5-HD or PIN on ATP production in isolated mitochondria from cardiomyocytes or pancreatic β-cells

There is a large difference in the availability of tissue between cardiomyocytes and pancreatic β-cells. In order to compare mitochondrial ATP production with the same technique a similar amount of mitochondria has to be

incubated. The amount of mitochondria was judged from ATP production induced by adenylate kinase, which is an intermembrane enzyme and as such was used as a marker for the amount of intact mitochondria.

It is essential for the experiments to exclude that the two chemicals for mK-ATP channel manipulation (PIN, 5-HD) may affect the adenylate kinase activity, which is used as a reference, or serve *per se* as substrates for mitochondrial ATP production. This is verified in Fig. 1. Since it is not possible to dilute mitochondria from different preparations to exactly the same adenylate kinase activity, the activity for several independent preparations is given and the effects of the test substances are calculated by linear regression.

The activity of adenylate kinase in mitochondria from cardiomycytes in the sole presence of ADP (50 μM) ranged from 0.075 to 0.23 nmol ATP during 10 min of incubation. There was no difference in adenylate kinase activity whether PIN (100 μM) or 5-HD (100 μM) were included in the assay (Fig. 1a). The slope of the regression line was 1.05 ± 0.14 in the presence of PIN and 0.98 ± 16 in the presence of 5-HD. The regression coefficient (r) was 0.93 with PIN and 0.88 with 5-HD.

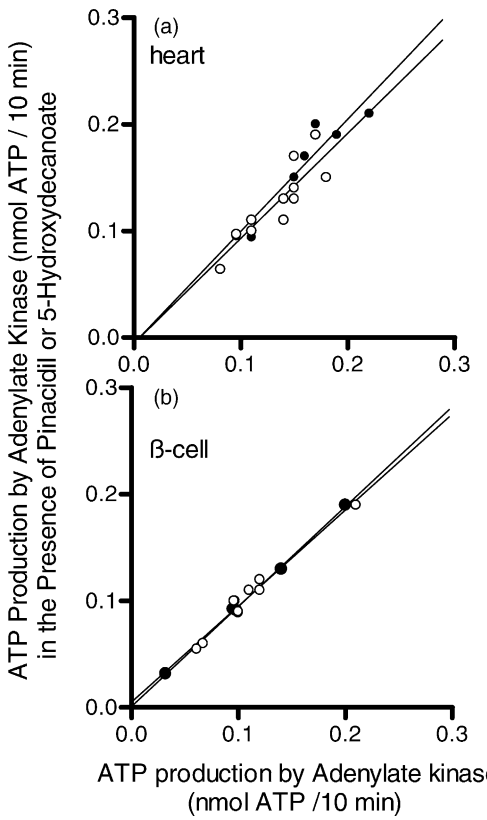

Fig. 1. Effect of PIN or 5-HD on ATP production by adenylate kinase activity in isolated mitochondria from cardiomyocytes or pancreatic β-cells. Isolated mitochondria from mouse cardiomyocytes or pancreatic β-cells are incubated for 10 min in the presence of ADP (50 μM) as the sole substrate. The effect of PIN (100 μM, open circles) or 5-HD (100 μM, closed circles) on ATP production by adenylate kinase activity was detected in parallel incubations. Lines are calculated by linear regression.

The activity of adenylate kinase in mitochondria isolated from pancreatic β-cells in the sole presence of ADP (50 μM) ranged from 0.03 to 0.19 nmol ATP during 10 min of incubation. There was no difference in adenylate kinase activity whether PIN or 5-HD were included in the assay (Fig. 1b). The slope of the regression line ranged from 0.93 ± 0.02 in the presence of PIN to 0.90 ± 0.05 in the presence of 5-HD. The regression coefficient (r) in the presence of PIN or 5-HD was 0.99.

3.2. Correlation between oxidative phosphorylation and adenylate kinase activity in isolated mitochondria from cardiomyocytes or pancreatic β-cells

In order to express ATP production in normalized dimensionless units the ratio of ATP production by oxidative phosphorylation or by adenylate kinase must be constant for each substrate and for both mitochondrial types. This is shown in Fig. 2. There was a linear relation between the ATP production by adenylate kinase activity and the

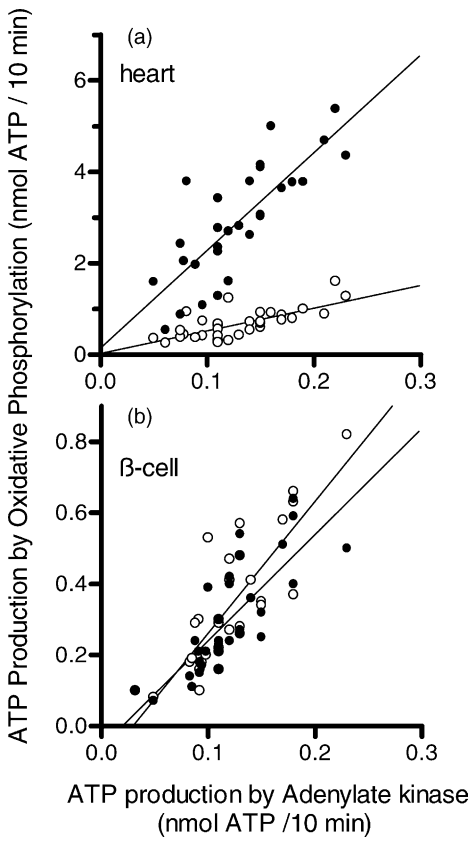

Fig. 2. Effect of pyruvate/malate or succinate on ATP production by oxidative phosphorylation in isolated mitochondria from cardiomyocytes or pancreatic β-cells. ATP production by adenylate kinase was determined by incubation of isolated mitochondria from cardiomyocytes or pancreatic β-cells in the presence of ADP (50 μM) as the sole substrate. The effect of succinate (5 mM, open circles) or pyruvate/malate (1 mM/1 mM, closed circles) on ATP production by oxidative phosphorylation is determined in parallel incubations in the presence of ADP (50 μM) and DAPP (1 μM) to inhibit adenylate kinase activity. Lines are calculated by linear regression.

ATP production by oxidative phosphorylation in the presence of pyruvate/malate or succinate in cardiomyocyte mitochondria (Fig. 2a). In 30 mitochondrial preparations the slope of the regression line for pyruvate/malate was 21.4 ± 3.1 and in 35 mitochondrial preparations the slope of the regression line for succinate was 4.9 ± 0.9. The correlation coefficient (r) was 0.80 for pyruvate/malate and 0.72 for succinate. Addition of antimycin A (0.5 μM) in the presence of substrates, DAPP and ADP completely prevented mitochondrial ATP production by oxidative phosphorylation (data not shown).

Also in isolated mitochondria from pancreatic β-cells there was a linear relation between the ATP production by adenylate kinase activity and ATP production by oxidative phosphorylation in the presence of pyruvate/malate or succinate (Fig. 2b). In 31 mitochondrial preparations the slope of the regression line with pyruvate/malate was 3.0 ± 0.4 and with succinate the slope was 3.7 ± 0.5. The correlation coefficient (r) was 0.79 for pyruvate/malate and 0.81 for succinate. Addition of antimycin A (0.5 μM) in the presence of substrates, DAPP and ADP completely prevented mitochondrial ATP production by oxidative phosphorylation (data not shown).

3.3. Effect of PIN on mitochondrial ATP production

3.3.1. Cardiomyocyte mitochondria

In five preparations of mitochondria from cardiomyocytes the normalized mitochondrial ATP production in the presence of pyruvate/malate amounted to 23.8 ± 2.2. Addition of PIN inhibited ATP production in a dose-dependent manner with half maximal effects at 365 μM (Fig. 3a). In 11 preparations of mitochondria from cardiomyocytes the normalized mitochondrial ATP production in the presence of succinate amounted to 4.6 ± 0.3. Addition of PIN stimulated ATP production in a dose-dependent manner with half maximal effects at 100 μM and maximal effects at 200 μM where the maximal ATP production reached 11.9 ± 1.3 (Fig. 3b). In the presence of 1000 μM PIN the ATP production was reduced again to the activity observed in the absence of PIN.

3.3.2. β-Cell mitochondria

In three preparations of mitochondria from pancreatic β-cells the normalized mitochondrial ATP production in the presence of pyruvate/malate reached 3.9 ± 0.4. Addition of PIN inhibited the mitochondrial ATP production in a

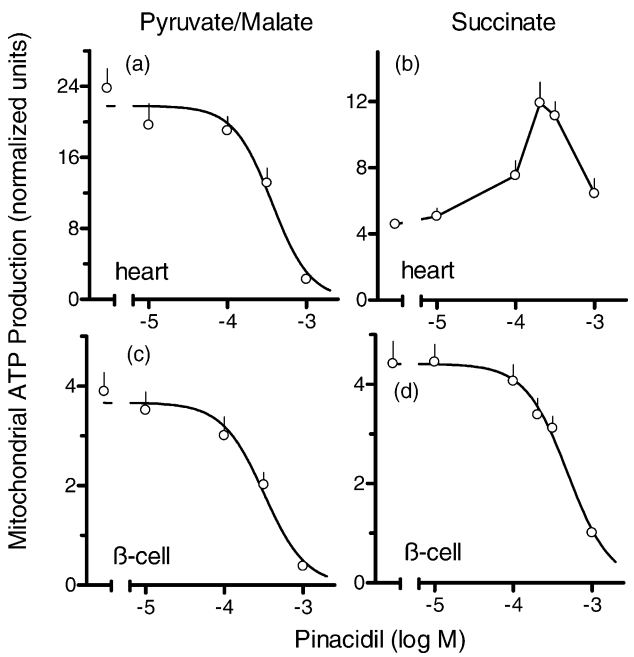

Fig. 3. Effect of PIN on normalized ATP production in cardiomyocytes or pancreatic β-cells. Concentrations of substrates (mM) were: pyruvate 1, malate 1, and succinate 5. Values are means ± SEM of 5 experiments for pyruvate/malate and 11 experiments for succinate in cardiomyocyte and of 3 experiments in β-cell mitochondria for each substrate. Curves are calculated by nonlinear regression.

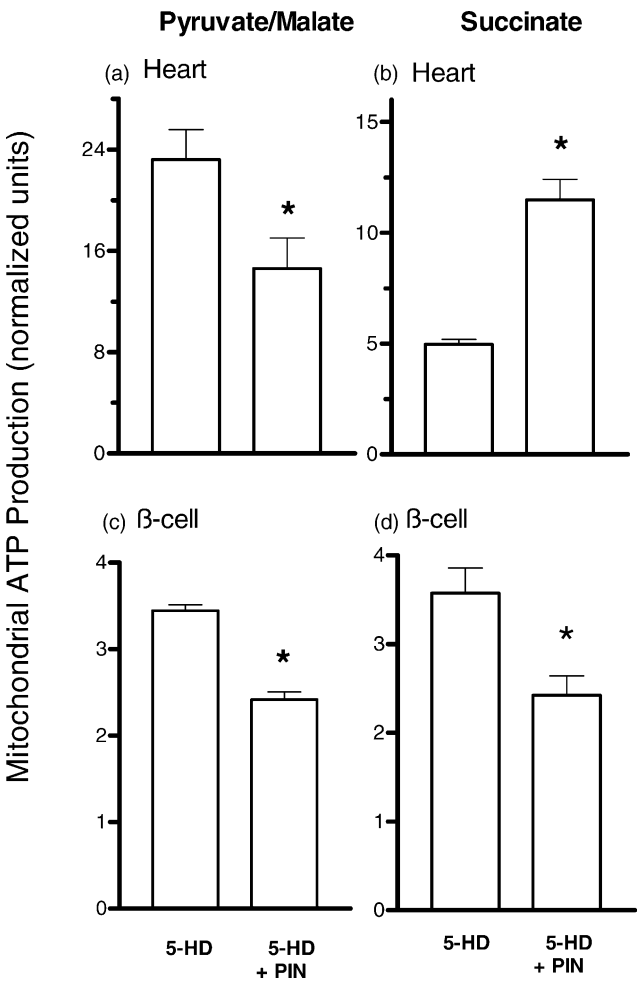

Fig. 4. Effect of 5-HD or PIN on normalized ATP production in cardiomyocytes or pancreatic β-cells. Concentrations of substrates (mM) were: pyruvate 1, malate 1, and succinate 5. Values are means ± SEM of three experiments. The concentration of 5-HD was 100 μM in experiments with pyruvate/malate and 300 μM in experiments in the presence of succinate. $^{*}P < 0.05$ compared with values observed in the absence of PIN.

concentration-dependent manner with half maximal effect at 320 μM (Fig. 3c). Succinate-induced ATP production was 4.4 ± 0.4 and was inhibited by PIN in a concentration-dependent manner and half maximal inhibition was observed at 510 μM PIN (Fig. 3d).

3.4. Effect of 5-HD on ATP production in the presence of PIN

3.4.1. Cardiomyocyte mitochondria

5-HD (300 μM) is not a substrate for mitochondrial ATP production in cardiomyocytes. In three incubations the normalized mitochondrial ATP production was only 0.03 ± 0.01 (data not shown). In the presence of 5-HD the normalized ATP production reached 23.3 ± 2.4 with pyruvate and malate as substrates. Addition of PIN in the presence of 5-HD still inhibited the mitochondrial ATP production to 14.6 ± 2.4 (Fig. 4a). The mitochondrial ATP production in the combined presence of 5-HD and succinate reached 5.0 ± 0.2 and addition of PIN in the presence of 5-HD still stimulated ATP production to 11.5 ± 0.9 (Fig. 4b).

3.4.2. β-Cell mitochondria

With pyruvate and malate as substrates and in the presence of 5-HD the normalized ATP production from pancreatic β-cell mitochondria reached 3.5 ± 0.1. PIN still inhibited ATP production in the presence of 5-HD to 2.4 ± 0.1. With succinate and in presence of 5-HD the mitochondrial ATP production reached 3.6 ± 0.3. PIN still inhibited the mitochondrial ATP production in the presence of 5-HD to 2.4 ± 0.2.

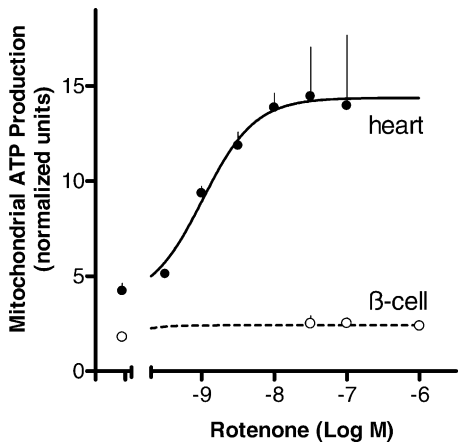

Fig. 5. Effect of rotenone on succinate-induced ATP production in isolated mitochondria from cardiomyocytes or pancreatic β-cells. Values are means ± SEM of three to five experiments. Succinate concentration was 5 mM. The solid curve is the calculated best fit for rotenone stimulated ATP production in cardiomyocyte mitochondria. The dotted line is the calculated best fit for ATP production in β-cell mitochondria. Calculation is based on nonlinear regression fitting a hyperbolic equation with a preset of basal activity in the presence of succinate and in the absence of rotenone.

3.5. Effect of rotenone on succinate-induced ATP production

Rotenone stimulated succinate-induced ATP production in cardiomyocyte mitochondria but not in β-cell mitochondria (Fig. 5). In cardiomyocyte mitochondria half maximal stimulation was observed in the presence of 1 nM rotenone and maximal stimulation (13.9 ± 0.8) was reached with 0.1 μM rotenone. Rotenone (0.1 μM) completely inhibited pyruvate/malate-induced ATP production in cardiomyocyte or β-cell mitochondria (data not shown).

4. Discussion

IPC or transient incubation of the heart with potassium channel openers protects the tissue from reperfusion damage [1,2]. During IPC the mitochondrial matrix swells and respiration increases which may provide more ATP to the recovering heart [2]. Incubation with diazoxide also induces matrix swelling, but in contrast to IPC, respiration is inhibited, thus other, yet unknown, mechanisms may be activated [2]. The use of PIN with mitochondria obtained from cardiomyocytes or pancreatic β-cells allowed us to identify another mechanism which may be cardioprotective in the heart, but which may be ineffective in β-cells.

In order to determine whether inhibition of pyruvate/malate-induced ATP production by PIN is mediated by a mK-ATP channel-dependent mechanism 5-HD was used as a specific mK-ATP channel blocker [18]. Since 5-HD is a fatty acid derivative it was essential for the analysis to establish that 5-HD is not used for ATP production. Enzymatic measurements demonstrated that 5-HD is activated by acyl-CoA synthase to 5-HD-CoA [2,7] and metabolized by the β-oxidation pathway [19]. Isolated cardiomyocyte mitochondria convert 5-HD to 5-HD-CoA in the presence of acyl-CoA synthase but formation of 5-HD-CoA does not increase oxygen consumption indicating that it is not metabolized [2]. In agreement with these observations direct addition of 5-HD to isolated mitochondria did not induce ATP production. Furthermore, the addition of 5-HD in the presence of additional substrates did not alter mitochondrial ATP production.

PIN inhibited pyruvate/malate-induced ATP production in cardiomyocytes or β-cells with half maximal effects at 300 μM very similar to the concentration dependency observed for the increase of respiration [10]. This PIN effect was blocked by glyburide and was explained as a specific mK-ATP channel activation [11], or as an uncoupling due to diffusion of the lipophilic PIN molecule at toxic concentrations [12]. In the present study, the inhibition of mitochondrial ATP production by PIN could not be antagonized by 5-HD even at lower PIN concentrations (100 μM), which indicates that mitochondrial ATP production may not depend on mK-ATP channel activity. PIN

may thus inhibit mitochondrial ATP production either by uncoupling or by direct inhibition of the respiratory chain as described before [7,12].

PIN induced opposite effects on succinate-induced ATP production in cardiomyocytes or β-cell mitochondria. Neither stimulation in cardiomyocytes nor inhibition in β-cells could be prevented by the inclusion of 5-HD. Substrate specific effects which are insensitive to mK-ATP channel blocking indicate that the action of PIN is not mediated by mK-ATP channel activation. Uncoupling by PIN in a concentration range between 100 and 300 μM may be excluded as well since succinate-induced ATP production is stimulated in cardiomyocytes in that concentration range. Thus, PIN may interact with an intramitochondrial site involved in substrate metabolism, which may be the respiratory chain.

Since PIN may not mediate its effects by activation of mK-ATP channels, rotenone was applied to further characterize the PIN effects on mitochondrial ATP production. The complex 1 inhibitor rotenone has two known effects on mitochondrial metabolism. Rotenone inhibits NADH oxidation and blocks pyruvate metabolism in all mitochondria. Complex 1 inhibition by PIN in disrupted cardiomyocyte mitochondria has been proposed recently, although the direct effect on mitochondrial ATP production remained undetermined [7]. In the present study, complex 1 inhibition could explain the observed PIN-dependent inhibition of ATP production in the presence of pyruvate/malate in cardiomyocyte or β-cell mitochondria.

Rotenone stimulates succinate-induced ATP production in skeletal muscle mitochondria due to relief of the metabolic inhibition of succinate dehydrogenase by oxaloacetate [20]. Rotenone also stimulated ATP production by 300% in cardiomyocyte mitochondria indicating the presence of a metabolic inhibition by oxaloacetate also in this tissue. β-Cell mitochondria producing ATP in the presence of succinate are not stimulated by PIN. This may be due to the fact that β-cell mitochondria export much more malate than heart mitochondria thereby reducing the concentration of oxaloacetate in the matrix [21].

In conclusion, PIN provokes opposite effects in mitochondria from cardiomyocytes or β-cells indicating a tissue specialization of mitochondria. The use of potassium channel openers or blockers could not identify an effect mediated by mK-ATP channel activation or inhibition on mitochondrial ATP production. PIN may act as a complex 1 inhibitor thus stimulating ATP production in tissues whose mitochondria have a metabolically inhibited succinate dehydrogenase. This indicates that the metabolic specialization of mitochondria is decisive for the successful pretreatment of tissues with PIN to promote survival after ischemia. The heart may fulfil that prerequisite whereas the β-cell, which may also be exposed to longer ischemic periods prior to transplantation, may not be protected by treatment with PIN.

Acknowledgments

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References

[1] Schwarz ER, Whyte WS, Kloner RA. Ischemic preconditioning. Curr Opin Cardiol 1997;15:475–81.
[2] Lim KH, Javadov SA, Das M, Clarke SJ, Suleiman MS, Halestrap AP. The effects of ischaemic preconditioning, diazoxide and 5-hydroxydecanoate on rat heart mitochondrial volume and respiration. J Physiol 2002;545:961–74.
[3] Halestrap AP. The regulation of the matrix volume of mammalian mitochondria *in vivo* and *in vitro* and its role in the control of mitochondrial metabolism. Biochim Biophys Acta 1989;973:355–82.
[4] Inoue I, Nagase H, Kishi K, Higuti T. ATP-sensitive K^+ channel in the mitochondrial inner membrane. Nature 1991;352:244–7.
[5] Grover GJ, Garlid KD. ATP-sensitive potassium channels: a review of their cardioprotective pharmacology. J Mol Cell Cardiol 2000;32:677–95.
[6] Sato T, Marban E. The role of mitochondrial K(ATP) channels in cardioprotection. Basic Res Cardiol 2000;95:285–9.
[7] Hanley PJ, Mickel M, Löffler M, Brandt U, Daut J. KATP channel-independent targets of diazoxide and pinacidil in the heart. J Physiol 2002;542:735–41.
[8] Grimmsmann T, Rustenbeck I. Direct effects of diazoxide on mitochondria in pancreatic B-cells and on isolated liver mitochondria. Br J Pharmacol 1998;123:781–8.
[9] Das M, Parker JE, Halestrap AP. Matrix volume measurements challenge the existence of diazoxide/glibencamide-sensitive KATP channels in rat mitochondria. J Physiol 2003;547:893–902.
[10] Armstrong SC, Liu GS, Downey JM, Ganote CE. Potassium channels and preconditioning of isolated rabbit cardiomyocytes: effects of glyburide and pinacidil. J Mol Cell Cardiol 1995;27:1765–74.
[11] Holmuhamedov EL, Jovanovic S, Dzeja PP, Jovanvic A, Terzic A. Mitochondrial ATP-sensitive K^+ channels modulate cardiac mitochondrial function. Am J Physiol Heart Circ Physiol 1998;44:H1567–76.
[12] Kowaltowski AJ, Seetharaman S, Paucek P, Garlid KD. Bioenergetic consequences of opening the ATP sensitive K^+ channel in heart mitochondria. Am J Physiol Heart Circ Physiol 2001;280:H649–57.
[13] Kopustinsiene DM, Jovaiense J, Liobikas J, Toleikis A. Diazoxide and pinacidil uncouple pyruvate/malate-induced mitochondrial respiration. J Bioenerg Biomembr 2002;34:49–53.
[14] Idahl LA, Lembert N. Glycerol 3-phosphate-induced ATP production in intact mitochondria from pancreatic B-cells. Biochem J 1995;312:287–92.
[15] Lembert N, Idahl LA. α-Ketoisocaproate is not a true substrate for ATP production by pancreatic β-cell mitochondria. Diabetes 1998;47:339–44.
[16] Lembert N, Joos HC, Idahl LA, Ammon HPT, Wahl MA. Methyl pyruvate initiates membrane depolarization and insulin release by metabolic factors other than ATP. Biochem J 2001;354:350–4.
[17] Hellman B. Studies in obese-hyperglycemic mice. Ann NY Acad Sci 1965;131:541–58.
[18] Hu H, Sato T, Seharaseyon J, Liu Y, Johns DC, O'Rourke B, Marban E. Pharmacological and histochemical distinctions between molecularly defined sarcolemmal KATP channels and native cardiac mitochondrial KATP channels. Mol Pharamcol 1999;55:1000–5.

[19] Hanley PJ, Gopalan KV, Lareau RA, Srivastava DK, Meltzer Mv M, Daut J. {Beta}-Oxidation of 5-hydroxydecanoate, a putative blocker of mitochondrial ATP-sensitive potassium channels. J Physiol 2003;547:387–93.

[20] Wibom R, Hultman E. ATP production in mitochondria isolated from microsamples of human muscle. Am J Physiol 1990;259:E204–9.

[21] MacDonald MJ. Feasibility of a mitochondrial pyruvate malate shuttle in pancreatic islets. J Biol Chem 1985;270:20051–8.