Mitochondrial ATP-sensitive K^+ channel opening decreases reactive oxygen species generation

Renato Ferranti, Mirian M. da Silva, Alicia J. Kowaltowski*

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, SP, 05508-900, Brazil

Received 20 November 2002; revised 26 December 2002; accepted 30 December 2002

First published online 14 January 2003

Edited by Vladimir Skulachev

Abstract Mitochondrial ATP-sensitive K^+ channel (mitoK_{ATP}) opening was shown previously to slightly increase respiration and decrease the membrane potential by stimulating K^+ cycling across the inner membrane. Here we show that mitoK_{ATP} opening reduces reactive oxygen species generation in heart, liver and brain mitochondria. Decreased H_2O_2 release is observed when mitoK_{ATP} is active both with respiration stimulated by oxidative phosphorylation and when ATP synthesis is inhibited. In addition, decreased H_2O_2 release is observed when mitochondrial ΔpH is enhanced, an effect expected to occur when mitoK_{ATP} is open. We conclude that mitoK_{ATP} is an effective pathway to trigger mild uncoupling, preventing reactive oxygen species release.

Key words: Free radicals; Ischemia; Preconditioning; Oxidative stress; Diazoxide

1. Introduction

Mitochondria continuously generate small amounts of superoxide radical anions through monoelectronic reduction of oxygen at intermediate steps of the electron transport chain. These superoxide radicals then produce other reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), through dismutation catalyzed by superoxide dismutases; hydroxyl radicals, from the reaction of H_2O_2 with Fe^{2+}; or peroxynitrite, through combination of superoxide radicals with nitric oxide, generated by the mitochondrial nitric oxide synthase [1]. Mitochondrially generated ROS have been implicated in cellular damage occurring in a variety of pathologies including ischemia/reperfusion, Parkinson's disease, Huntington's disease and aging [1,2]. Thus, in order to establish better therapeutic approaches for these conditions, it is important to understand how mitochondrial ROS release is regulated.

› Respiratory rates and the inner membrane potential (ΔΨ) are well-established regulators of ROS release. Low ΔΨ, accompanied by enhanced respiration, prevents mitochondrial ROS release, while low respiratory rates strongly increase ROS generation [3,4]. This seemingly contradictory finding, in which ROS release is lowest when oxygen consumption is highest, is justified by the fact that low respiratory rates result in longer life times for electron transport chain intermediates capable of monoelectronically reducing oxygen, such as semiquinone radicals in the ubiquinone pool [3,4]. Indeed, the activation of pathways which decrease ΔΨ and increase respiration, such as uncoupling proteins and plant alternative oxidases, strongly prevent mitochondrial ROS release [5,6].

A recently described inner mitochondrial membrane channel, the ATP-sensitive K^+ channel (mitoK_{ATP}), is another mechanism through which respiration and ΔΨ are regulated [7]. This channel promotes K^+ entrance into the mitochondrial matrix in a manner stimulated by ΔΨ. Enhanced K^+ uptake through mitoK_{ATP} leads to increased mitochondrial volume, faster respiration, lower ΔΨ and, in the absence of adequate levels of phosphate, increased ΔpH, with matrix alkalinization (for a review, see [8]). These mitoK_{ATP} effects could promote a decline in mitochondrial ROS production. However, because K^+ transport rates through mammalian mitoK_{ATP} are low, the respiratory and ΔΨ effects of this channel are limited. In fact, the ΔΨ effect of mitoK_{ATP} in heart is so small it cannot be detected using conventional measurements [7]. The result of such a small decrease in ΔΨ and respiratory increment on mitochondrial ROS release had not been determined before this study.

Although mitoK_{ATP} activity could decrease mitochondrial ROS release due to its respiratory effect, studies using intact cells have suggested the opposite: that mitoK_{ATP} opening leads to increased ROS release [9–11]. This finding is compatible with the hypothesis that ROS participate in ischemic preconditioning (the protective effect of short ischemic periods on a subsequent longer ischemic insult), in which mitoK_{ATP} activation is an essential step (for a review, see [12]). The mechanism through which ROS may increase with mitoK_{ATP} opening has not been determined, but may be related to matrix alkalinization [8].

In order to uncover the direct effect of mitoK_{ATP} on mitochondrial ROS release and understand better the functional role of mitoK_{ATP}, we measured H_2O_2 and total ROS generation in isolated mitochondria incubated under conditions in which mitoK_{ATP} activity, respiration, ΔΨ and ΔpH were manipulated.

*Corresponding author. Av. Lineu Prestes, 748, Cidade Universitária, São Paulo, SP, 05508-900, Brazil. Fax: (55)-11-3815 5579.
E-mail address: alicia@iq.usp.br (A.J. Kowaltowski).

Abbreviations: BSA, bovine serum albumin; DCFDA, dichlorofluorescein diacetate; DZX, diazoxide; FCCP, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone; H_2DCFDA, dichlorodihydrofluorescein diacetate; HRP, horseradish peroxidase; mitoK_{ATP}, mitochondrial ATP-sensitive K^+ channel; P_i, inorganic phosphate; ROS, reactive oxygen species; S.E.M., standard error; ΔΨ, mitochondrial membrane potential

0014-5793/03/$22.00 © 2003 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00007-3

2. Materials and methods

2.1. Mitochondrial isolation

Brain mitochondria were isolated as described by Andreyev and Fiskum [13], except protease was omitted. Heart and liver mitochondria were isolated as described previously [7,14]. All experiments were conducted within 3 h of mitochondrial isolation. Protein concentrations were determined using the Biuret method.

2.2. H_2O_2 release measurements

Amplex Red (Molecular Probes) oxidation in the presence of extramitochondrial horseradish peroxidase (HRP) bound to H_2O_2 generates resorufin, a highly fluorescent compound, with a 1:1 stoichiometry [15]. Resorufin fluorescence was monitored using a temperature-controlled Hitachi 4010 fluorescence spectrophotometer operating at excitation and emission wavelengths of 563 nm and 587 nm, respectively, with continuous stirring. Control experiments conducted in the absence of Amplex Red or HRP indicated that changes in mitochondrial morphology or direct interactions between mitochondrial components and Amplex Red do not alter resorufin fluorescence measurements. In the absence of mitochondria, fluorescence increment rates were negligible (not shown). In order to calibrate the results, a plot relating fluorescence to the concentration of commercial resorufin was constructed. Resorufin formation was then quantified in the experimental traces, and assumed to be equal to released H_2O_2.

2.3. ROS measurements

Dichlorodihydrofluorescein diacetate (H_2DCFDA) is a membrane-permeable reagent oxidized by ROS such as H_2O_2 and nitric oxide, generating fluorescent dichlorofluorescein diacetate (DCFDA) [16]. DCFDA fluorescence was monitored using a temperature-controlled Hitachi 4010 fluorescence spectrophotometer operating at excitation and emission wavelengths of 488 nm and 525 nm, respectively, with continuous stirring.

2.4. Data analysis

Figs. 1A,C, 2 and 3 are representative data of at least three similar repetitions using different mitochondrial preparations. Fig. 1B represents the average ± S.E.M. (standard error) of three different determinations. Multiple pairwise Tukey tests performed by SigmaStat were used to determine statistical significance.

3. Results

Brain mitochondria exhibit the highest levels of mitoK_{ATP} protein and K^+ transport activity of any mammalian tissue studied to date [17]. Predicting that any possible effect of mitoK_{ATP} on ROS release would be intensified in brain, we initially used mitochondria isolated from this tissue to study the effects of mitoK_{ATP} on ROS release. In Fig. 1A, brain mitochondrial H_2O_2 release was measured by following the formation of the highly fluorescent compound resorufin due to the oxidation of Amplex Red in the presence of HRP (see Section 2 and [15]). Oligomycin was present in all traces to prevent ATP synthesis or degradation through the ATP synthase, and bovine serum albumin (BSA) was added to avoid mitochondrial uncoupling due to contaminating fatty acids. We found that under control conditions (line a), H_2O_2 release rates were lower than those observed in the presence of ATP (line b), which inhibits K^+ transport though mitoK_{ATP} [1,8,17,18]. When the inhibitory effect of ATP was overcome by the concomitant presence of diazoxide (DZX), a mitoK_{ATP} agonist [7,17], H_2O_2 release was lowered (line c) to levels similar to those observed in control mitochondria. Confirming that mitoK_{ATP} activity decreased H_2O_2 release, mitochondria incubated in media in which K^+ was substituted by Na^+ (which is not transported by mitoK_{ATP} [19]) presented higher H_2O_2 release rates (line d). The subsequent addition of the H^+ ionophore FCCP (carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone) decreased H_2O_2 release rates to levels which

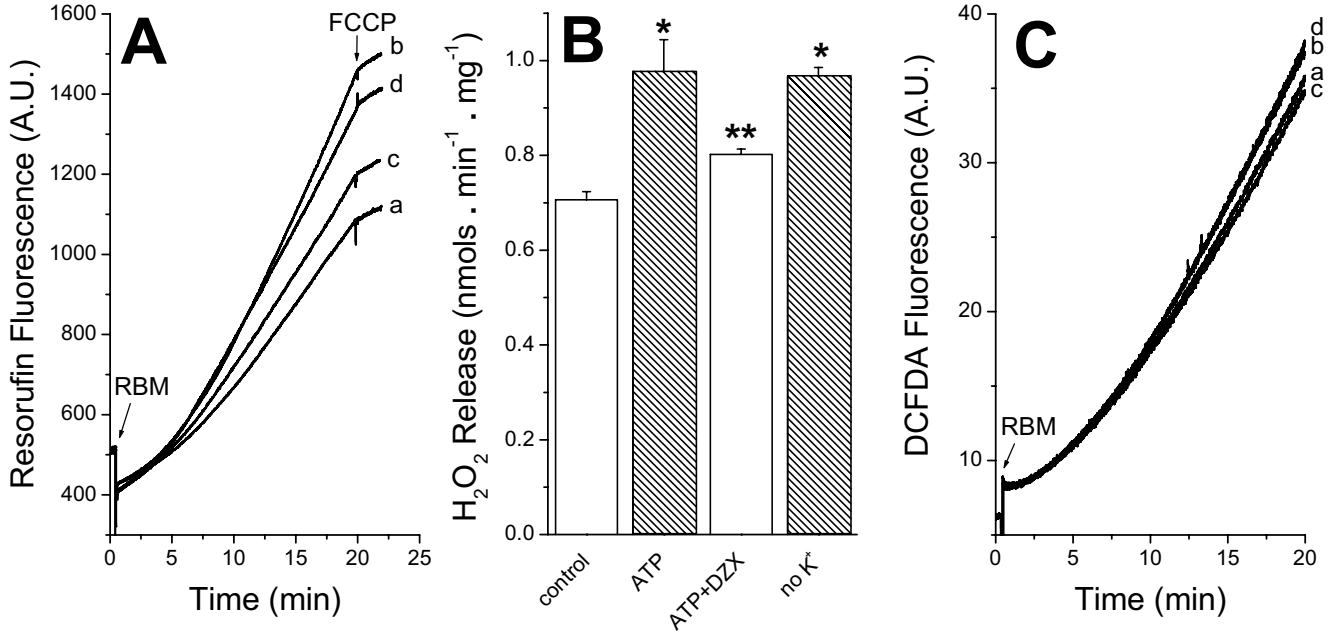

Fig. 1. K^+ transport through mitoK_{ATP} decreases ROS release in brain mitochondria. Rat brain mitochondria (RBM, 0.2 mg/ml) were added to reaction media at 37°C containing 150 mM KCl, 10 mM HEPES, 2 mM Mg^{2+}, 1 mM EGTA, 1 mM P_i, 2 mM malate, 2 mM glutamate, 1 mg/ml BSA, 2 μg/ml oligomycin, pH 7.2 (KOH). Amplex Red (50 μM) and 1 U/ml HRP (panels A and B) or 10 μM H_2DCFDA (panel C) were present to measure ROS production under control conditions (no further additions, lines a) or in the presence of 1 mM ATP (lines b), 1 mM ATP and 10 μM DZX (lines c), or no further additions, in a media in which all K^+ salts were substituted for Na^+ (lines d). FCCP (2 μM) was added where indicated. Panel B represents average ± S.E.M. H_2O_2 release rates, observed between 10 and 20 min after mitochondrial addition using three different mitochondrial preparations. *$P < 0.05$ compared to control, **$P < 0.05$ compared to the experiment conducted in the presence of ATP.

were not significantly different under any of the conditions tested.

Fig. 1B shows average H_2O_2 release rates under the conditions described above, and indicates that a lack of mitoK_{ATP} activity (hatched columns) results in a statistically significant increment (approximately 35%) in mitochondrial H_2O_2 release rates, compared to mitochondria in which mitoK_{ATP} is active (empty columns).

In order to ensure the accuracy of our results, we measured ROS generation using a second fluorescent product, DCFDA, which is formed by the oxidation of H_2DCFDA promoted by H_2O_2 and nitric oxide (and possibly other ROS). Despite the lower sensitivity of DCFDA, we observed (Fig. 1C) that mitochondria with mitoK_{ATP} open in the absence of ATP or the presence of ATP plus DZX (lines a and c, respectively) presented lower rates of ROS production than those in which mitoK_{ATP} activity was inhibited by ATP or the lack of K^+ (lines b and d, respectively).

In order to verify if the decrease in mitochondrial ROS release promoted by mitoK_{ATP} activity is related to the higher activity of this channel in brain [17], we performed similar measurements in mitochondria isolated from heart and liver (Fig. 2). Despite the lower mitoK_{ATP}-dependent K^+ transport rates in these tissues (in heart, rates are so low $\Delta\Psi$ changes are not measurable [7,17]), we found that mitoK_{ATP} opening (open columns) lead to a decrease in H_2O_2 release compared to conditions in which mitoK_{ATP} activity is inhibited (hatched columns). On average, heart mitochondrial H_2O_2 release was increased by 28%, while liver mitochondria displayed 18% more H_2O_2 release when mitoK_{ATP} was closed. Thus, even low K^+ transport rates through liver and heart mitoK_{ATP} are sufficient to significantly reduce ROS generation.

The experiments in Figs. 1 and 2 were conducted in the presence of oligomycin, in order to prevent changes in respiratory rates promoted by the addition of ATP, which often contains low quantities of contaminating ADP. However, mitochondria within intact cells usually phosphorylate ADP, a process that increases respiratory rates, reduces $\Delta\Psi$ and decreases ROS release. In Fig. 3, we measured the effect of mitoK_{ATP} activation in brain mitochondria in which ATP synthesis was induced by adding excess ADP. In the presence of ADP, which inhibits mitoK_{ATP} [18], mitochondria oxidized Amplex Red at slower rates than in the absence of oxidative phosphorylation, as expected due to elevated respiratory rates and lower $\Delta\Psi$ [3,4]. Interestingly, even under these conditions, mitoK_{ATP} activation by DZX leads to a measurable decrease in H_2O_2 formation. This effect of mitoK_{ATP} activation was not observed in the absence of K^+ ions, confirming that increases in K^+ cycling through mitoK_{ATP} activity decrease H_2O_2 release.

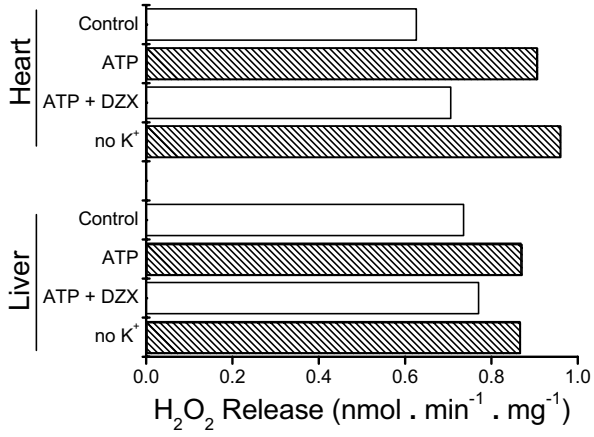

Fig. 2. K^+ transport through mitoK_{ATP} decreases H_2O_2 release in heart and liver mitochondria. Rat heart or liver mitochondria (0.2 mg/ml) were added to the reaction media described in Fig. 1, in the presence of 50 μM Amplex Red, 1 U/ml HRP and (control) no further additions, (ATP) 1 mM ATP, (ATP+DZX) 1 mM ATP and 10 μM DZX, or (no K^+) no further additions, in a media in which all K^+ was substituted for Na^+. H_2O_2 release rates were determined as described in Section 2, between 10 and 20 min after mitochondrial addition.

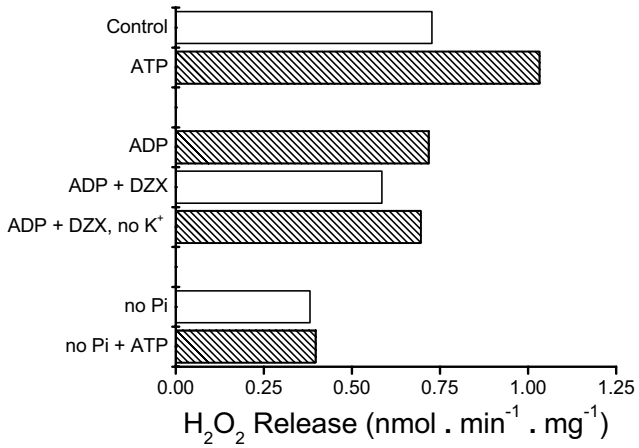

Fig. 3. Effect of oxidative phosphorylation and lack of P_i on mitoK_{ATP}-regulated H_2O_2 release. Rat brain mitochondria (0.2 mg/ml) were added to the reaction media described in Fig. 1, in the presence of 50 μM Amplex Red, 1 U/ml HRP and (control) no further additions; (ATP) 1 mM ATP; (ADP) 2 mM ADP in the absence of oligomycin; (ADP+DZX) 2 mM ADP and 10 μM DZX, in the absence of oligomycin; (ADP+DZX, no K^+) 2 mM ADP and 10 μM DZX in the absence of oligomycin, in a media in which all K^+ was substituted for Na^+; (no P_i) no further additions, in media devoid of P_i; (no P_i+ATP) 1 mM ATP, in the absence of P_i. H_2O_2 release rates were determined as described in Section 2, between 10 and 20 min after mitochondrial addition.

In intact cells, a relative lack of the membrane-permeable anion inorganic phosphate (P_i) may occur, and mitoK_{ATP} activity can generate a ΔpH gradient across the inner mitochondrial membrane [8], with intense matrix alkalinization. This alkalinization has been suggested to be the cause of possible ROS increases observed in intact cells when mitoK_{ATP} was activated [8,11]. We investigated the effect of matrix alkalinization on mitochondrial H_2O_2 release. Mitochondria incubated in the presence of excess P_i, a condition in which matrix alkalinity is minimal, present H_2O_2 release rates higher than those incubated in the absence of added P_i (Fig. 3), a condition in which ΔpH and matrix alkalinity are increased. Under these conditions, mitoK_{ATP} inhibition by ATP does not significantly affect H_2O_2 release.

4. Discussion

Using isolated mitochondria from brain (Figs. 1 and 3), heart and liver (Fig. 2), we found that mitochondrial ROS generation is significantly decreased when mitoK_{ATP} is active. Increased mitochondrial ΔpH, an effect expected to occur within intact cells when mitoK_{ATP} is highly active [8], also decreased ROS release (Fig. 3). The decreased ROS produc-

tion promoted by mitoK$_{ATP}$ occurs both in the absence (state 4, Figs. 1 and 2) and presence (state 3, Fig. 3) of oxidative phosphorylation, and is in line with the finding that mild uncoupling between respiration and oxidative phosphorylation strongly prevents mitochondrial ROS formation [3–6]. However, these results differ from findings using intact cells, in which the addition of DZX promoted an apparent increase in mitochondrial ROS [9–11].

The reason for these conflicting results is not immediately clear, but may reflect differences in the experimental protocols. While previous papers measured ROS within intact vascular cells, this study used isolated mitochondria, an approach which we find interesting, since it allows us to activate mitoK$_{ATP}$ both by omitting ATP from the reaction medium and by adding DZX to counteract the ATP effect. In isolated mitochondria, it is also possible to test if the effects of these additions can be directly attributed to mitoK$_{ATP}$ by repeating the tests in media devoid of K^+ salts. In intact cells, mitoK$_{ATP}$ can only be regulated using pharmacological tools, which may present artifacts [7]. In addition, mitoK$_{ATP}$ opening in intact cells may not *directly* enhance mitochondrial ROS generation, but may activate other intracellular pathways responsible for ROS production.

Another possible reason for the discrepancy between our results and previous measurements of mitoK$_{ATP}$-stimulated ROS release in intact cells is the use of different fluorescent probes. We used primarily Amplex Red in our measurements, which is oxidized specifically by HRP-bound H_2O_2 to form the fluorescent product resorufin. We find the specificity, high sensitivity and low noise of this probe very useful in H_2O_2 measurements. We also find that Amplex Red measurements respond as expected to conditions known to increase and decrease mitochondrial ROS, such as respiratory inhibition with antimycin A (results not shown) and respiratory stimulation with FCCP (Fig. 1A), respectively. Another finding supporting the idea that our measurements accurately measure mitochondrial H_2O_2 are the similar results obtained using the less specific and sensitive indicator H$_2$DCFDA (Fig. 1C).

H$_2$DCFDA was previously used by Forbes and co-authors [9] to measure ROS in intact cardiac myocytes treated with DZX and pinacidil to induce mitoK$_{ATP}$ opening. In contrast to our results, these authors observed an increase in fluorescence increment of this probe when mitoK$_{ATP}$ was open. Again, this result may reflect indirect effects of mitoK$_{ATP}$ opening on ROS generation in intact cells, not observed in isolated mitochondria. Another possibility for these differing results may be that the authors loaded the cells with H$_2$DCFDA before their measurements, allowing the generation of intracellular dichlorodihydrofluorescein, a membrane-impermeable metabolite of H$_2$DCFDA [9]. Due to its negative charge, this compound may redistribute within the cell depending on mitochondrial ΔpH and $\Delta\Psi$, parameters that are altered by mitoK$_{ATP}$ opening [7,17].

Other previous investigations of mitoK$_{ATP}$ effects on ROS generation used reduced Mitotracker probes (Mitotracker Red and Orange, [10,11]) in vascular cell lines. In one of these studies, the authors found that neither the uncoupler dinitrophenol nor the respiratory inhibitor myxothiazol cause a change in the probe's fluorescence under control conditions [11]. This unusual result may indicate that these probes are not, in fact, accurately measuring ROS. Moreover, these authors found that the mitoK$_{ATP}$ effect in intact cells was not observed using more traditional ROS indicators such as H$_2$DCFDA (ref. [11] as opposed to the results of ref. [9]) and dihydroethidium [10]. According to the maker (Molecular Probes), reduced Mitotracker Red and Orange do not fluoresce until they enter a respiring cell, where they are oxidized by ROS and sequestered in mitochondria as fluorescent products. It is possible that mitochondrial sequestration of these dyes is affected by other factors controlled by mitoK$_{ATP}$, including $\Delta\Psi$, ΔpH and, most importantly, volume [7]. We have previously shown that changes in mitochondrial volume may strongly affect uncalibrated fluorescent measurements using many different dyes [20], and mitoK$_{ATP}$ opening is known to significantly increase mitochondrial volume [7,10,17].

The hypothesis that mitoK$_{ATP}$ opening could increase mitochondrial ROS release was developed based on experiments showing that ischemic preconditioning in heart involves both the activation of mitoK$_{ATP}$ and increased cellular ROS [12,21]. Since ROS generation appeared to occur downstream of mitoK$_{ATP}$ activation [22,23], it seemed logical to propose that it was mediated by this channel. However, there is solid evidence today that ROS are increased in preconditioned hearts upstream of mitoK$_{ATP}$ activation [24], through yet undetermined mechanisms. In view of this data, it is possible that mitoK$_{ATP}$ opening is not directly involved in the increase in ROS observed after preconditioning. However, the results in this manuscript may explain the preventive effect of ischemic preconditioning on ROS release observed after ischemia/reperfusion, an effect related to mitoK$_{ATP}$ activation [25].

Independently of the controversy regarding ischemic preconditioning and ROS, the finding that mitoK$_{ATP}$ decreases mitochondrial ROS release suggests many interesting possibilities. We find the effect of this channel an ideal method to regulate mitochondrial ROS generation, since K^+ transport through mitoK$_{ATP}$ is limited by the low quantity and transport rates of this channel, and does not seriously affect other mitochondrial functions such as oxidative phosphorylation, Ca^{2+} uptake and $\Delta\Psi$ [7]. Thus, mitoK$_{ATP}$ opening may have a physiological role as an endogenous prevention mechanism against excessive mitochondrial ROS formation, without hampering mitochondrial function. The finding that mitoK$_{ATP}$ activity is stimulated by oxidation promoted by ROS [26,27] further supports this hypothesis. Also, unlike uncoupling proteins (which promote mild mitochondrial uncoupling and prevent ROS release [5]), mitoK$_{ATP}$ has well known pharmacological regulators. These mitoK$_{ATP}$ regulators may be effective tools in the prevention of diseases related to mitochondrial ROS accumulation.

Acknowledgements: This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). R.F. and M.M.S. are students supported by FAPESP scholarships. A.J.K. is supported in part by a Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) 'Produtividade em Pesquisa' fellowship. The authors would like to thank Professor Luis E.S. Netto and Dr. Mario H. Barros for critical reading of the manuscript.

References

[1] Kowaltowski, A.J. and Vercesi, A.E. (2001) in: Mitochondria in Pathogenisis (Lemasters, J.J. and Nieminen, A.L., Eds.), pp. 281–300, Plenum Publishing Corporation, New York.
[2] Nicholls, D. (2002) Int. J. Biochem. Cell. Biol. 34, 1372–1381.
[3] Skulachev, V.P. (1996) Quart. Rev. Biophys. 29, 169–202.

[4] Korshunov, S.S., Skulachev, V.P. and Starkov, A.A. (1997) FEBS Lett. 416, 15–18.
[5] Nègre-Salvayre, A., Hirtz, C., Carrera, G., Cazenave, R., Troly, M., Salvayre, R., Pénicaud, L. and Casteilla, L. (1997) FASEB J. 11, 809–815.
[6] Popov, V.N., Simonian, R.A., Skulachev, V.P. and Starkov, A.A. (1997) FEBS Lett. 415, 87–90.
[7] Kowaltowski, A.J., Seetharaman, S., Paucek, P. and Garlid, K.D. (2001) Am. J. Physiol. 280, H649–H657.
[8] Garlid, K.D. and Paucek, P. (2001) IUBMB Life 52, 153–158.
[9] Forbes, R.A., Steenbergen, C. and Murphy, E. (2001) Circ. Res. 88, 802–809.
[10] Carroll, R., Gant, V.A. and Yellon, D.M. (2001) Cardiovasc. Res. 51, 691–700.
[11] Krenz, M., Oldenburg, O., Wimpee, H., Cohen, M.V., Garlid, K.D., Critz, S.D., Downey, J.M. and Benoit, J.N. (2002) Basic Res. Cardiol. 97, 365–373.
[12] Grover, G.J. and Garlid, K.D. (2000) J. Mol. Cell. Cardiol. 32, 677–695.
[13] Andreyev, A. and Fiskum, G. (1999) Cell Death Differ. 6, 825–832.
[14] Netto, L.E., Kowaltowski, A.J., Castilho, R.F. and Vercesi, A.E. (2002) Methods Enzymol. 348, 260–270.
[15] Zhou, M., Diwu, Z., Panchuk-Voloshina, N. and Haugland, R.P. (1997) Anal. Biochem. 253, 162–168.
[16] Kehrer, J.P. and Paraidathathu, T. (1992) Free Radic. Res. Commun. 16, 217–225.
[17] Bajgar, R., Seetharaman, S., Kowaltowski, A.J., Garlid, K.D. and Paucek, P. (2001) J. Biol. Chem. 276, 33369–33374.
[18] Paucek, P., Mironova, G., Mahdi, F., Beavis, A.D., Woldegiorgis, G. and Garlid, K.D. (1992) J. Biol. Chem. 267, 26062–26069.
[19] Belisle, E. and Kowaltowski, A.J. (2002) J. Bioenerg. Biomembr. 34, 285–298.
[20] Kowaltowski, A.J., Cosso, R.G., Campos, C.B. and Fiskum, G. (2002) J. Biol. Chem. 277, 42802–42807.
[21] Vanden Hoek, T.L., Becker, L.B., Shao, Z., Li, C.Q. and Schumacker, P.T. (1998) J. Biol. Chem. 273, 18092–18098.
[22] Yue, Y., Qin, Q., Cohen, M.V., Downey, J.M. and Critz, S.D. (2002) Cardiovasc. Res. 55, 681–689.
[23] Patel, H.H. and Gross, G.J. (2001) Cardiovasc. Res. 51, 633–636.
[24] Lebuffe, G., Schumacker, P.T., Shao, Z.H., Anderson, T., Iwase, H. and Vanden Hoek, T.L. (2002) Am. J. Physiol. (in press) epub ahead of print: Sep 26.
[25] Vanden Hoek, T., Becker, L.B., Shao, Z.H., Li, C.Q. and Schumacker, P.T. (2000) Circ. Res. 86, 541–548.
[26] Pastore, D., Stoppelli, M.C., Di Fonzo, N. and Passarella, S. (1999) J. Biol. Chem. 274, 26683–26690.
[27] Zhang, D.X., Chen, Y.F., Campbell, W.B., Zou, A.P., Gross, G.J. and Li, P.L. (2001) Circ. Res. 89, 1177–1183.