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Redox regulation of the mitochondrial K_{ATP} channel in cardioprotection ☆

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ABSTRACT

The mitochondrial ATP-sensitive potassium channel (mK_{ATP}) is important in the protective mechanism of ischemic preconditioning (IPC). The channel is reportedly sensitive to reactive oxygen and nitrogen species, and the aim of this study was to compare such species in parallel, to build a more comprehensive picture of mK_{ATP} regulation. mK_{ATP} activity was measured by both osmotic swelling and Tl^+ flux assays, in isolated rat heart mitochondria. An isolated adult rat cardiomyocyte model of ischemia–reperfusion (IR) injury was also used to determine the role of mK_{ATP} in cardioprotection by nitroxyl. Key findings were as follows: (i) mK_{ATP} was activated by $O_2^{\bullet-}$ and H_2O_2 but not other peroxides. (ii) mK_{ATP} was inhibited by NADPH. (iii) mK_{ATP} was activated by S-nitrosothiols, nitroxyl, and nitrolinoleate. The latter two species also inhibited mitochondrial complex II. (iv) Nitroxyl protected cardiomyocytes against IR injury in an mK_{ATP}-dependent manner. Overall, these results suggest that the mK_{ATP} channel is activated by specific reactive oxygen and nitrogen species, and inhibited by NADPH. The redox modulation of mK_{ATP} may be an underlying mechanism for its regulation in the context of IPC. This article is part of a Special Issue entitled: Mitochondria and Cardioprotection.

1. Introduction

The past 25 years has witnessed much investigation into the phenomenon of ischemic preconditioning (IPC), in which short non-lethal periods of ischemia and reperfusion (IR) can elicit protection against prolonged ischemia–reperfusion (IR) injury [1]. Despite this effort, the mechanism by which IPC protects organs such as the heart and brain from IR injury is still debated.

One proposed mechanism of IPC-induced cardioprotection is the opening of a mitochondrial ATP-sensitive potassium channel (mK_{ATP}), which elicits mild swelling of the mitochondrial matrix. This in turn is thought to impact on mitochondrial Ca^{2+} loading, reactive oxygen species (ROS) generation, metabolic efficiency, and assembly of the permeability transition pore [2], and these downstream events bring about protection via unclear mechanisms. Although the molecular identity of the mK_{ATP} channel remains unknown, several pharmacologic mK_{ATP} modulators mimic IPC, and many IPC signaling pathways are thought to converge on mK_{ATP} as an end effector [3].

Mitochondria are a quantitatively significant source of ROS, which contribute to tissue damage during ischemia, but are also mediators of IPC signaling [4]. Accumulating evidence suggests that redox signaling pathways play an important role in IPC [5–9], and can promote mK_{ATP} activation [10–15]. The primary ROS generated by mitochondria is superoxide ($O_2^{\bullet-}$) [16,17], while hydrogen peroxide (H_2O_2) or lipid peroxides can be formed secondarily [17]. Both $O_2^{\bullet-}$ and H_2O_2 are thought to activate mK_{ATP} [13,18–20], although conflicting reports exist regarding $O_2^{\bullet-}$ [18,20]. The effect of other peroxides on mK_{ATP} is not known. Furthermore, it is apparent that some but not all types of antioxidants can inhibit IPC and mK_{ATP} activity [6,13], warranting further investigation. Table 1 summarizes the disparate results to date regarding redox regulation of the mK_{ATP} channel.

Nitric oxide ($NO^{\bullet}$) is also implicated in IPC, and elicits a large variety of cardioprotective effects [21]. $NO^{\bullet}$ has been detected in isolated mitochondrial preparations [22], and can secondarily generate many reactive nitrogen species (RNS) [23–25], which can serve either damaging or beneficial signaling roles [17,21,25,26]. mK_{ATP} is a potential target for such RNS, and while high doses (10 mM) of an S-nitrosothiol have been shown to activate the channel in intact mitochondria [20], evidence for more subtle physiologically relevant effects of $NO^{\bullet}$ has mostly relied on indirect measures of channel activity [27] or study of the channel removed from its mitochondrial environment [28]. Thus, it is not clear whether the levels of NO• that would be experienced inside mitochondria are capable of modulating mK_{ATP} activity.

The one electron reduction product of $NO^{\bullet}$, nitroxyl (HNO) may also modulate the mK_{ATP} channel. Nitroxyl is protective in IR injury [29], and while it shares some signaling pathways with $NO^{\bullet}$, it also possesses distinct biochemistry from $NO^{\bullet}$, such as a direct interaction

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Table 1
Previous studies on the effects of oxidants, reactive nitrogen species, antioxidants and reducing agents on mK_{ATP} channel activity.

Reagent	Conc.	Effects	Experimental conditions	References
Ascorbate	1 mM	No effect	Isolated mitochondria	[12]
DTE	10 mM	Inhibits glyburide binding	Submitochondrial particles	[46]
DTNB	500 μM	Inhibits glyburide binding	Submitochondrial particles	[46]
DTT	100 μM	Inhibits activation by DZX	Isolated mitochondria	[12,13]
	1 mM	Activates run-down channels Loss in selectivity	Reconstituted channels	[66]
	10 mM	Inhibits glyburide binding	Submitochondrial particles	[46]
Mersalyl	100 μM	Inhibits glyburide binding	Submitochondrial particles	[46]
MPG	200 μM	Inhibits activation by DZX	Isolated mitochondria	[12,13,31]
NAC	4 mM	Inhibits activation by DZX	Isolated mitochondria	[12,13]
NEM	2 mM	Inhibits activation by $O_2^{\bullet -}$ Inhibits glyburide binding	Reconstituted channels Submitochondrial particles	[18] [46]
	60 nmol/mg	Decreases selectivity	Isolated mitochondria	[67]
Thimerosal	500 μM	Inhibits glyburide binding	Submitochondrial particles	[46]
X/XO	0.038 U/mL	Activates	Reconstituted channels	[18]
	6 mU/mL	No Effect	Isolated mitochondria	[20]
H_2O_2	1 μM	Activates	Isolated mitochondria	[13]
	1 μM	Activates	Isolated mitochondria	[19]
	6 mU/mL X/XO + 30 U SOD	Activates	Isolated mitochondria	[20]
SNAP	10 mM	Activates	Isolated mitochondria	[20]

with thiols. In this regard, the nitroxyl donor Angeli's salt (AS) inhibits mitochondrial complex II in a manner sensitive to glutathione and independent of S-nitrosation [30]. mK_{ATP} activity is exquisitely sensitive to complex II modulation [31,32], and herein we explored the concept that nitroxyl may regulate mK_{ATP} via effects on complex II.

Despite a collection of studies to date examining the effect of single redox agents on mK_{ATP}, often at high doses, a comparative study across a wide range of doses is lacking. In addition, unique chemical properties of certain $NO^{\bullet}$ derived species have precluded their use to date in studying mK_{ATP}. The current study aimed to address such issues, and the collective results suggest that mitochondrial redox state is an important regulator of mK_{ATP} channel activity, mitochondrial function, and cardioprotection in the context of IPC.

2. Materials and methods

Full experimental details are in the online supplement.

2.1. Animals, chemicals and supplies

Male Sprague–Dawley rats, 200–300 g, were purchased from Harlan (Indianapolis, IN) or bred at the *Biotério do Conjunto das Químicas* (*Universidade de São Paulo*) housed on a 12 h light/dark cycle with food and water available *ad libitum*. All procedures were performed in accordance with the US National Institutes of Health "Guide for the care and use of laboratory animals" and the *Colégio Brasileiro de Experimentação Animal*, and were approved by the appropriate university animal ethics committees. Linoleic peroxide was a kind gift from Sayuri Miyamoto (São Paulo) and stored under argon in methanol [33]. Nitro-linoleate was synthesized and analyzed as previously described [34]. All other reagents used were analytical grade or higher, obtained from Sigma (St. Louis MO) or EMD (Gibbstown NJ).

2.2. Mitochondrial isolation, Cx-II, and mK_{ATP} assays

Heart mitochondria were rapidly isolated as previously described [31,35]. Cx-II activity was measured as previously described [31,32]. mK_{ATP} activity was measured by osmotic swelling as previously described [32,36]. All channel modulating agents (reactive oxygen and nitrogen species, antioxidants, etc.) were present in the assay buffer prior to mitochondrial addition. The nature of the mK_{ATP} osmotic swelling assay, requiring mitochondrial addition last of all, precludes its use to study highly reactive species such as HNO [37]. In such cases a fluorescence-based Tl^+ flux assay for mK_{ATP} activity [38] was used, permitting incubation of mitochondria prior to assay initiation by Tl^+ addition.

2.3. Cardiomyocyte model of IR injury

Adult rat ventricular myocytes were isolated, and a model of simulated IR (SIR) injury was as previously described [32]. Cells were incubated in anoxic glucose-free Krebs Henseleit (KH) buffer at pH 6.5 for 30 min, followed by reoxygenation in glucose-replete KH at pH 7.4. Where indicated, compounds were present 20 min prior to the onset of simulated ischemia. At the end of all protocols, viability was determined by Trypan blue exclusion.

2.4. Statistics

All experiments were performed on at least 3 independent mitochondrial or cell preparations, and results are presented as mean ± SEM. Statistical significance between groups was determined by ANOVA.

3. Results and discussion

3.1. mK_{ATP} is activated by some but not all peroxides

While the ability of ROS to modulate mK_{ATP} activity has been reported [13,18–20], the differential action of various classes of ROS is less well understood. In particular, ROS such as H_2O_2 may initiate chemistry that generates secondary peroxides such as lipid hydroperoxides (LOOH) [17]. We therefore hypothesized that peroxides may modulate mK_{ATP} activity. In Fig. 1A, the effects of H_2O_2, *t*-butyl hydroperoxide (*t*-BuOOH), and linoleic hydroperoxide (LOOH) on mK_{ATP} were tested. Interestingly, while H_2O_2 robustly opened the channel at 1 μM, higher concentrations of *t*-BuOOH and LOOH did not. The differential hydrophobicity of *t*-BuOOH, LOOH, and H_2O_2 suggests that the peroxide sensor of mK_{ATP} may be in a hydrophilic region of the molecule. Alternatively, it has been suggested that H_2O_2 activation of the mK_{ATP} may occur via a PKCε dependent mechanism [20], although the location of the kinase in this particular mitochondrial preparation is unknown.

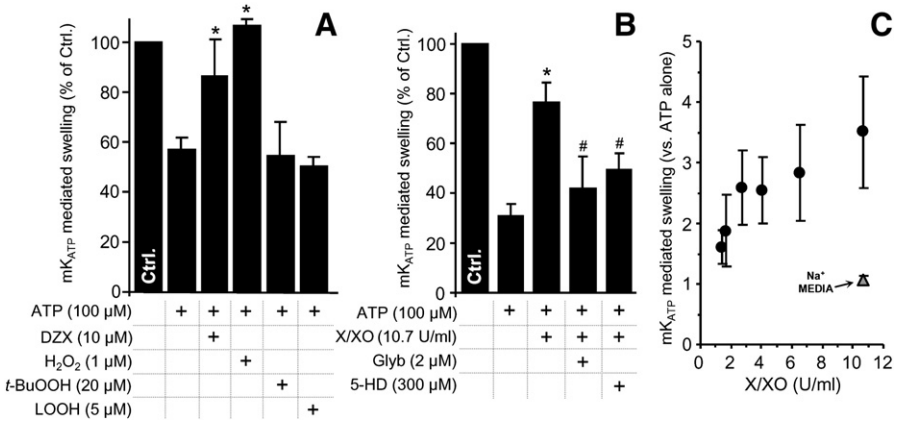

Fig. 1. Selective mK$_{ATP}$ activation by ROS: mK$_{ATP}$ activity was measured by osmotic swelling as detailed in Materials and methods. Controls (Na$^+$ based media) are in Fig. S1. (A): mK$_{ATP}$ is activated by H$_2$O$_2$ but not by *t*-BuOOH or LOOH. Diazoxide (DZX) opening of mK$_{ATP}$ was used as a positive control. (B): mK$_{ATP}$ activation by $O_2^{\bullet-}$ generated by the X/XO system. Glyburide (Glyb) and 5-hydroxydecanoate (5-HD) are mK$_{ATP}$ antagonists. (C): Dose response of mK$_{ATP}$ activation by X/XO in K$^+$ (black circles) or Na$^+$ (gray triangles) based media. *p<0.05 vs. ATP alone. #p<0.05 vs. ATP + H$_2$O$_2$ or X/XO. Experimental conditions are listed below the X axis.

3.2. mK$_{ATP}$ is activated by supra-physiological levels of superoxide

Activation of mK$_{ATP}$ by $O_2^{\bullet-}$ has been reported previously [18], but at relatively high doses. It is unclear whether the amounts of $O_2^{\bullet-}$ made by mitochondria are capable of modulating mK$_{ATP}$ activity. To investigate this, a xanthine/xanthine oxidase (X/XO) system was used to modulate ROS flux. Fig. 1B and C shows that XO levels as low as 1.4 U/mL (approximating to a $O_2^{\bullet-}$ flux of 1.2 μM/min) activated the channel. Reported maximal rates of $O_2^{\bullet-}$ generation by heart mitochondria range from 30 nM/min to 1 μM/min [39,40]. Thus, it is unlikely that mitochondrial $O_2^{\bullet-}$ generation under normal conditions approaches levels required for mK$_{ATP}$ channel activation. This finding suggests that the channel would only be activated in situ with ROS originated from non-mitochondrial sources or conditions that increase ROS production such as pre- and post-conditioning. Also under conditions of reverse electron flow, $O_2^{\bullet-}$ in the mitochondrial microenvironment may reach levels capable of activating the channel [13]. No effect of H$_2$O$_2$ or X/XO on mitochondrial swelling was observed in Na$^+$ based media (supplemental Fig. 1).

3.3. mK$_{ATP}$ is inhibited by some but not all reductants/antioxidants

While IPC is inhibited by both thiol antioxidants and catalase (suggesting a role for H$_2$O$_2$ [12]), cardioprotection by mK$_{ATP}$ agonists such as diazoxide is prevented only by thiol antioxidants [13], suggesting that antioxidant-sensitive proteins distinct from mK$_{ATP}$ may be important in IPC (e.g. SERCA, [41]). Thus, a detailed understanding of the selective regulation of mK$_{ATP}$ by reductants is a key step toward understanding its role in IPC.

The effect of several reducing agents on mK$_{ATP}$ activity was tested under baseline conditions and conditions of maximal channel opening (presence of ATP and diazoxide). Fig. 2 shows that while most reducing agents had a mild inhibitory effect on mK$_{ATP}$ activity, NADPH was a strong inhibitor, almost completely preventing channel activity. Compiling these data with those from our previous study [13], supplemental Fig. 2 shows the ability of reductants to inhibit mK$_{ATP}$ channel activity correlated with redox potential ($r^2 = 0.73$). A notable outlier to this correlation was NADPH (inclusion of NADPH in the linear regression fit lowers r^2 to 0.43). The underlying mechanism for a difference in the effect of NADH vs. NADPH, despite their identical redox potentials, is elusive at this stage. Interestingly, while both surface K$_{ATP}$ channels [42–45] and the mK$_{ATP}$ [12,13,18,46,47] do have redox active thiols, NADPH does not directly reduce thiols, suggesting that its direct redox activity is not the mechanism of channel regulation.

Nevertheless, NADPH does play an important role in overall mitochondrial redox status; transhydrogenases reduce mitochondrial NADP$^+$ using electrons from NADH and the electrochemical proton gradient as an energy source [48]. The resulting NADPH is used as an electron source for thiol peroxidase removal systems, including glutathione and thioredoxin peroxidase/reductase [17]. Thus, the finding that NADPH can regulate mK$_{ATP}$ activity suggests that this channel may play a role in sensing both energy metabolism and redox status [13,49].

Notably, surface K$_{ATP}$ channel activity has been shown to be sensitive to pyridine nucleotides [50], possibly at the same site as adenine nucleotides modulate channel activity. Furthermore, insulin secretion in pancreatic β cells, which occurs secondarily to K$_{ATP}$ closure, correlates with NADPH/NADP$^+$ ratios [51]. Thus, a direct modulation of the mK$_{ATP}$ channel by NADPH binding may occur. Another possibility might be that NADPH competes with DZX for a binding site on the mK$_{ATP}$ channel. However, we consider this unlikely since NADPH was also able to inhibit channel opening by the structurally unrelated opener atpenin A5 [32] (supplemental Fig. 3).

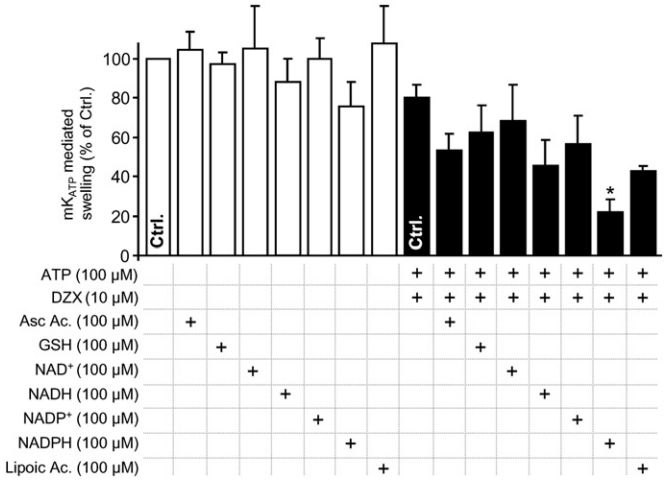

Fig. 2. Inhibition of DZX-activated mK$_{ATP}$ by reductants: mK$_{ATP}$ activity was measured by osmotic swelling as detailed in Materials and methods. Data are shown for the baseline condition (open bars) or maximal swelling in the presence of both ATP and DZX (filled bars). Reductants were present in the media before mitochondrial addition, at the concentrations indicated. *p<0.05 vs. the appropriate control (bar marked Ctrl.) in the absence of reductant.

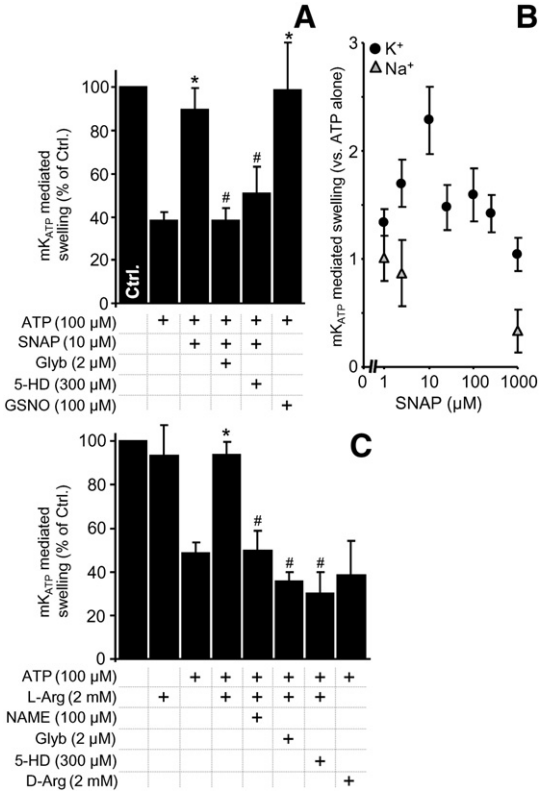

Fig. 3. mK_{ATP} activation by S-nitrosothiols and mitochondrial-associated NOS: mK_{ATP} activity was measured by osmotic swelling as detailed in Materials and methods. Controls (Na^+ based media) are in Fig. S2. (A): mK_{ATP} was activated by S-nitrosoacetylpenacillamine (SNAP) and S-nitrosoglutathione (GSNO), at the indicated doses. (B): Dose response of mK_{ATP} activation by SNAP in K^+ (black circles) or Na^+ (gray triangles) based media. *$p<0.05$ vs. ATP alone. #$p<0.05$ vs. ATP plus SNAP. (C): mK_{ATP} activation by NOS modulators. L- or D-Arginine, L-nitroarginine methyl ester (NAME), Glyb and 5-HD were present at the concentrations indicated. *$p<0.05$ vs. *$p<0.05$ vs. ATP alone. #$p<0.05$ vs. ATP plus L-arginine.

3.4. mK_{ATP} is activated by S-nitrosothiols and L-arginine

In addition to ROS, much recent interest has focused on mK_{ATP} as a possible target for NO• or its redox congeners [20]. While NO• effects on mK_{ATP} activity in intact cells are thought to be mediated via cGMP signaling [52,53], direct effects of NO• on the purified channel have been measured in planar bilayer studies [28]. In addition NO• was shown to activate mK_{ATP} in mitochondria by using flavoprotein fluorescence as a read-out [27]. However, the dose response of mK_{ATP} to NO• in intact mitochondria is unknown. Fig. 3A and B shows that S-nitrosoacetylpenacillamine (SNAP) dose-dependently activated mK_{ATP}, in a glybenclamide and 5-HD sensitive manner. Notably, >10 μM SNAP led to mK_{ATP} inhibition, presumably due to NO• inhibition of cytochrome *c* oxidase [54] leading to mitochondrial de-energization, removing the driving force for K^+ uptake. SNAP did not activate swelling in Na^+ based buffers (supplemental Fig. 4). Notably, the optimal SNAP concentration for mK_{ATP} channel opening in this study (10 μM) is 3 orders of magnitude lower than previously reported (Table 1). We are unsure as to the origin of this very large discrepancy [20].

There has been much interest in the possibility that mitochondria may contain a nitric oxide synthase, termed "mtNOS". Despite recent developments including retraction of some work [55–57], NOS is a common contaminant of isolated mitochondrial preparations [58]. This may be particularly applicable to mK_{ATP} studies, since a rapid and crude mitochondrial isolation is required to effectively measure channel activity (full experimental details are in the online supplement) [32,36]. The data in Fig. 3C show that the NOS substrate L-arginine stimulates mK_{ATP} opening, in a manner sensitive to the NOS inhibitor L-NAME. No effect was seen with D-arginine, suggesting that the origin of this effect resides at the level of a mitochondrially associated NOS or L-NAME-sensitive enzyme. Unfortunately, further purification of mitochondria that would be required to assert a mitochondria-resident NOS in mediating these effects, also results in loss of mK_{ATP} channel activity, in a manner mechanistically related to classical channel "run-down" [38].

3.5. Certain RNS can activate mK_{ATP}, via a mechanism involving mitochondrial complex II

In addition to "classical" RNS such as NO•, RNS such as nitro-lipids and nitroxyl may regulate mK_{ATP}. Nitro-lipids are an emerging class of anti-inflammatory signaling lipids which can mediate NO• signaling, [59,60] and are known to be generated during IPC [34,61]. One example of a nitro-lipid, nitrolinoleate (LA-NO_2), can elicit cardioprotection in a cGMP-independent manner [34,62]. Fig. 4A shows that, low doses (0.5 μM) of LA-NO_2 opened the mK_{ATP} channel in a 5-HD- and glyburide-sensitive manner, while native linoleic acid (LA) did not. Complex II of the mitochondrial respiratory chain has been proposed as an important regulator of mK_{ATP} activity [31,32,63], and in this regard Fig. 4B shows that LA-NO_2, but not LA, inhibited complex II in a dose-dependent manner. Notably however, the amount of LA-NO_2 required to open the channel was significantly lower than that required to inhibit complex II (see below for discussion).

The disparate effects of LA-NO_2 (stimulation mK_{ATP} activity), and lipid hydroperoxide (no effect on mK_{ATP}, see section 3.1) highlight the different chemical properties of these species. While both are hydrophobic reactive lipids, only LA-NO_2 possesses an electrophilic moiety that can adduct thiols by Michael addition [61]. Thus it is suggested that the mechanism of LA-NO_2 mediated mK_{ATP} opening may involve modification of thiols on the channel (c.f. section 3.3).

The levels of LA-NO_2 generated inside mitochondria during IPC may reach 1 μM [34], raising the possibility that LA-NO_2 is an important endogenous mK_{ATP} regulator. However, we previously showed that cardioprotection induced by exogenously added LA-NO_2 was insensitive to mK_{ATP} blockers [34], suggesting that other mechanisms of LA-NO_2 action (e.g. mild uncoupling) may account for its cardioprotective effects.

Similar to nitro-lipids, the importance of nitroxyl in cardiovascular signaling has also been the subject of recent attention [29,37]. In agreement with previous findings, we showed that the nitroxyl donor Angeli's salt (AS) dose-dependently inhibited complex II in rat heart mitochondria (Fig. 5A). Consistent with an interaction between complex II and the mK_{ATP} channel, we also found that Angeli's salt

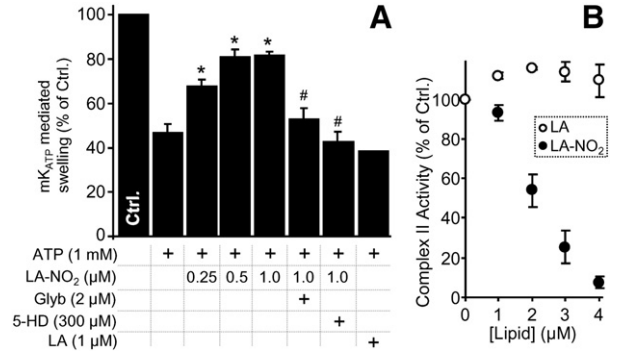

Fig. 4. LA-NO_2 Opens mK_{ATP} and inhibits complex II: (A): mK_{ATP} activity was measured by osmotic swelling as detailed in Materials and methods. LA-NO_2, native linoleate (LA), Glyb, and 5-HD were present at the indicated concentrations. *$p<0.05$ vs. ATP alone. #$p<0.05$ vs. ATP plus LA-NO_2. (B): Complex II activity in the presence of LA-NO_2 was determined as detailed in Materials and methods. Values are expressed as percentage of control complex II rate (128 ± 26 nmol DCPIP min^{-1} mg $protein^{-1}$).

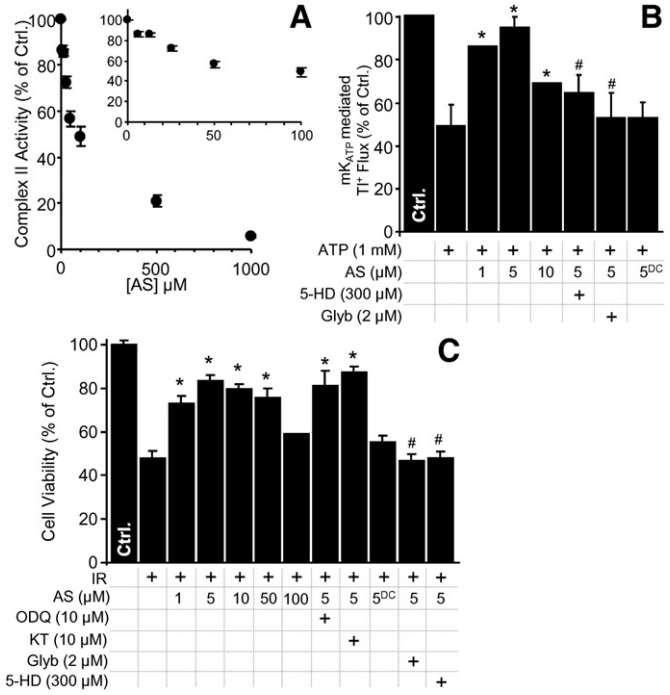

Fig. 5. Nitroxyl inhibits complex II, opens mK$_{ATP}$ and is cardioprotective: (A): Complex II activity was measured, following nitroxyl exposure of mitochondria, as described in Materials and methods. Values are expressed as percentage of control complex II rate (108 ± 8 nmol DCPIP min^{-1}mg protein^{-1}). (B): Nitroxyl activation of mK$_{ATP}$ was monitored using a novel Tl$^+$ flux assay as described in Materials and methods. Data show the magnitude of change in intra-mitochondrial Tl$^+$ based fluorescence following Tl$^+$ addition, relative to control. 5-HD, Glyb, the nitroxyl donor Angeli's salt (AS) and decomposed AS (AS^{DC}) were present at the indicated concentrations. *$p<0.05$ vs. ATP alone. #$p<0.05$ vs. ATP plus 5 μM AS. (C): Nitroxyl protects against cardiomyocyte IR injury. Cell viability was measured via Trypan blue exclusion at the end of reoxygenation, as described in Materials and methods, and expressed as percentage of control (normoxic) cell viability. 5-HD, Glyb, Angeli's salt (AS), decomposed AS (AS^{DC}), the PKG inhibitor KT-5823 (KT) or the soluble guanylate cyclase inhibitor ODQ were present at the indicated concentrations. *$p<0.05$ vs. IR alone. #$p<0.05$ vs. IR plus 5 μM AS.

opened mK$_{ATP}$ in a manner sensitive to 5-HD and glyburide (Fig. 5B). Furthermore, in agreement with previous studies [29,64] Angeli's salt was protective in a cardiomyocyte model of IR injury. This protection was sensitive to 5-HD and glyburide, but insensitive to the guanylate cyclase inhibitor ODQ or the protein kinase G inhibitor KT-5823 (Fig. 5C). The role of other known components of the preconditioning signaling pathway (e.g. PKC, ERK, PI3 kinase) in mediating the effects of Angeli's salt is currently unknown, although none of these signals has previously been linked to nitroxyl. Together, these data suggest that Angeli's salt mediated cardioprotection proceeds via non-PKG mediated activation of mK$_{ATP}$, possibly involving an inhibition of complex II.

Both nitroxyl and LA-NO$_2$ inhibit mitochondrial complex II, activate mK$_{ATP}$, and are known to react with complex II thiols [30,34]. This suggests that modification of complex II thiols may underlie the mechanism of mK$_{ATP}$ activation. However, the concentrations of nitroxyl and LA-NO$_2$ which activated mK$_{ATP}$ did not significantly inhibit complex II enzymatic activity (Figs. 4 and 5). In this regard, nitroxyl and LA-NO$_2$ are similar to other species which activate the mK$_{ATP}$ at doses far below those at which they inhibit complex II, including atpenin A5 [32], malonate [31] and diazoxide [65]. Thus, the regulation of mK$_{ATP}$ activity appears to be mechanistically divorced from the bulk enzymatic activity of complex II itself. The fact that 5 unrelated compounds which inhibit complex II by distinct mechanisms all activate mK$_{ATP}$ at lower concentrations, collectively suggests that a small sub-population of complex II may play an important role in regulating mK$_{ATP}$ activity, while not impacting total complex II enzymatic activity.

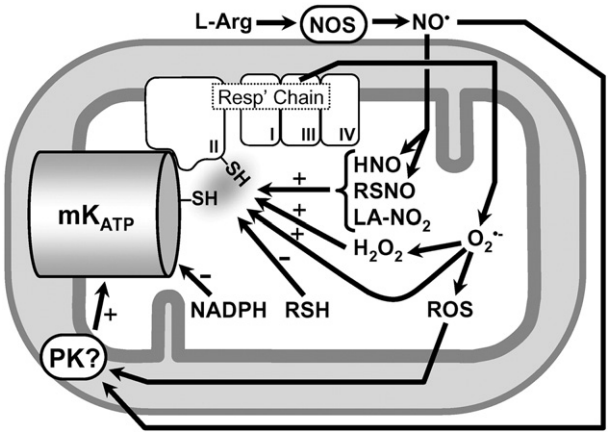

Fig. 6. Schematic showing redox regulation of mK$_{ATP}$. Nitroxyl (HNO), RSNO, and LA-NO$_2$ activate the channel, possibly via thiols on the channel itself or on complex II of the respiratory chain. The ability of low molecular weight thiols (RSH) to inhibit the channel may also be mediated via thiols on the channel or on complex II. In contrast, the effects of NADPH are likely no mediated via thiols. The ability of NO$^•$ to activate the channel may be mediated via the generation of secondary RNS (e.g. RSNO, LA-NO$_2$, HNO), which can activate the channel via PKG-independent mechanisms, or via classical NO$^•$ protein kinase signaling. ROS (in particular H$_2$O$_2$) can also activate the channel, via mechanisms that may include thiol modification or protein kinase signaling. The nature of the interaction between complex II and the subunits of the mK$_{ATP}$ channel itself remains to be elucidated.

4. Conclusions

In summary, a variety of redox active species, including ROS, RNS, antioxidants, and reductants, all act on the mK$_{ATP}$. While a broad conclusion of this work can be summarized as "oxidants activate, reductants inhibit", it is apparent that many species do not conform to this simple model. Key examples include NADPH, which may regulate the channel via direct binding, and LA-NO$_2$ and nitroxyl, which are thought to mediate their effects via complex II. A summary of the potential mechanisms of mK$_{ATP}$ channel modulation by various species is shown in Fig. 6. Clearly, further work in this area, including the molecular identification of the mK$_{ATP}$ itself, and the redox-sensitive residues within it, will facilitate a better understanding of the role that channel regulation by redox plays in events such as IPC.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bbamcr.2010.11.005.

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