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The role of mitochondrial K_{ATP} channels in cardioprotection

Introduction

Ischemic preconditioning (IPC) is a phenomenon whereby brief periods of prior ischemia protect the myocardium against subsequent lethal ischemia (35). Although we do not yet understand the cellular mechanisms responsible for IPC, compelling evidence suggest that ATP-sensitive K^+ (K_{ATP}) channels are central players in this process. Gross and Auchampach (18) first reported that K_{ATP} channel blockers abolish, and K_{ATP} channel openers mimic, the protection afforded by IPC in dogs. Subsequently, a number of studies in various species, including humans, confirmed the idea that K_{ATP} channels play a key role in IPC (17). The cardioprotective effects were initially attributed to K_{ATP} channels in plasma membrane (surfaceK_{ATP} channels) (36). Opening of surfaceK_{ATP} channels shortens the action potential, thereby attenuating energy consumption and calcium overload. However, several studies indicate that abbreviation of action potential duration as a result of surfaceK_{ATP} channel opening may not be necessary for IPC (19, 28, 49, 51). Instead, K_{ATP} channels in the mitochondrial inner membrane (mitoK_{ATP} channels) have emerged as effectors of cardioprotection (12, 31). This brief review outlines the current understanding of the involvement of mitoK_{ATP} channels in ischemic cardioprotection.

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Pharmacology of mitoK_{ATP} channels

In 1991, Inoue et al. (24) first identified the mitoK_{ATP} channel in patch-clamp single channel recordings of the inner membrane of rat liver mitochondria. The molecular identity of cardiac mitoK_{ATP} channels remains unclear, although cardiac surfaceK_{ATP} channels have been molecularly defined as an octameric complex of four pore-forming Kir6.2 subunits and four SUR2A sulfonylurea receptors (6, 23). The mitoK_{ATP} channels share similar properties to surfaceK_{ATP} channels, notably modulation by adenine nucleotides and blockage by the antidiabetic sulfonylurea glibenclamide (24). However, the emerging evidence indicates that the pharmacological profile of mitoK_{ATP} channels is distinct from that of surfaceK_{ATP} channel. Garlid's laboratory demonstrated that diazoxide opens mitoK_{ATP} channels > 2000-fold more potently than surfaceK_{ATP} channels, using reconstituted mitochondrial vesicles or isolated mitochondria in heart (13, 38). In a complementary approach to assay the function of mitoK_{ATP} channels, Liu et al. (31) measured flavoprotein fluorescence in rabbit ventricular cells, with simultaneous measurement of surfaceK_{ATP} channel current. They found that diazoxide reversibly increased only flavoprotein oxidation without affecting surfaceK_{ATP} channel currents: diazoxide targets only mitoK_{ATP} channels in intact heart cells. Unlike diazoxide, exposure to pinacidil increased both flavoprotein oxidation and membrane current, indicating that pinacidil targets both mitoK_{ATP} and surfaceK_{ATP} channels. Furthermore, nicorandil, an orally efficacious antianginal drug, primarily activates mitoK_{ATP} channels; a 10-fold higher concentration recruits both surfaceK_{ATP} and mitoK_{ATP} channels (43).

Glibenclamide is not a useful probe of mitoK_{ATP}-induced mitochondrial oxidation, because the drug uncouples respiration from ATP synthesis and independently oxidizes mitochondria (22, 47). Liu et al. (31) reported that diazoxide-induced flavoprotein oxidation is inhibited by 5-hydroxyde-

Table 1 Effects of mitoK$_{ATP}$ and surfaceK$_{ATP}$ channel-selective agents on early and delayed phase of IPC

Drugs	Species	Phase	Effects	References
Diazoxide (mitoK$_{ATP}$ channel opener)	Rat (Langendorff)	Early	Contractile function improved	Garlid et al. (12)
	Rat (in vivo)	Early	Infarct size reduced	Fryer et al. (10)
	Rat (in vivo)	Delayed	Infarct size reduced	Takashi et al. (48)
	Rabbit (in vitro)	Early	Osmotic fragility improved	Liu et al. (31) Sato et al. (45)
	Rabbit (Langendorff)	Early	Infarct size reduced	Miura et al. (34)
	Rabbit (in vivo)	Early	Infarct size reduced	Baines et al. (2) Ockaili et al. (37)
	Rabbit (in vivo)	Delayed	Infarct size reduced	Ockaili et al. (37)
	Human (in vitro)	Early	Creatine kinase release and tissue viability improved	Ghosh et al. (14)
5-Hydroxydecanoate (mitoK$_{ATP}$ channel blocker)	Rat (Langendorff)	Early	Improved contractile function by diazoxide abolished	Garlid et al. (12)
	Rat (in vivo)	Early	Infarct size-limiting effect by IPC or by diazoxide abolished	Schultz et al. (46) Fryer et al. (10)
	Rat (in vivo)	Delayed	Infarct size-limiting effect by opioid (TAN-67) abolished	Fryer et al. (11)
	Rabbit (in vitro)	Early	Improved osmotic fragility by diazoxide abolished	Liu et al. (31)
	Rabbit (Langendorff)	Early	Infarct size-limiting effect by diazoxide abolished	Miura et al. (34)
	Rabbit (in vivo)	Early	Infarct size-limiting effect by IPC or by diazoxide abolished	Hide et al. (20) Baines et al. (2) Ockaili et al. (37)
	Rabbit (in vivo)	Delayed	Infarct size-limiting effect by diazoxide abolished	Ockaili et al. (37)
	Rabbit (in vivo)	Delayed	Infarct size-limiting effect by IPC abolished	Bernado et al. (4)
	Dog (in vivo)	Early	Infarct size-limiting effect by IPC abolished	Auchampach et al. (1)
	Chick embryo (in vitro)	Early	Improved cellular injury by opioid (morphine) abolished	Liang and Gross (29)
	Human (in vitro)	Early	Improved CK release and tissue viability by IPC abolished	Ghosh et al. (14)
HMR 1098 (surfaceK$_{ATP}$ channel blocker)	Rat (in vivo)	Early	Infarct size-limiting effect by IPC intact	Fryer et al. (10)
	Rabbit (in vitro)	Early	Improved osmotic fragility by IPC intact	Sato et al. (45)
	Rabbit (in vivo)	Early	Infarct size-limiting effect by IPC intact	Lung et al. (32)
	Human (in vitro)	Early	Improved CK release and tissue viability by IPC intact	Ghosh et al. (14)

CK creatine kinase, *IPC* ischemic preconditioning

canoate (5HD). Moreover, in the presence of 5HD, pinacidil failed to increase flavoprotein oxidation, whereas surfaceK$_{ATP}$ channel current turned on without impediment (42). These studies established that 5HD selectively inhibits mitoK$_{ATP}$ channels without affecting surfaceK$_{ATP}$ channels. This notion was further supported by the fact that 5HD could not inhibit the cardiac surfaceK$_{ATP}$ channel reconstituted by coexpression of Kir6.2 and SUR2A in HEK293 cells (22).

MitoK$_{ATP}$ channel and cardioprotection

The studies to address the role of mitoK$_{ATP}$ channels were facilitated by application of mitoK$_{ATP}$ channel-selective agents. The experimental studies listed in Table 1 clearly demonstrate the protective effect of diazoxide and antagonistic effect of 5HD in IPC. The mitoK$_{ATP}$ channel opener diazoxide protects rabbit ventricular cells in a pelleting model of ischemia (31, 45), improves functional recovery in isolated rat hearts subjected to ischemia/reperfusion (12), and reduces infarct size in rat (10) and rabbit hearts (2, 34, 37). Conversely, the selective mitoK$_{ATP}$ channel blocker 5HD prevents the cardioprotective effects of diazoxide (2, 12, 31, 34, 37), and can block genuine IPC (1, 10, 20, 46).

IPC occurs in a biphasic pattern of myocardial protection, an early phase (classic IPC), which develops immediately and lasts approximately two hours after the IPC stimulus, and a delayed phase (late IPC or second window of protection), which reappears after 24 hours and lasts at least 72 hours (27, 33). The underlying pathophysiology and mechanisms between early and delayed phases of cardioprotection are likely to differ. Nevertheless, previous studies with 5HD suggest that the mitoK$_{ATP}$ channel appears to feature prominently in both phases of protection. Bernardo et al. (4) have reported that 5HD abolishes late IPC in the rabbit heart. Fryer et al. (11) also found that 5HD abolished opioid-induced delayed protection in the rat heart. More recent studies provide direct

evidence that the mitoK$_{ATP}$ channel opener diazoxide mimics late IPC and reduces infarct size after 24 hours in rat (48) and rabbit hearts (37). Thus, mitoK$_{ATP}$ channels may be the site of action responsible for the cardioprotective effect of both classic and late IPC.

To clinch the idea that mitoK$_{ATP}$ rather than surfaceK$_{ATP}$ channels are involved in cardioprotection, surfaceK$_{ATP}$ channel-selective agents are desirable. HMR1098 is a novel sulfonylurea which inhibits K$_{ATP}$ channels in cardiac cells with 50-fold higher potency than in pancreatic β-cells (15). We confirmed that HMR1098 inhibited surfaceK$_{ATP}$ channel activated by exposure to 2,4-dinitrophenol (45). Conversely, HMR1098 did not affect the diazoxide-induced flavoprotein oxidation (45), indicating that HMR1098 targets only surfaceK$_{ATP}$ channels without suppressing mitoK$_{ATP}$ channels. Studies in a pelleting model of simulated ischemia have revealed that HMR1098 does not prevent the cardioprotection afforded by IPC and by diazoxide (45). In addition, we have succeeded in identifying the selective surfaceK$_{ATP}$ channel opener P-1075, and found that this compound could not protect myocytes subjected to simulated ischemia (45). Other studies in vivo clearly divorce the surfaceK$_{ATP}$ channels from IPC; HMR1098 did not abolish the cardioprotection afforded by IPC in rat (10) and rabbit hearts (32). Moreover, Ghosh et al. (14) recently demonstrated that the protective effect of IPC in isolated human right atrium was not abolished by HMR1098.

Signaling in IPC and the mitoK$_{ATP}$ channel

A number of signaling pathways have been proposed to be involved in mediating the cardioprotective effect of IPC. It is well known that G-protein coupled receptors, such as adenosine (A_1, A_3), bradykinin (B_2) and opioid (δ_1), constitute the trigger of IPC, and downstream protein kinase C (PKC) plays a key role in the induction and maintenance of IPC (9). If mitoK$_{ATP}$ channels are the dominant effectors of IPC, mitoK$_{ATP}$ channels should be linked to these mediators of IPC. Sato et al. (42) first addressed the links between PKC and mitoK$_{ATP}$ channels. Phorbol 12-myristate 13-acetate (PMA), an activator of PKC, had no effect on flavoprotein fluorescence by itself but potentiated and accelerated the diazoxide-induced opening of mitoK$_{ATP}$ channels. These effects of PMA were blocked by 5HD, and the inactive control compound 4α-phorbol did not alter the effect of diazoxide. A more recent study from the Marbán laboratory has also demonstrated that adenosine potentiates the oxidative effects of diazoxide and abbreviates the latency to mitoK$_{ATP}$ channel activation on application of diazoxide (44). These effects of adenosine were prevented by the A_1-receptor agonist 8-(*p*-sulfophenyl)-theophyline and the PKC inhibitor polymyxin B. Therefore, the adenosine-PKC sequence is linked to mitoK$_{ATP}$ channels. Furthermore, the results from Gross's laboratory that 5HD abolished the protective effects of the opioid receptor agonists of morphine and TAN-67 reveal that mitoK$_{ATP}$ channel activation is involved in opioid-induced cardioprotection (11, 29).

MitoK$_{ATP}$ channels are located downstream of PKC. Indeed, Miura et al. (34) reported that the PKC inhibitor calphostin C abolished the infarct size-limiting effects afforded by the A_1-receptor agonist R-phenylisopropyladenosine but not by diazoxide. PKC-isozyme translocation occurs during IPC. Wang and Ashraf (50) recently reported that PKC-δ is translocated to mitochondria in rat myocytes. However, in another study, PKC-ε but not PKC-δ has been argued to be responsible for IPC in rabbit cardiomyocytes (30). In connection with this issue, it also remains unclear whether PKC directly activates mitoK$_{ATP}$ channels or does so indirectly through a downstream tyrosine kinase-mediated pathway. Protein tyrosine kinase is reported to be downstream of PKC for early and late IPC (3, 39). Further studies are necessary to determine which PKC isozymes and what other kinases might be responsible for the activation of mitoK$_{ATP}$ channels.

Bolli et a1. (5) have addressed a possible role for nitric oxide (NO) in mediating late IPC. Later on, they showed that protein tyrosine kinase signaling is essential for the augmentation of inducible NO synthase (iNOS) activity during the late phase of IPC, indicating that iNOS is involved as a downstream element of protein tyrosine kinase (7). Sasaki et al. (41) reported a link between NO and mitoK$_{ATP}$ channels, in which they demonstrated that exposure of myocytes to an NO donor directly activates mitoK$_{ATP}$ channels as well as potentiates the ability of diazoxide to open these channels. These findings, taken together, provide tangible links between various key elements in the IPC cascade, and implicate mitoK$_{ATP}$ channels as the effectors of both early and late IPC.

Mechanism of cardioprotection

Despite extensive pharmacological evidence that mitoK$_{ATP}$ channels are crucial for IPC, the question remains as to how the opening of mitoK$_{ATP}$ channels might protect myocytes against ischemic damage. It has been proposed that inner membrane depolarization produced by the increased K^+ conductance may reduce mitochondrial Ca^{2+} entry through the calcium uniport, which blunts mitochondrial Ca^{2+} overload. Consistent with this hypothesis, Holmuhamedov et al. (21) have demonstrated that mitoK$_{ATP}$ channel openers release Ca^{2+} from Ca^{2+}-loaded mitochondria. Other possibilities are that "mild uncoupling" and oxidation of flavoproteins induced by diazoxide will lower free radical production in mitochondria, and changes of mitochondrial membrane potential could alter glycolytic pathways during ischemia in favor of myocyte survival. However, these hypotheses have not yet been addressed.

Takashi et al. (48) recently proposed the attractive hypothesis that the activation of mitoK_{ATP} channels is antiapoptotic. They demonstrated that diazoxide decreased the TUNEL-positive cells in the border zone of infarct myocardium, an effect which was antagonized by 5HD. However, it has been pointed out that the validity of the TUNEL assay as a method to detect apoptosis has been questioned (26). Holmuhamedov et al. (21) reported that, in isolated cardiac mitochondria, mitoK_{ATP} channel opening by cromakalim and pinacidil increased matrix volume and released cytochrome c, which may counteract the postulated beneficial action of mitoK_{ATP} channel. Perhaps crucial aspects of the apoptotic signaling pathways are disrupted in the process of mitochondrial isolation. These disparate results need to be reconciled by complementary studies on intact cells. Although the cardioprotection by IPC cannot be explained solely by apoptosis inhibition, it has been reported that IPC reduces ischemic injury by decreasing apoptosis (16, 40). The role of mitoK_{ATP} channels in myocyte apoptosis merits further scrutiny.

Conclusion and future perspectives

Evidence is rapidly accumulating that the mitoK_{ATP} channel may be responsible for cardioprotection. However, most of the results in support of the mitoK_{ATP} channel hypothesis were obtained by the application of pharmacological tools. D'hahan et al. (8) recently reported that diazoxide activates cardiac SUR2A/Kir6.2 currents in the presence of ADP, suggesting diazoxide may open not only mitoK_{ATP} channels but also surfaceK_{ATP} channels in ischemic myocardium. Nevertheless, it is apparent that diazoxide can protect myocardium independently of action potential abbreviation (31, 45). More recently, Jovanovic et al. (25) demonstrated that transfected COS-7 cells with surfaceK_{ATP} channel subunits (SUR2A and Kir6.2) exert tolerance against hypoxic injury. This study directly supports the idea that action potential abbreviation is not necessary for cardioprotection. In addition, an important question arises: what is the action potential-independent mechanism of surfaceK_{ATP} channel for cardioprotection? Future studies of mitoK_{ATP} channels are essential in elucidating just how activation of mitoK_{ATP} channels protects against lethal ischemic injury. In addition, possible crosstalk between surfaceK_{ATP} and mitoK_{ATP} channels should be evaluated.

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