Role of energy metabolism in the preconditioned heart — a possible contribution of mitochondria

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Abstract

A brief period of ischemia and reperfusion has been shown to protect the myocardium against subsequent sustained ischemia and reperfusion injury, which is called "preconditioning". A great number of investigators have explored the mechanisms underlying this preconditioning-induced cardioprotection. This article dealt with possible mechanisms of energy metabolism and mitochondrial activity for preconditioning-induced cardioprotection. Particularly, the contribution of energy metabolites produced during a brief period of ischemia and reperfusion injury, as well as mitochondrial function that is modified by changes in mitochondrial ATPase activity, opening of mitochondrial ATP-dependent potassium channels and production of free radicals in mitochondria, to ischemic preconditioning is discussed.

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1. Introduction

A brief period of reversible ischemia before sustained ischemia and reperfusion causes cardioprotection against subsequent ischemia/reperfusion injury, as first reported by Murry et al. [1]. This tolerance to ischemia is termed "ischemic preconditioning". The mechanisms of ischemic preconditioning are of great concern for cardiovascular researchers and clinicians. The period required for preconditioning effects is so short that the effects were at first thought to be attributable to physiological events rather than to pathological ones. Despite extensive investigations in the past decade, the mechanisms responsible for ischemic preconditioning still remain elusive. Preconditioning effects are produced in animals of different species and humans, and thus a variety of the mechanisms have been proposed in different animals. On account of the species differences, it appears to be impossible to define a universal mechanism for all the events afforded by ischemic preconditioning. Recently, three aspects of the process of ischemic preconditioning have been distinguished: the initial triggers, activation of signal transduction and/or biochemical alterations (the mediators), and action of the end-effectors [2,3]. Several G protein-coupled receptor agonists such as adenosine [4–7], bradykinin [8,9], angiotensin II [10], norepinephrine [11,12], opioids [13] and endothelin-1 [14,15] have been proposed as the initial triggers. The stimulation of the respective receptors of these agonists leads to activation of signal transduction for ischemic preconditioning. Activation of protein kinase C (PKC) is, in most cases, observed in preconditioned hearts of a variety of animals such as dogs, rabbits, and rats [16–21]. Therefore, the signal transduction relating to activation of PKC is expected to be a part of mechanisms responsible for ischemic preconditioning. In contrast, some reports failed to demonstrate the association of activation of PKC with ischemic preconditioning [22,23]. Furthermore, there is still no concrete evidence for the presence and role of proteins that are phosphorylated by PKC. Recently, roles of tyrosine kinase [24] and p38 mitogen-activated protein kinase [25–27] in ischemic preconditioning have been proposed. Despite such proposals, the role

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of PKC activation in the ischemic preconditioning should still be discussed further. The manifestation of action of the end-effectors includes reduction or attenuation of the development of myocardial infarct area, posts ischemic cardiac dysfunction and incidence of arrhythmias. The latter two events have been shown to be undetectable in some cases [1,28–30]. On the contrary, preconditioning-induced reduction in myocardial infarct size is generally detected as a manifestation of the end-effector in most of the studies.

The role of energy metabolism in preconditioned hearts has been extensively studied from the early stage of the discovery of ischemic preconditioning [31,32]. Several possible mechanisms of energy metabolite contribution to preconditioning effects have been proposed, including reduction of acidosis [33–35], maintenance or facilitation of glycolytic flux rate [36,37], contribution of adenosine [4,5,7] and preservation of high-energy phosphates [31,38]. It still remains inconclusive which mechanism(s) plays a key role in preconditioning effects. Recently, several factors relating to cardiac mitochondria have been focused on, including energy-producing ability in mitochondria [37], mitochondrial ATPase [39–41], mitochondrial ATP-sensitive K⁺-channels [42–44], and contribution of oxygen free radicals generated in mitochondria [45,46]. In this article, recent progress in the field dealing with cardiac energy metabolism, particularly the mitochondrial function, as triggers and/or mediators for ischemic preconditioning is described.

2. Role of energy metabolites in preconditioning hearts

Since ischemic preconditioning is produced by a transient ischemia, it is conceivable that some energy metabolites produced during ischemic preconditioning would play a role in the cardioprotection. In this respect, H⁺, glycolytic intermediates, and adenosine that are produced during ischemia would be plausible mediators of ischemic preconditioning.

2.1. Acidosis

It is well recognized that H⁺ is formed under ischemic conditions predominantly by glycolytic ATP turnover, CO₂ accumulation and net ATP breakdown [47]. Acidosis plays a critical role in cardiac contractile function [48], as a result of causing decreased sensitivity of myofibrils to calcium [49] and decreased function of the cardiac sarcoplasmic reticulum [50], and/or the following disturbance in the intracellular sodium and calcium homeostasis maintained by ionic pumps and Na⁺/H⁺ and Na⁺/Ca²⁺ exchangers [51,52]. That preconditioning reduced acidosis during sustained ischemia is universally documented in the reports on preconditioning. The NMR study gives us real-time information concerning energy metabolites in the intracellular milieu of ischemic/reperfused hearts. Several NMR studies on perfused rat hearts have shown that preconditioning reduced acidification during the prolonged ischemia, which was associated with improvement of posts ischemic contractile function [35,53–56]. Preconditioning seems to delay the onset of acidosis in the ischemic heart [57]. Moreover, there is a good relationship between suppression of the fall in intracellular pH and recovery of posts ischemic contractile function in rat hearts [35]. However, sustained ischemia-induced acidosis is not completely prevented by preconditioning. Such partial reduction of acidosis may be, in some sense, suitable to exert beneficial effects on the ischemic/reperfused heart, since mild acidosis has been shown to protect the myocardium against ischemia/reperfusion injury [58,59].

Glycogen, irrespective of the benefit to produce glucose under anaerobic conditions, can produce metabolites detrimental to biochemical and physiological events during ischemia in cardiac cells, including H⁺ [47] and lactate [60]. Acidosis obviously aggravates cardiac function and metabolism during ischemia and reperfusion as described above. Glycogen depletion as a mechanism of ischemic preconditioning is an attractive hypothesis, although it is considered to be deleterious for the heart due to depletion of energy sources [25]. In advance of the first report of preconditioning by Murry et al. [1], Neely and Grottyohann [61] showed that depletion of glycogen induced by anoxic perfusion was beneficial for hearts subjected to subsequent ischemic insult. This sequence is exactly comparable to ischemic preconditioning. Preconditioning elicited a reduction in glycogen content in the preischemic heart, attenuated the accumulation of harmful glycolytic metabolites such as H⁺ and lactate during sustained ischemia, and enhanced the recovery of posts ischemic contractile function during the following ischemia/reperfusion [33]. Furthermore, there are reports demonstrating the relationship between preconditioning-induced cardioprotection and myocardial glycogen content [34,62]. Preconditioning-induced depletion of glycogen results in less acidosis in the ischemic myocardium, leading to prevention of ionic disturbance induced by the acidosis-induced activation of Na⁺/H⁺ exchange and the following increase in Na⁺/Ca²⁺ exchange [53,54]. These sequences may eventually lead to prevention of impairments of cell membrane integrity and ionic disturbance such as massive calcium overload in the reperfused myocardium.

The significant role of glycogen depletion in cardioprotection is also supported by the finding that transient β-adrenoceptor stimulation with norepinephrine or isoproterenol elicited preconditioning-like cardioprotection associated with glycogen depletion [63,64]. Thus, attenuation of acidosis caused by glycogen depletion is one of the plausible mechanisms for the benefit to cardiac function and energy metabolism in preconditioned hearts. However, several reports [25,55,65] have cautioned that although
glycogen depletion limited the fall in pH during ischemia, glycogen depletion alone did not protect the heart against ischemic injury. Other factors such as activation of PKC may be involved in the preconditioning effects. In accord with this, it is considered that the effect of preconditioning on the fall in intracellular pH during sustained ischemia appeared to be independent of activation of PKC [56].

Acidosis or low pH is known to decrease mitochondrial ATPase activity [66,67] and oxidative phosphorylation [68]. However, the relationship between acidosis and mitochondrial function in preconditioned hearts has not been elucidated yet.

2.2. Glycolytic flux

A brief period of ischemia [32] or hypoxic perfusion [69] increases anaerobic glycolytic flux, a Pasteur effect. This may lead to increased production of ATP in the glycolytic pathway, although a marked reduction in ATP production in the mitochondria occurs due to lack of oxygen. It is generally admitted that glycolytically derived ATP is of particular importance in the preservation of ionic pump function and thereby membrane integrity whereas ATP from oxidative phosphorylation preferentially supports contractile function [70]. Thus, enhancement of glycolytical production of ATP may serve preferentially as an energy source for ionic pump activity during sustained ischemia, leading to maintenance of ionic homeostasis and eventually to cardioprotection. Contradictorily, the increased anaerobic glycolysis accumulates tissue H⁺ and lactate, resulting in myocardial acidosis and cell injury. Ischemic contracture that occurs when glycolytic ATP production is abolished [71–73] develops earlier in the preconditioned heart than in the non preconditioned one [33,74–76], probably due to faster depletion of myocardial glycogen. This finding suggests that preconditioning decreases, but does not enhance, anaerobic glycolytic flux during ischemia and that this may confer a benefit to the heart.

Phosphofructokinase (PFK) is a rate-limiting enzyme of the glycolytic pathway. A certain period of acidosis is known to inhibit PFK activity [77,78]. There is a report showing that ischemic preconditioning attenuated ischemia- as well as reperfusion-induced reduction in the ratio of [fructose 1,6-biphosphate]/([glucose 6-phosphate]+[fructose 6-phosphate]), a suggestive marker of PFK activity, without any alteration in glycolytic metabolites positioned in the downstream of the Embden–Mayerhof pathway in association with a better recovery of posts ischemic cardiac function [37]. The findings suggest that preconditioning-induced attenuation of acidosis as a result of glycogen depletion may prevent impairment of PFK per se and/or its activity during sustained ischemia. Thus, immediately upon reperfusion, glycolytic flow may be restored, resulting in an enhancement of glycolytic ATP production.

2.3. Preservation of myocardial energy during ischemia

Murry et al. [31] have shown that although ATP was decreased during preconditioning itself, myocardial ATP levels were higher after 10 min of subsequent sustained ischemia in preconditioned dogs than in non preconditioned ones. This higher level of high-energy phosphates of preconditioned hearts was considered to be due to slowing of the rate of ATP consumption during ischemia [79]. Slowing of the rate of ATP consumption of preconditioned hearts during sustained ischemia was also detected by an NMR study in pigs [80]. Similarly, preservation of creatine phosphate during sustained ischemia was seen in preconditioned pig hearts in another NMR study [80]. Thus, one of the proposed mechanisms underlying ischemic preconditioning is preservation of myocardial high-energy phosphates during a certain period of ischemia. Generally, ATP may be consumed under physiological conditions for muscular contraction, ionic pump function, and metabolic consequences. Particularly, 60–70% of myocardial high-energy phosphates is utilized for muscular contraction [81,82]. Ischemic hearts, irrespective of preconditioning or non preconditioning, revealed a poor contractile activity as a result of severe acidosis. Since ischemic hearts do not require so much energy for contraction, there may be no difference in consumption of ATP during sustained ischemia between preconditioned and non preconditioned hearts. In fact, ATP levels later in ischemia became similar in both preconditioned and non preconditioned hearts [31,34,35,54]. Several reports have shown that preconditioning, irrespective of ATP levels after sustained ischemia, enhanced recovery of the posts ischemic contractile function [34,37,39,53,54]. Furthermore, studies in perfused rat hearts have shown diverse results: preconditioning slows [35,36,40,83,84], accelerates [33,41,55,57], or does not affect [85,86] the rate of myocardial ATP consumption during prolonged ischemia. These conflicting results appear to be due to differences in preconditioning procedure used. That is, when a brief period of ischemia was performed one or two times, myocardial ATP levels after preconditioning still remained considerable. In such a case, the rate of myocardial ATP consumption during the following prolonged ischemia was slowed [36,40,83,84]. In contrast, when a brief period of ischemia was repeated several times, myocardial ATP levels during subsequent sustained ischemia were reduced to a greater degree. In this case, the decline of myocardial ATP levels was not detected [41,55]. Irrespective of the amount of ATP that remained after preconditioning, posts ischemic functional recovery was observed in these studies. Thus, the decline of myocardial ATP levels during sustained ischemia appears to be independent of the occurrence of preconditioning effects.

In addition, several reports have shown that ischemic contracture, a marker of glycolytic ATP depletion of perfused rat hearts, occurred earlier in the preconditioned
heart than in the nonpreconditioned ones, as described previously. Since ischemic contracture is considered to represent rigor formation of myofibrils, this finding implies that depletion of glycolytically derived ATP in preconditioned hearts may precede that in nonpreconditioned hearts. Taken together, the data indicate that higher levels of myocardial energy store during ischemia are not always accompanied by cardioprotection in preconditioned hearts.

2.4. Adenosine

It is commonly admitted that ischemia induces an abrupt release of adenosine from the myocardium. Adenosine is one of the plausible mediators of ischemic preconditioning [4–7]. Adenosine is considered to exert its effects by stimulating A<sub>1</sub> and/or A<sub>2</sub> receptors [4,87]. An adenosine A<sub>1</sub> receptor agonist is known to decrease the activity of adenylate cyclase and thereby to antagonize the action of catecholamines [88,89]. Thus, it is conceivable that adenosine may reduce the intracellular calcium concentration under conditions of catecholamine stimulation caused by a brief period of ischemia. Vander Heide et al. [90] suggested that adenosine acting via an A<sub>1</sub> receptor slows ischemic metabolism and mimics preconditioning via an inhibition of norepinephrine release from synaptic nerve during ischemia in dog hearts. A contribution of A<sub>1</sub> receptor stimulation to the preconditioning effects was reported in dog and rabbit hearts [4,91], but not in rat hearts [76,92,93]. It is also proposed that activation of the adenosine A<sub>1</sub> receptor coupled with inhibitory GTP-binding proteins (Gi proteins) [94] stimulates sarcolemmal K<sub>ATP</sub> channels, resulting in shortening of action potential duration during ischemia [6]. This is followed by the blockade of Ca<sup>2+</sup> influx through voltage-gated calcium channels, and thus leads to cardioprotection. Furthermore, it has been shown that preconditioning-induced cardioprotection and increase in the intracellular calcium concentration were abolished by adenosine antagonists that have the ability to increase glucose uptake, but not by an adenosine antagonist that lacks this ability [95]. Considering these complicated results, further examination is required to conclude the mechanism of contribution of adenosine to ischemic preconditioning.

3. Mitochondrial function in preconditioned hearts

3.1. Preservation of mitochondrial function

Alterations in mitochondrial ATP synthesis, including qualitative changes in ATP synthetase, are recognized to occur at the initial phase of ischemia [96]. Ischemia induces a decrease in the activities of ADP-supported state 3 respiration by inhibiting complex I, NADH–CoQ reductase system, and complex V, a mitochondrial ATPase [97]. Ischemia also induces a decrease in the amount of the atracyloside-inhibitable ADP/ATP translocator across the mitochondrial inner membrane [98,99]. It has been shown that preconditioning enhanced recovery of posts ischemic contractile function and effectively preserved the mitochondrial oxygen consumption rate (Fig. 1) with paralleled preservation of the atracyloside-inhibitable ADP/ATP translocator during sustained ischemia [37]. This observation suggests the importance of the preservation of mitochondrial function for preconditioning-induced improvement of posts ischemic cardiac function. The exact mechanism for the preservation of mitochondrial function is unknown at present.

3.2. Inhibition of F<sub>1</sub>F<sub>0</sub>-ATPase

Mitochondrial ATP synthetase is a macromolecular complex of proteins comprising the F<sub>1</sub>–F<sub>0</sub> particles of mitochondria [100,101]. This enzyme has a unique biochemical profile. Under normoxic conditions, this ATP synthetase phosphorylates ADP with the driving force of electrochemical proton gradients across the mitochondrial inner membrane, and thus this enzyme is considered to play an important role in ATP synthesis in mitochondria. In contrast, under ischemic conditions where the mitochondrial membrane potential falls, this enzyme catalyzes the reverse reaction from ATP to ADP and serves as a major consumer of ATP, resulting in ATP wastage. On the basis of these properties, this enzyme is also called as mitochondrial F<sub>1</sub>F<sub>0</sub>-ATPase. Experimental observation showed that 35–50% of the overall high-energy phosphates in the myocardium were consumed by this ATPase during ischemia [102,103]. Thus, activation of this enzyme

![Fig. 1. Mitochondrial oxygen consumption capacity of saponin-treated bundles from preconditioned (■) and nonpreconditioned hearts (□) prior to ischemia (Basal), 40 min ischemia (I40), 5 min (R5) and 30 min (R30) of reperfusion. Values are the means±SEM of eight experiments.* Significantly different from the corresponding nonpreconditioned group and # significantly different from the basal value (p<0.05). (Reproduced from Ref. [37] with permission from Elsevier Science.)](https://academic.oup.com/cardiovascres/article-abstract/43/1/32/356302)
may be an undesirable consumer of ATP under ischemic conditions. Oligomycin, an inhibitor of the F, F, -ATPase, has been shown to attenuate the rate of ATP consumption during ischemia [67,102,104]. If preconditioning affects the F, F, -ATPase activity, it may play an important role in ATP preservation of the ischemic heart.

Vuorinen et al. [40] have shown that preconditioning of isolated rat hearts caused inhibition of the mitochondrial F, F, -ATPase, slowed the rate of decrease in myocardial ATP, and attenuated the development of tissue acidosis during subsequent prolonged ischemia. They suggested that the inhibition of the ATPase might account for sparing of high-energy phosphates and improvement of the time-averaged energy state of the preconditioned myocardium. This hypothesis focused on the role of mitochondrial F, F, -ATPase in the mechanism of ischemic preconditioning. In contrast, Vander Heide et al. [105] reported that preconditioning was not associated with inhibition of this mitochondrial ATPase activity in the canine myocardium. In addition, Green et al. [41] also failed to demonstrate inhibition of F, F, -ATPase in the preconditioned isolated rat hearts before or during prolonged ischemia. Rather, they observed that the preconditioning enhanced the rate of ATP consumption, suggesting that preconditioning accelerated ATP consumption. In this study, they also showed that preconditioning was associated with shortening of the onset of ischemic contracture, which is commonly seen during ischemia in the perfused heart as a result of glycolytic ATP depletion, as described above. Thus, they concluded that the mechanism for ischemic preconditioning was independent of the inhibition of the F, F, -ATPase activity.

In relation to this, there is evidence for the existence of ATPase inhibitor protein (IF, ) that inhibits the ATP hydrolysis activity of the F, F, -ATPase [106]. This hydrolysis inhibitor is a small protein with molecular weight of 9500 Da [107] found in the matrix space of the mitochondria. This protein binds to the ATPase and inhibits its energy-wasting activity under acidic conditions [108–110] and thereby might play a role in the inhibition of F, F, -ATPase in ischemia. Animals with a fast heart rate such as rats do not have very much of this inhibitory protein [109,111]. Nevertheless, cardioprotective effects of preconditioning on myocardial energy metabolism have been clearly established in rat hearts [35,37,40,53,54,83]. This suggests that although inhibition of the ATPase activity is a possible explanation of conservation of high-energy phosphates after sustained ischemia/reperfusion [40], the contribution of the F, F, -ATPase to cardioprotection of ischemic preconditioning in rat hearts is unlikely.

3.3. Activation of mitochondrial ATP-sensitive K+ channel

The presence of ATP-regulated potassium (K,ATP) channel in hearts was first demonstrated by Noma [112]. Opening of this channel is inhibited by not only ATP but also sulfonylurea derivatives such as glibenclamide and is accelerated by several K,ATP channel openers [113]. Potassium channel openers such as cromakalim, pinacidil and nicorandil improved postischemic recovery of cardiac function in isolated rat [114,115] and guinea-pig hearts [116]. Several reports have shown that infarct-limiting effects by preconditioning in dog hearts were abolished by the K,ATP channel blocker glibenclamide [117,118]. Thus, it is suggested that the preconditioning effect was attributable to opening of sarcolemmal K,ATP channels. It is generally believed that opening of sarcolemmal K,ATP channels results in an enhancement of membrane repolarization in cardiac cells and thereby shortening of action potential duration, leading to reduction in calcium entry into cardiac cells through voltage-gated calcium channels [6]. Such reduction in calcium entry may decrease cardiac work and prevent calcium-dependent pathological events. This notion tempted the conclusion of a definitive role of K,ATP channel opening in preconditioning effects against ischemic/reperfusion injury [6]. However, there is increasing evidence that physiologically and pharmacologically contradict this hypothesis. That is, cardioprotection afforded by K,ATP channel openers such as cromakalim and bimakalim was not accompanied by shortening of monophasic action potential duration [119,120]. Cardioprotection by K,ATP channel openers and ischemic preconditioning occurs even in unstimulated cardiomyocytes, in which action potential abbreviation cannot be observed [121,122]. Cancellation of preconditioning effects by the K,ATP channel blocker glibenclamide was not detected in rabbit hearts [123]. In isolated rat hearts, the K,ATP channel blocker did not abolish the preconditioning-induced protective effect [76,92,124,125], whereas it blocked the cardioprotective effects afforded by K,ATP channel openers [42,114]. Furthermore, ischemic preconditioning shortens the time to onset of contracture, a marker of ATP depletion as described above, in ischemic hearts whereas K,ATP channel openers prolong the time [126]. Thus, further study is needed concerning the relationship between preconditioning effects and activation of K,ATP channels.

There is evidence that K,ATP channels, in addition to being in the sarcolemma, are present in the inner membrane of mitochondria where the channels regulate mitochondrial volume and energetics [127,128]. This channel is blocked by the K,ATP channel blocker glibenclamide and by ATP [127]. This channel regulates electron transport in mitochondria and appears to be little dependent on pH [128]. Garlid et al. [42] have shown that K,ATP channel openers diazoxide and cromakalim at concentrations of 30 and 10 μM, respectively, exerted protective effects against ischemia/reperfusion injury in isolated perfused rat hearts (Fig. 2). The protective effects of these agents were abolished by K,ATP channel blockers such as glibenclamide [42]. Diazoxide and cromakalim at the concentrations exerting the cardioprotective effect as described above did
not affect, or affected only to a minor degree, sarcolemmal $K_{\text{ATP}}$ channels of isolated rat ventricular myocytes (Fig. 3). These results suggest that the cardioprotective effect of these drugs is attributed to opening of mitochondrial $K_{\text{ATP}}$ channels.

Opening of mitochondrial $K_{\text{ATP}}$ channels tends to dissipate the membrane potential established by the proton pump and thus accelerates electron transfer by the respiratory chain and leads to net oxidation in the mitochondrial matrix [129]. On the basis of this notion, Liu et al. [43] determined the mitochondrial oxidative activity of isolated rabbit cardiomyocytes. They found that diazoxide enhanced the mitochondrial oxidative activity, an index of opening of $K_{\text{ATP}}$ channels, without any change in $I_\text{c,ATP}$, an index of opening of sarcolemmal $K_{\text{ATP}}$ channels (Fig. 4). Diazoxide-induced increase in the mitochondrial oxidative activity was abolished by treatment with a mitochondrial $K_{\text{ATP}}$ channel blocker, 5-hydroxydecanoate (5-HD). They also showed that diazoxide used at the same concentration as for opening of mitochondrial $K_{\text{ATP}}$ channels elicited cardioprotection of isolated rabbit cardiomyocytes against simulated ischemia and that this effect was virtually cancelled by 5-HD (Fig. 5). These results appear to allow the conclusion that opening of mitochondrial $K_{\text{ATP}}$ channels may accelerate mitochondrial oxidative phosphorylation, which may lead to cardioprotection of quiescent cardiomyocytes, without any effect on sarcolemmal $K_{\text{ATP}}$ channels. Although the above results indicate a possible contribution of mitochondrial $K_{\text{ATP}}$ channels to cardioprotection against ischemia/reperfusion injury, it is so far unknown how opening of mitochondrial $K_{\text{ATP}}$ channels is achieved under ischemia/reperfusion conditions, particularly in ischemic preconditioned hearts. Furthermore, the link between opening of mitochondrial $K_{\text{ATP}}$ channels and protection of cardiomyocytes against ischemic damage remains unclear. That is, there is no evidence that opening of $K_{\text{ATP}}$ channels leads to either protection of mitochondrial energy producing ability or preservation of myocardial high-energy phosphates during ischemia. During ischemia, mitochondrial ATP production would be limited due to lack of oxygen, whereas during reperfusion $K_{\text{ATP}}$ channels would not be operative due to the increased amount of ATP produced [127]. Unless this discrepancy can be resolved, it would be impertinent to conclude that opening
of $K_{\text{ATP}}$ channels directly leads to enhancement of ATP production in the cardiac mitochondria under ischemic or reperfused conditions.

$K_{\text{ATP}}$ channel openers have been shown to preserve high-energy phosphates and inhibit accumulation of AMP in the ischemic myocardium [130,131]. As described above, $K_{\text{ATP}}$ channel openers prolonged the time to ischemic contracture, a marker of ATP depletion of perfused hearts [126,130]. These findings suggest that ATP breakdown is suppressed in cardiac cells when $K_{\text{ATP}}$ channels are activated. In addition, as described above, mitochondrial function of preconditioned hearts has been shown to be preserved at the end of sustained ischemia, which lasted during reperfusion [37]. This observation gives an idea that one of the beneficial roles for the $K_{\text{ATP}}$ channel, if it exerts cardioprotective effects in preconditioned hearts, would be attributable to preservation of mitochondrial function during ischemia. The exact link between opening of $K_{\text{ATP}}$ channels and preservation of mitochondrial function still remains unclear. Recently, Sato et al. [44] have shown that the mitochondrial matrix redox potential, an index of mitochondrial $K_{\text{ATP}}$ channel activity, was enhanced by a mitochondrial $K_{\text{ATP}}$ channel opener diazoxide, whose enhancement was cancelled by a mitochondrial $K_{\text{ATP}}$ channel blocker, 5-HD. The enhancement of the matrix redox potential was potentiated by the PKC activator phorbol 12-myristate 13-acetate [44]. On the basis of the findings they suggested a direct mechanistic link between the signal transduction of ischemic preconditioning and pharmacological cardioprotection targeted at $K_{\text{ATP}}$ channels.

### 3.4. Contribution of reactive oxygen species in mitochondria

It is generally admitted that reactive oxygen species can be generated in ischemic/reperfused hearts through the xanthine/xanthine oxidase reaction of purine metabolism in the cytosol [132], the activation of NADPH oxidase and myeloperoxidase of leukocytes in the circulatory system [133], and/or one-electron reduction process of the respiratory chain in mitochondria [134]. The oxygen radicals may induce a lipid peroxidation of the cellular as well as mitochondrial membranes, and thus their formation may result in reperfusion injury including cardiac cell necrosis. In accord with this concept, expression of the mRNA of several stress-related genes such as HSP70, Mn-SOD, catalase, and glutathione peroxide has been shown to increase in the preconditioned myocardium of rats [135]. Furthermore, it has been observed that preconditioning reduced the ability of mitochondria to produce oxygen radical species in posts ischemic hearts, which was associated with improvement of the respiratory function of mitochondria [136]. However, this preconditioning did not alter the activities of scavengers of reactive oxygen species, such as superoxide dismutase, catalase, and gluta-
thione peroxidase. Thus, the close relationship between free radical formation in mitochondria and protection of cardiac function in preconditioned hearts remains inconclusive.

Paradoxically, several reports have proposed a possible role of reactive oxygen species as a trigger of the initiation of preconditioning effects. Reactive oxygen species have been shown to be generated during a brief period of hypoxia or ischemia [137]. Thus, the produced reactive oxygen species may play a role in triggering cardioprotection. Murry et al. [138] have shown that free radical scavengers given to dogs prior to preconditioning caused attenuation of the response of preconditioning in 50% of the cases. Ambrosio et al. [139] proposed a role of low levels of oxygen radicals as potent mediators of preconditioning. Thus, low levels of free radicals might be beneficially involved in some way in the preconditioning of animals in vivo. Several studies have shown that exposure of cardiomyocytes, which can eliminate the contribution of the circulatory system, to superoxide or H₂O₂ caused preconditioning-like protection in hearts [45,140,141]. In addition, antioxidants abolished the induction of preconditioning in coronary artery occlusion/reperfusion rabbit and rat hearts [142,143]. Recently, a more interesting observation has appeared. Vanden Hoek et al. [46] have shown in chick embryonic ventricular myocytes that hypoxic preconditioning or exposure to H₂O₂ decreased subsequent hypoxia/reoxygenation-induced cell death. During hypoxic preconditioning, an increase in 2′,7′-dichlorofluorescein oxidation, a suggestive indicator of generation of H₂O₂ or hydroxy radicals, was also detected. The preconditioning elicited cardioprotection, which was blocked by a mitochondrial anion channel blocker, 4,4′-diisothiocyanato-stilbene-2,2′-disulfonate (Fig. 6). From these results, they concluded that hypoxia may increase the generation of mitochondrial superoxide and then enhance its release through the mitochondrial anion channel, resulting in the initiation of preconditioning cardioprotection. If observations similar to this finding can be detected in other animal species, this mechanism would be worth considering for cardioprotection by preconditioning.

4. Conclusion

In conclusion, several possible mechanisms responsible for ischemic preconditioning have been discussed in this article with respect to myocardial energy metabolism and mitochondrial function. Recent studies have progressively explored the role of mitochondrial function in preconditioning cardioprotection. Although energy metabolites have been taken as classic mediators of preconditioning, such substantial findings as referenced in this article provide us suggestive cues for the mechanisms of cardioprotection against ischemia and reperfusion injury.

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