札幌医学雑誌　**75**（4 － 6）19 ～ 28（2006）

INVITED REVIEW

Ischemic preconditioning, an endogenous protective mechanism in the cardiomyocyte, and its clinical application

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ABSTRACT

To develop novel strategies for protection of cardiomyocytes from ischemia/reperfusion injury, we have analyzed cytoprotective mechanisms of ischemic preconditioning (PC). PC, a form of adaptation, refers to enhancement of myocardial tolerance against ischemia/reperfusion-induced necrosis by exposing the myocardium to a brief episode of non-lethal ischemia. PC induces the generation of agonists of Gi/Gq protein-coupled receptors and release of TNF-α and HB-EGF. Stimulation of receptors of these agonists provokes activation of multiple signal pathways, including PKC-ε and PI3K/Akt pathways, which ultimately reach to the "end-effector" of cardiomyocyte protection. There are three possible effectors in PC, mitochondrial ATP-sensitive K^+ channel (mK_{ATP} channel), glycogen synthase kinase-3β (GSK-3β) and connexin-43 (Cx43). Activation of the mK_{ATP} channel attenuates mitochondrial dysfunction presumably by suppressing mitochondrial Ca^{2+} overload during ischemia/reperfusion. The level of phospho-(Ser9)-GSK-3β, a suppressor of mitochondrial permeability transition upon reperfusion, was found to be a determinant of infarct size *in vivo*. Accelerated closure of the gap junction after the onset of ischemia by PKC- and ERK-mediated Cx43 phosphorylation is suggested to be an adjunctive mechanism of protection, which suppresses propagation of myocyte injury within the area at risk. However, the cytoprotective signaling of PC can be impaired by concurrent diseases such as post-infarct ventricular remodeling. An opener of the mK_{ATP} channel is currently being used for myocardial protection, and selective GSK-3β inhibitors are candidates for novel therapy. Further characterization of cytoprotective signaling and effector molecules and elucidation of their modifications by co-morbidity are necessary for the development of clinically applicable agents.

(Received December 13, 2006)

Key words: Signal transduction, Cytoprotection, Ischemia, Reperfusion, Myocardial infarction

1 Introduction

Acute myocardial infarction (AMI) is a leading cause of death in industrialized countries. The major determinant of prognosis after AMI is size of infarct, and current therapy for reducing infarct size is restoration of blood flow in occluded coronary arteries by percutaneous coronary interventions and coronary bypass surgery. However, efficacy of these reperfusion therapies in terms of infarct size limitation strongly depends on the time interval between onset of coronary occlusion and restoration of coronary blood flow. Although coronary reperfusion within 3 hrs after the onset of AMI symptoms is a goal in recommendations, coronary reperfusion within such a short time-frame is currently achieved in less than 30% of patients. Thus, there are three approaches to reduce infarct size in AMI patients: 1) improvement in the medical communication/transport system to shorten the time interval between onset of AMI symptoms and arrival of patients to medical facilities, 2) development of instruments and agents that can achieve coronary reperfusion faster than the current ones, and 3) development of agents that can reduce infarct size independent of duration of myocardial ischemia.

In addition to duration of ischemia, level of coronary collateral blood flow is a major determinant of infarct size after coronary occlusion[1]. In contrast to the common belief, myocardial oxygen consumption in the ischemic myocardium is a minor factor influencing infarct size. Thus, rational approaches to infarct size limitation are enhancement of the development of coronary collateral arteries and augmentation of the myocardial tolerance against ischemia/reperfusion[1,2]. The second

approach was taken in this laboratory, and we have aimed to identify target molecules for myocyte protection by clarifying intracellular mechanisms of ischemic preconditioning (PC). PC refers to enhancement of myocardial tolerance against infarction afforded by exposing the myocardium to brief sublethal ischemia[3]. A number of critical signaling steps in PC have been identified in the past decade, and series of studies in this laboratory have indicated that there are at least three target molecules of myocyte protection. From these results, several agents are promising as agents for clinical use to attenuate ischemia/reperfusion injury. These agents include openers of the mitochondrial ATP-sensitive K^+ channel (mK_{ATP} channel) and inhibitors of glycogen synthase kinase-3β (GSK-3β). In this article, we provide a brief overview of our PC research and future perspectives of application of PC biology to clinical therapy.

2 Phenomenological Features of PC

In experimental animals, myocardial protection by PC is achieved by exposing the myocardium to an episode of 2～10 min of ischemia (i.e., PC ischemia)[3-6]. PC with a shorter period of ischemia is not cardioprotective, and a longer period of ischemia can in fact increase vulnerability to subsequent ischemic injury. Repetition of PC ischemia generally does not augment the infarct size-limiting effect. However, repetitive PC makes the myocardial protection more resistant to inhibitors of PC signaling, since the levels of signal inputs from the redundant receptors relevant to PC are increased (below)[4,7]. PC primarily delays progression of myocardial injury after the onset of ischemia, and thus reperfusion is necessary for PC to be protective against infarction. PC induces stunning in the preconditioned myocardium, but the stunning is neither a determinant of infarct size-limiting effect nor a requisite for development of protection of PC[8]. Anti-infarct tolerance induced by PC decays during the subsequent 1～2 hrs, though a second phase of anti-infarct tolerance (so-called "second window protection" or "late protection") reportedly re-appears from 24 hrs to 72 hrs.

3 Receptors Involved in Triggering Mechanisms of PC Protection

The PC mechanism is triggered by activation of Gi/Gq-coupled receptors (GPCR) and cytokine receptors in cardiomyocytes by their ligands generated or released in the myocardium subjected to PC ischemia (Fig. 1). Such ligands include adenosine, bradykinin, enkephalins and angiotensin II, and the importance of each ligand in PC differs depending on the protocol of PC (single vs. repetitive PC) and animal species[5,9-14]. Adenosine is the most extensively studied ligand as a trigger of PC[6]. Myocardial protection by PC is abolished by adenosine receptor blockade and mimicked by agonists of the adenosine A_1/A_3 receptor. By use of an *in vivo* microdialysis technique, interstitial adenosine level during PC was calculated to be approximately 1 micromolar in the rabbit heart[15,16]. Furthermore, inhibition of the nucleotide transporter by dipyridamole, dilazep, or R75231 significantly reduced the threshold of PC ischemia for triggering cardioprotective mechanisms[10,12,16]. On the other hand, inhi-

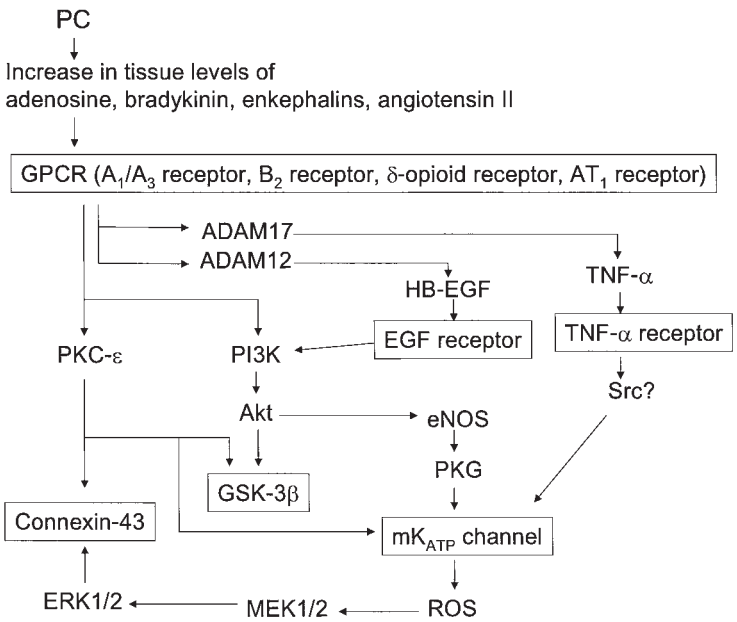

Fig. 1 Trigger mechanisms of PC.
PC = ischemic preconditioning, GPCR = G-protein-coupled receptor, ADAM=a disintegrin and metalloprotease, HB-EGF=heparin-binding EGF-like growth factor, TNF-α=tumor necrotic factor-α, PKC = protein kinase C, PI3K = phosphoinoside 3-kinase, PKG = protein kinase G, GSK-3β = glycogen synthase kinase-3β, mK_{ATP} channel = mitochondrial ATP-sensitive K^+ channel, ROS = reactive oxygen species

bition of ecto-5'-nucleotidase eliminated neither elevation of interstitial adenosine level during PC nor PC-induced infarct size limitation[17]. These results suggest that PC ischemia increases generation of adenosine by cytosolic 5'-nucleotidase within cardiomyocytes, resulting in elevation of interstitial adenosine level and activation of adenosine receptors to a level sufficient for triggering PC protection. Subtypes of adenosine receptors involved in PC are still controversial, but our findings indicate that the A_1 receptor is responsible for PC at least in rabbit hearts[5,6].

In addition to the adenosine receptor, the bradykinin B_2 receptor, the δ-opioid receptor and the angiotensin type 1 receptor participate in triggering PC-induced cytoprotection[11,13,14]. This redundancy in the GPCR as trigger mechanisms of PC is presumably a safety factor that assures development of PC protection, an adaptation to ischemia, in different and possibly interfering circumstances. However, simultaneous activation of multiple receptors by repetitive PC makes it difficult to analyze signaling pathways in PC mechanisms[4,7].

Cytokine receptors also participate in triggering cardioprotection of PC. Based on a report that ADAMs are activated by GPCR and cleave membrane-anchored cytokines[18], we postulated that TNF-α released by ADAM17 plays a role in PC. TNF-α was indeed released from hearts during PC, and inhibition of ADAM17 by KB-R7785 inhibited PC-induced TNF-α release and attenuated the infarct size-limiting effect of PC[19]. On the other hand, Krieg et al.[20] reported that activation EGF receptor by HB-EGF produced by ADAM12 contributes to myocardial protection by ligands of Gi-protein-coupled receptors (acetylcholine and the δ-opioid agonist [D-Ala2, D-Leu5]-enkephalin acetate [DADLE]). Contribution of other cytokines to PC is possible but has not been systematically examined yet.

4 Signal Pathways from GPCR in PC Mechanisms

Multiple signal pathways are activated by activation of Gq, Gi and $\beta\gamma$ subunits of GPCR in PC. Jak2 tyrosine kinase may also be activated by its binding to cytoplasmic domain of Gq-coupled receptors. However, the primarily important protein kinase in PC is protein kinase C-ε (PKC-ε)[6,21-23]. Contribution of this kinase to PC was first suggested by studies showing that PKC inhibitors abolished infarct size limitation by PC and that pretreatment with PKC activators could mimic PC. We found that PKC-ε is activated by PC and translocated to mitochondria, where it presumably interacts with the mK_{ATP} channel[22,23]. Furthermore, a mK_{ATP} channel opener did not induce PKC-ε translocation, and infarct size limitation by diazoxide was insensitive to PKC inhibitors[22,23]. Together with results of a study showing positive regulation of the mK_{ATP} channel by PKC[24], our findings indicate that the mK_{ATP} channel is downstream of PKC-ε in PC mechanisms.

Whether PKC requires some intracellular machinery to receptors of activated C kinases (RACKs) or simply diffuses to RACKs in the cell remains controversial. However, we found that depolymerization of microtubules by colchicines or nocodazole prevented induction by PC of PKC-ε translocation and anti-infarct tolerance[25]. It remains unclear whether microtubules play roles in transportation of PKC to the membrane or in localization of RACKs. Nevertheless, the findings indicate that integrity of microtubles is necessary for translocation of PKC-ε to RACKs relevant to myocyte protection.

Akt (PKB) is also activated by PC, presumably as a result of activation of PI3K-γ by activated $\beta\gamma$ subunits of GPCR. The role of Akt in the trigger phase of PC has been extensively studied by Downey's group[20,26,27], and they proposed that Akt is upstream of eNOS, which transmits signals to the mK_{ATP} channel via PKG. However, since they used activation of the muscarinic receptor, a Gi-coupled receptor, as a model of PC-induced protective signaling, the role of Akt and its downstream signaling may not be predominant in entire mechanisms of PC. In fact, this pathway from Akt to the mK_{ATP} channel does not seem to be activated by stimulation of the adenosine receptor[26].

Phosphorylation of ERK and p38MAPK by PC has been observed in several preparations. Association of the activation of these kinases with PC-induced myocardial protection has been suggested in some models of myocardial infarction but not in other models. Thus, although we cannot totally exclude the possibility of their roles in PC, their activation does not appear to be predominant and crucial in PC.

5 Cytoprotective Effectors in PC

Although the direct cause of cardiomyocyte death during ischemia/reperfusion has not been fully elucidated, rupture of sarcolemma and irreversible dysfunction of mitrohondria are cardinal features of irreversibly injured cardiomyocytes[28]. A major cause of mitochondrial dysfunction is believed to be mitochondrial Ca^{2+} overload secondary to Ca^{2+} overload in the cytosolic compartment. A main route for Ca^{2+} influx during ischemia/reperfusion

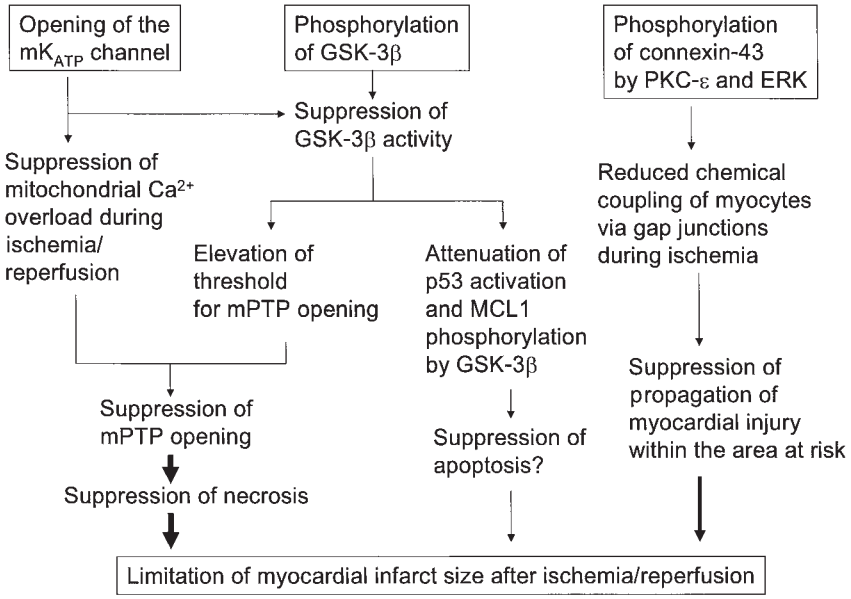

Fig. 2. The proposed functions of effector molecules in the mechanism of infarct size limitation by PC.
mPTP = mitochondrial permeability transition pore. See figure 1 legend for the other abbreviations.

is reverse mode Na^+-Ca^{2+} exchange, which is driven by Na^+ overload due to Na^+-H^+ exchange, "persistently opened" Na^+ channel and suppressed Na^+-K^+-ATPase. The Na^+ overload in a cardiomyocyte can propagate to adjacent cardiomyocytes via gap junctions. Thus, a plausible explanation for protective effects of PC is presence of the effector molecules, which prevent the lethal events in the sarcolemma, mitochondria and/or gap junctions, downstream of PC-induced cell signaling. The effector molecules in PC have not been unequivocally identified, but we have characterized roles of the mK_{ATP} channel, GSK-3β and connexin-43 (Cx43), a gap junction subunit, as possible effectors in PC (Fig. 2).

5.1 The mK_{ATP} Channel

Contribution of the mK_{ATP} channel to PC was first suggested by the finding that inhibitors of the K_{ATP} channel abrogated infarct size-limitation of PC. Presence of the K_{ATP} channel in the mitochondrial inner membrane has been indicated by electrophysiological features of a class of the potassium channel in a mitoplast preparation. Unfortunately, the mK_{ATP} channel is the only K_{ATP} channel that has not been cloned, and its molecular structure remains unknown. However, a recent study[29] suggested that this channel may consist of mitochondrial ATP-binding cassette protein 1 (mABC1), succinate dehydrogenase, phosphate carrier, adenine nucleotide translocator and ATP synthase, unlike other types of K_{ATP} channel, which are complexes of sulfonylurea receptors and Kir6.x proteins. The notion that PKC positively regulates the mK_{ATP} channel is supported by the use of change in NADH oxidation level as an index of mK_{ATP} channel activity, in isolated cardiomyocytes[24] and potassium flux across the mK_{ATP} channel reconstituted from isolated mitochondria.

We examined the role of the mK_{ATP} channel in PC-induced infarct size limitation by use of activation of the adenosine A_1 receptor, a major trigger receptor in PC[23]. Infarct size limitation by R-phenylisopropyl-adenosine, an A_1 receptor agonist, was abolished by a PKC inhibitor (calphostin C) and a mK_{ATP} channel selective inhibitor (5-hydroxydecanote, 5-HD), whereas protection afforded by a mK_{ATP} channel opener, diazoxide, was abrogated by 5-HD but not by calphostin C. These results were consistent with the findings that activation of the mK_{ATP} channel opener did not induce translocation of PKC-ε and that 5-HD did not prevent PKC-ε translocation by PC[22]. Furthermore, pretreatment with diazoxide significantly suppressed mitochondrial respiratory dysfunction after ischemia/reperfusion[23]. Mechanisms by which opening of the mK_{ATP} channel protects mitochondrial functions from ischemia/reperfusion injury have not been clarified, but suppression of mitochondrial Ca^{2+} overload by slight depolarization of mitochondrial membrane potential and suppression of outer membrane permeabilization by induction of mitochondrial swelling are possibilities[30]. Nevertheless, our observations[22,23] support the notion that the mK_{ATP} channel is an effector downstream of PKC-ε, affording cytoprotection against ischemia/reperfusion injury.

The critical timing of mK_{ATP} channel opening for suppression of myocardial infarction was investigated by activating or blocking the mK_{ATP} channel before or during ischemia or upon reperfusion in isolated rabbit hearts[31]. The myocardium was protected by activation of the mK_{ATP} channel before or 15 min after the onset of ischemia, whereas such protection was not detected when its opener was administered from 25 min after ischemia or from 5 min before reperfusion. The protective effects of diazoxide were eliminated by 5-HD. Since myocardial necrosis starts to develop at 15~20 min after the onset of ischemia in rabbit hearts, these results suggest that the mK_{ATP} channel needs to be open at the reversible phase of ischemia for enhancing myocardial tolerance against infarction. An explanation for this timing of the mK_{ATP} channel activation is that the role of this channel is suppression of priming of mitochondrial permeability transition pores (mPTP)[32] during ischemia, the opening of which upon reperfusion leads to cell death.

5.2 GSK-3β

GSK-3β is a protein kinase involved in a variety of functions besides regulation of glycogen synthase. Based on four lines of evidence, we hypothesized that GSK-3β is a major effector in the mechanism of infarct size limitation by PC. First, GSK-3β is a substrate of protein kinases activated by PC (i.e., PKC, Akt). Second, not only PC but also other interventions that limit infarct size have been shown to induce phosphorylation of GSK-3β at Ser-9[33,34]. Third, it was recently found that the threshold for mPTP opening in response to reactive oxygen species (ROS) was markedly elevated by inhibition of GSK-3β or by reduction of its protein expression[35]. Fourth, suppression of GSK-3β phosphorylation by FrzA overexpression attenuated infarct size limitation by PC[36].

To test our hypothesis in the myocardium *in vivo*, we compared the effects of PC, erythropoietin (Epo) receptor activation and a combination of PC and Epo on infarct size and on levels of phosphorylation of protein kinases, including GSK-3β[37]. Epo was selected for combination with PC, since signal pathways downstream of its receptor include a pathway to GSK-3β but do not entirely overlap those of PC. The infarct size-limiting effect of PC was additive to that of Epo, and the effects of PC and Epo on level of GSK-3β phosphorylation at 5 min after reperfusion were also additive. Such an additive effect was not observed for phosphorylation of Akt or STAT3. Phosphorylation of GSK-3β by PC plus Epo was partly eliminated by blockade of PKC or PI3K. Most importantly, there was a strong negative correlation between the level of phospho-GSK-3β and percentage of ischemic myocardium infarcted ($r = -0.809$, $p < 0.05$). Furthermore, administration of a GSK-3β inhibitor (SB216763) to mimic the effect of GSK-3β phosphorylation resulted in a significant reduction in infarct size. These results support the notion that phospho-GSK-3β is a determinant of myocyte tolerance against necrosis during ischemia/reperfusion *in vivo*[37].

The mechanism by which phospho-GSK-3β protects cardiomyocytes from necrosis after ischemia/reperfusion remains unclear. Suppression of mPTP opening by phospho-GSK-3β is obviously a possible mechanism, and our preliminary study showed that there is physical interaction between phospho-GSK-3β and adenine nucleotide translocase, a major subunit protein of mPTP. However, suppression of pro-apoptotic functions of GSK-3β, such as GSK-3β-p53 interaction and MCL-1 phosphorylation by GSK-3β, may also be involved in cytoprotection by GSK-3β phosphorylation.

5.3 Cx43

Gap junctions are responsible for electrical and chemical coupling of cells in tissues, including the myocardium, and gap junctions in the ventricular myocardium mainly consist of Cx43. It was almost a decade ago that contribution of gap junctions as routes for propagation of myocardial injury within ischemia/reperfused region was shown; Garcia-Dorado et al.[38] found that infusion of a gap junction blocker upon reperfusion reduced infarct size and changed the pattern of infarcted areas from confluent infarct to patchy and scattered areas of infarcts. Also, a study has shown that chemical coupling via the gap junction persists for a long time in the ischemic myocardium even after electrical coupling is lost[39]. Furthermore, as mentioned above, gap junctions provide routes for extension of Na^+ overload, which leads to Ca^{2+} overload by Na^+-Ca^{2+} exchange, within ischemic regions of the heart. Gap junction communication is under control of protein kinases, and phosphorylation of Cx43 by PKC is known to reduce gap junction permeability. Based on these earlier observations, we hypothesized that accelerated closure of the gap junction by Cx43 phosphorylation at a PKC-dependent site (Ser368) by PC suppresses gap junction-mediated propagation of myocardial injury, leading to infarct size limitation[40].

To examine this hypothesis, we assessed the effect of PC on gap junction permeability in the ischemic myocardium by use of Lucifer yellow (LY) as a tracer of

gap junction permeability[40]. PC significantly reduced gap junction permeability to LY to a level similar to that to heptanol in the ischemic myocardium, and structurally different blockers of gap junction (heptanol, 18β-glycyrrhetinic acid, 2,3-butanedione monoxime) infused after the onset of ischemia limited infarct size, mimicking PC. PC attenuated dephosphorylation of Cx43 during ischemia, and this effect was abolished by pretreatment with calphostin C, a PKC inhibitor[40]. Furthermore, we found that activation of PC signaling by DADLE, a δ-opioid agonist, reduced gap junction permeability to LY, like PC, and this change in the gap junction permeability was abrogated by a PKC-ε selective blocker (PKC-ε translocation inhibitory peptide). In the DADLE-pretreated myocardium, Cx43 was co-immunoprecipitated with PKC-ε, and phosphorylation of Cx43 at Ser368 was significantly enhanced after ischemia compared with that in untreated controls[41]. In addition to PKC-ε, ERK1/2 also can play a role in reduction of gap junction permeability by PC. PC induces ROS generation by activation of the mK_{ATP} channel during the trigger phase of protection[20]. We found that activation of the mK_{ATP} channel by diazoxide induces ROS-dependent phosphorylation of both MEK1/2 and ERK1/2 and phosphorylation of Cx43 at ERK-dependent sites (Ser279/Ser282)[42]. Diazoxide reduced gap junction permeability in the ischemic myocardium, which was abolished by PD98059, a MEK1/2 inhibitor. Taken together, these findings support our current hypothesis regarding the role of Cx43 in PC shown in Fig. 2.

It is difficult to determine the relative importance of suppressed gap junction permeability among multiple protective mechanisms of PC, since there is no selective agent that can re-open the gap junction. Circumstantial evidence, however, support the notion that accelerated closure of the gap junction is an adjunct mechanism of protection of PC. When the change in gap junction permeability caused by DADLE was prevented by PKC-ε translocation inhibitory peptides, approximately 40% of DADLE-induced protection was lost (unpublished observation). Theoretically, the importance of suppression of gap junction-mediated propagation of myocardial injury depends on the level of protection of each cardiomyocyte. If each cardiomyocyte within the area at risk (i.e., area subjected to ischemia/reperfusion) is fully protected, further protection cannot be expected for closure of the gap junction. On the other hand, prevention of gap junction-mediated extension of injury would be significant when PC cannot protect some cardiomyocytes. Consistent with this explanation, the potent infarct size-limiting effect of a high dose of diazoxide (100μM) was not inhibitable by PD98059 at a dose sufficient to abolish suppression of gap junction permeability by diazoxide, whereas protection by a lower dose of diazoxide (10 μM) was abolished by the same dose of PD98059[42].

6 Impairment of PC Mechanisms by Concurrent Diseases

Most of the experimental studies on PC and myocardial protection have been conduced using healthy normal animals. However, most patients with AMI have concurrent diseases such as hypertension and diabetes, and many of them have a history of previous myocardial infarction. Thus, there is concern about whether mechanistic insights obtained from normal animals or tissues are applicable for patients. For PC mechanisms, it is interesting to note that some of the signal pathways activated by PC are known to play roles in development of ventricular hypertrophy. Accordingly, we examined the possibility that mechanisms of PC are impaired by the process of myocardial remodeling after myocardial infarction.

In rabbit hearts, significant hypertrophy of the myocardium and interstitial fibrosis in the non-infarcted region are detectable 2 weeks after infarction. PC failed to protect this remodeled myocardium from necrosis due to acute ischemic insult, whereas the impairment in myocardial response to PC was prevented by suppression of post-infarct remodeling by the use of an AT_1 receptor blocker[43]. In the remodeled myocardium, the adenosine receptor and its G protein were intact, but PC failed to activate PKC-ε[43,44]. On the other hand, myocardial protection by activation of the mK_{ATP} channel, a downstream target of PKC, was similarly observed in normal and post-infarct remodeled myocardium. Taken together with the results of a study showing that down-regulation of phospholipase C was associated with post-infarct ventricular remodeling[45], our findings suggest that chronic activation of the AT_1 receptor makes cardiomyocytes refractory to PC due to disruption of signal transmission between G protein and PKC.

Post-infarct myocardial remodeling is not the only pathological factor that impairs PC mechanisms. Failure of PC to limit infarct size was reported in some models of aging and diabetes mellitus but not in the others. These contradictory results have not been clearly explained, though differences in models and duration of disease interfering with PC signaling may be responsible.

7 Application of PC Biology to Clinical Therapy

From the viewpoint of the general scheme of PC mechanisms (Fig.1 and Fig.2), manipulation of end-effectors in PC is a reasonable approach for protecting ischemic myocardium in patients. Of three effectors of PC indicated by basic research, the mK_{ATP} channel has been the most intensively studied as a target of drug therapy in patients with coronary artery disease. A double-blind randomized trial (IONA trial)[46] has demonstrated clinical benefit of a mK_{ATP} channel opener, nicroandil. There are currently no agents targeting GSK-3β or Cx43 for myocardial protection. Cx43 is a difficult target to manipulate for this purpose, since inhibition of electrical coupling by the gap junction would induce electrical derangements, leading to arrhythmias. In contrast, more than 30 inhibitors of GSK-3β have been synthesized as possible therapeutic agents for Alzheimer's disease and/or diabetes mellitus. A potential disadvantage of a GSK-3β inhibitor is promotion of myocardial hypertrophy by reduced phorphorylation of NF-AT and possible increase in risk of cancer by reduced degradation of β-catenin. However, these untoward effects of GSK-3β inhibition should be negligible when it is administered only once before reperfusion to reduce infarct size in a patient with AMI. Further investigation is needed to determine the efficacy and disadvantages of GSK-3β inhibitors as cardioprotective agents.

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虚血プレコンディショニングの心筋細胞保護機構とその臨床応用

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虚血再灌流障害に対する心筋保護法の開発という観点から，虚血プレコンディショニング（PC）の細胞保護機構を解析してきた．PC は適応反応の一つで，心筋を短時間の非致死性虚血に暴露することにより虚血再灌流障害に対する心筋細胞の耐性が顕著に増強する現象である．PC は複数の Gi/Gq 蛋白連関受容体作動物質の産生ならびに TNF-α, HB-EGF の放出をもたらし，それぞれの受容体を活性化することが PC 機構のトリガーとなっている．これら受容体の刺激により活性化される PKC-ε，PI3K/Akt を含む複数の細胞保護シグナル経路は最終的に細胞を保護する効果器に到達すると考えられている．そうした効果器として，ミトコンドリア ATP 感受性カリウムチャネル（mK_{ATP} channel），グリコーゲン合成酵素キナーゼ-3β（GSK-3β），コネキシン 43（Cx43）が挙げられる．mK_{ATP} channel の活性化は虚血再灌流におけるミトコンドリア Ca^{2+} 過負荷を抑制することによりミトコンドリア機能を温存する．リン酸化 GSK-3β は再灌流時のミトコンドリア透過性遷移の制御因子であり，そのレベルが in vivo における心筋梗塞サイズの規定因子であることが明らかとなった．また Cx43 の PKC，ERK によるリン酸化はギャップ結合閉鎖を促進することによって心筋細胞障害の伝播を抑制する機序であることが示唆された．しかしこうした細胞保護的なシグナル伝達は，梗塞後心筋リモデリングなどの病態の存在によって障害を受けうることが示された．PC 機構の応用として，mK_{ATP} channel 開口薬は心筋保護を目的として既に臨床の場で使用されている．選択的 GSK-3β 阻害薬は新たな心筋保護薬となる可能性は高い．今後の臨床応用可能な新規薬剤の開発には，心筋細胞保護を導く細胞内シグナル伝達のさらなる解明と，併存する病態によるその修飾についての解析が必要と思われる．