Reduction of Myocardial Ischemia-Reperfusion Injury with Pre- and Postconditioning: Molecular Mechanisms and Therapeutic Targets

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Abstract: Reduction of infarct size as well as alleviation of other ischemia- and reperfusion-associated injuries are the goals of primary importance in cardiology. One of the remedies is considered to be myocardial preconditioning (PreCon) referred usually to as an increased myocardial tolerance to prolonged ischemia following brief ischemic or non-ischemic challenge. In this review, PreCon stimuli tested to date are considered including a number of mildly noxious factors applied either locally to the myocardium or systemically. Recently, one more mode of heart protection against reperfusion injury termed postconditioning (PostCon) has been developed. On the basis of ample evidence published, along with our findings, a detailed comparative analysis of PreCon and PostCon is presented, with special emphasis on the cellular, molecular, and pharmacological aspects of the topic as well as clinical applications, both implemented and awaiting practical approval.

Key Words: Heart, ischemia-reperfusion injury, infarct size, cardioprotection, preconditioning, postconditioning.

1. INTRODUCTION

Coronary heart disease (CHD) is generally thought to be a major cause of morbidity and mortality. In particular, in the Russian Federation mortality rate due to CHD has been documented to be as high as 484 cases (330 males and 154 females) per 100.000 of the population [1]. It seems, therefore, that prevention and/or alleviation of myocardial ischemic injury remains to be among the most important goals of the medical care. For this, the development of the techniques aimed at heart protection in the setting of myocardial infarction (MI) is considered to be of prime significance. With the advent of reperfusion strategies, first of all, thrombolysis and percutaneous revascularization, both survival and prognosis rates have been improved in the patients with MI. Along with the apparent favorable effects, reperfusion therapy has brought to light the problem of irreversible reperfusion injury referred usually to as lethal one [2]. Although the underlying mechanisms have been extensively studied, the reliable treatment strategies are lacking as yet as far as clinical application is concerned. On the other hand, a promising approach can stem from the animal models as judged by the numerous data on myocardial pre- and postconditioning (PreCon and PostCon, respectively) procedures aimed at injury reduction. The main purpose of this review, therefore, was to analyze and update information available on the modes and mechanisms of myocardial PreCon and PostCon, with special emphasis on potential therapeutic targets.

2. HISTORICAL BACKGROUND

Phenomenon of ischemic PreCon has been discovered two decades ago when Murry *et al.* [3] observed the significant infarct size limitation in the open-chest dogs following four episodes of 5-min ischemia and 5-min reperfusion. Since then, the enormous body of papers on the mechanisms, end points, and distinct protocols of ischemic PreCon has been accumulated. Perhaps, the main cardioprotective effect of ischemic PreCon is infarct size limitation. Infarct-limiting effect of PreCon has been consistently reproduced in virtually all species of laboratory animals by a number of research teams. The important point is that the infarct-limiting effect of PreCon would be lost if the duration of test ischemia was extended beyond a certain period of time believed to be species-specific. For instance, ischemic PreCon did not protect against infarction in the rabbit when the duration of test ischemia exceeded 3 h [4]. In fast heart rate animals (e. g., rats and mice), ischemic PreCon was capable of affording protection against infarction caused by even shorter time intervals. This means that PreCon protection did not entirely prevent the development of ischemic necrosis; rather it delayed the occurrence of infarction. Therefore, PreCon can not substitute reperfusion therapy which remains the only treatment option able to terminate ischemic injury. However, PreCon may be considered to be a valuable adjunct to reperfusion therapy because of broadening the time range between the chest pain onset and revascularization procedure. It should be noted that American National Heart, Lung and Blood Institute regarded early reperfusion and ischemic PreCon as the most effective means in the prevention of myocardial ischemia-reperfusion injury (IRI) [5].

Apart from the overt infarct-limiting effect, PreCon has been shown to reduce ischemic [6] and reperfusion [7] arrhythmias, endothelial dysfunction [8], apoptosis [9], and myocardial stunning [10]. Thus, different aspects of ischemic and reperfusion injury can be prevented by PreCon. Depending on the time interval between the application of PreCon stimulus and induction of test ischemia, two phases of Pre-

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Con protection were described – early or classic and delayed. Early PreCon occurs when brief ischemic stimulus is separated from the test ischemia by 5-120 min while delayed one also referred to as the second window of protection (SWOP) develops 24 to 96 h after PreCon protocol [11]. Two major differences between early and delayed PreCon have been established. Firstly, early PreCon results in greater infarct-limiting effect than delayed. Second, in contrast to delayed, early PreCon does not involve *de novo* protein synthesis. In this review, we will mainly concentrate on the mechanisms and clinical applications of early PreCon.

In 2003, Zhao *et al.* [12] introduced the phenomenon of ischemic PostCon. The latter is viewed as a way of cardiac protection against reperfusion injury, which is achieved by the induction of several brief episodes of ischemia during the initial stage of reperfusion after prolonged ischemic insult. In the study by Zhao *et al.*, PostCon was induced by three 30-s episodes of ischemia separated by 30-s episodes of reperfusion started just after 60-min left anterior descending coronary artery occlusion in the open-chest dogs. As a result of PostCon, the significant infarct size limitation was observed as well as reduced myocardial edema, better endothelial function, and the lesser accumulation of polymorphonuclear neutrophils [12]. Subsequently, the cardioprotective effect of PostCon was observed in other species using different models of ischemia-reperfusion. For example, Kin *et al.* [13] showed the reduction in infarct size and creatine kinase (CK) release in the rat model ischemic PostCon. Besides, we described the abrogation of persistent reperfusion-induced ventricular fibrillation in the course of ischemic PostCon using the isolated rat heart model [14]. In the isolated either rat or rabbit heart, PostCon resulted in better postischemic left ventricular function and smaller infarct size [15, 16]. Furthermore, PostCon with twenty 10-s cycles of ischemia-reperfusion caused significant infarct size limitation in the conscious rats, although the effect was evident only in coronary occlusions of less than 45 min [17].

According to Heusch [18], the concept of PostCon is not entirely novel, and actually it stems from the observations on so-called «modified reperfusion». Aimed at the prevention of acute reperfusion injury mainly in cardiac surgery, this approach is based on both pharmacological and non-pharmacological modulation of the reperfusion conditions applied. The protection could be achieved with regional hypothermia [19] and cardioplegia [20], alkalinization of the (re)perfusion solution, enrichment of perfusate with antioxidants and cell membrane protectors [21], and, above all, gradual/staged reperfusion [22]. The latter implies progressive increase in the heart blood supply up to normal values. Gradual reperfusion offers certain benefit to the reperfused heart and, like PostCon, results in the decreased infarct size. Interestingly, PostCon may also be superior to gradual reperfusion, in particular, as far as the reduction of the neutrophil accumulation in the infarcted myocardium is concerned [23].

Both PreCon and PostCon are cardioprotective interventions resultant in the reduction of myocardial IRI. PreCon is believed to be a preventive and thereby prophylactic approach as that effective solely when performed before test ischemia, PostCon being a therapeutic intervention to be applied, along with a number of pharmacological agents, after the onset of prolonged ischemia.

3. DIVERSITY OF PRECONDITIONING STIMULI

Recent studies suggest that the cardioprotective response after ischemic PreCon can be elicited by a wide variety of factors distinct from myocardial ischemia. Thus, myocardial tolerance to IRI could be substantially improved with such non-ischemic stimuli as pharmacological agents, physical factors, and metabolic abnormalities. From this perspective, PreCon protection may represent a non-specific response to various kinds of sublethal stress [24]. In the present review, we find it possible to arrange different modes of PreCon protection on the basis of the particular stimuli relevant to the expression of a given cardioprotective phenotype (Fig. **1**). First of all, ischemic PreCon may be of two main types – local and remote (distant). Also of importance is the fact that certain pharmacological agents may mimic PreCon response, the phenomenon termed «pharmacological PreCon» [25]. Furthermore, such physical factors as mechanical myocardial stretch, increased temperature, etc., have been shown to induce PreCon. Besides, PreCon can emerge in chronic metabolic disorders like diabetes and thyroid dysfunction. Finally, even physical exercise can entail PreCon-like protection of the myocardium from ischemia-reperfusion. The factors just outlined need a detailed substantiation and are considered below.

3.1. Local Ischemic Preconditioning

Originally described by Murry *et al.* [3], local ischemic PreCon is probably the most extensively studied type of PreCon. A wealth of information about the mechanisms of local ischemic PreCon is available now (for review, see [26, 27]). It is generally held that local PreCon can produce the strongest possible protection against infarction and other manifestations of IRI. So, local PreCon is considered to be a «gold standard» of protection, and any newly developed cardioprotective intervention is compared with local PreCon.

Local ischemic PreCon occurs after one or several brief episodes of myocardial ischemia-reperfusion. The protocol of local PreCon may have a significant impact on the extent of cardioprotective effect. In other words, the number and/or duration of PreCon episodes are essential to achieve considerable infarct limitation. This fact has contributed to the concept of PreCon threshold, which could be illustrated by the following example. Such a subthreshold stimulus as a single 3-min episode of regional ischemia was shown to fail to protect canine heart from subsequent infarction. Unlike it, if the duration of PreCon ischemia was extended (10 min), the protection became significant [28]. One can speculate on how the magnitude of protection correlates with the strength stimulus used above the threshold level. A number of authors explain it in terms of the «on-off» mechanism, implicative of maximal protection elicited by stimulus exceeding the threshold [29]. However, our data showed a gradual augmentation of the infarct-limiting effect with the increased number of

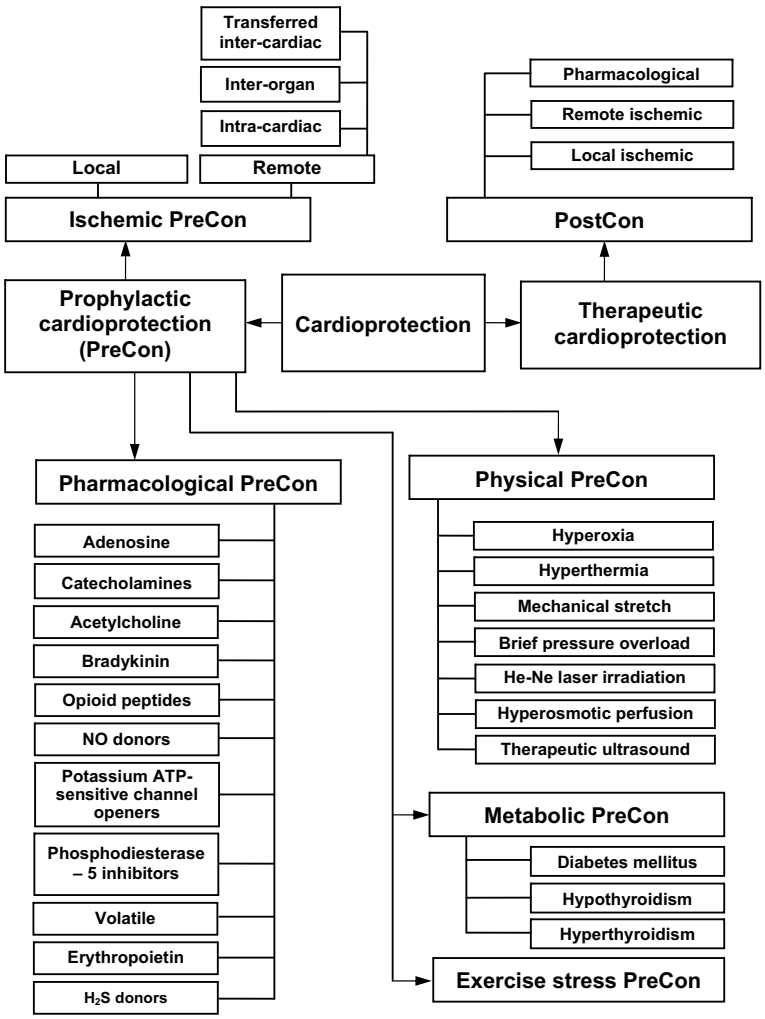

Fig. (1). Diversity of PreCon and PostCon stimuli.

PreCon cycles that can be accounted for somewhat different model used [unpublished observation]. Of note, different end points of protection with PreCon may have distinct thresholds. Thus, in the rat the antiarrhythmic effect of PreCon has lower threshold than the infarct-limiting one. Liu and Downey [30] showed that single 5-min episode of ischemia-reperfusion resulted in nearly complete abrogation of ischemic tachyarrhythmias but failed to protect against infarction in the anesthetized rat. Significant infarct limitation was observed after three episodes of PreCon alone. These observations point to the important conclusion that even a sub-threshold ischemic PreCon stimulus which can also happen in humans with CHD might be potentially enhanced pharmacologically thereby reaching the threshold of protection.

3.2. Remote Ischemic Preconditioning

Myocardial tolerance to ischemia could be raised not only by brief episodes of local ischemia-reperfusion but also with use of ischemia-reperfusion procedure in the distant targets, a phenomenon termed remote PreCon (=PreCon at a distance). It has been found that occlusion-reperfusion of the infrarenal aorta as well as the mesenteric, renal, and femoral artery can produce myocardial PreCon [31, 32]. Przyklenk *et al.* [33] offers to subdivide remote PreCon into three types, that is, intra-cardiac, inter-organ, and transferred inter-cardiac. Intra-cardiac remote PreCon has been first described by these authors a decade before [34]. In the open-chest dogs, the authors showed the brief episodes of occlusion-reperfusion of the left circumflex artery to protect virgin myocardium of the heart supplied with blood by left anterior descending artery from subsequent prolonged ischemia. Inter-organ remote PreCon occurs when brief ischemia-reperfusion of some of the organs (e. g., kidney, small intestine, etc.) leads to improved tolerance against the prolonged heart ischemia. Finally, Dickson *et al.* [35] demonstrated in the isolated two-rabbit heart model that the intact heart could be successfully preconditioned by providing it with the coronary effluent collected from the other *ex vivo* heart subjected to ischemic PreCon. The same team showed that the intact *in vivo* heart could be rendered ischemia-tolerant by means of whole blood transfusion from a preconditioned animal [36].

The most intriguing question about inter-organ remote PreCon is on the nature and underlying mechanisms impli-

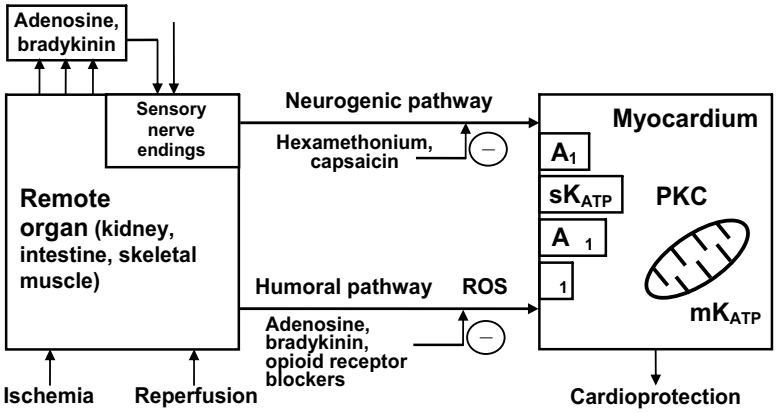

Fig. (2). Hypothetical mechanisms of remote ischemic PreCon. ROS – reactive oxygen species, A_1 – adenosine A_1 receptors, sK_{ATP} – sarcolemmal ATP-sensitive potassium channels, AT_1 – angiotensin II AT_1 receptors, δ_1 – opioid δ_1 receptors, PKC – protein kinase C, mK_{ATP} – mitochondrial ATP-sensitive potassium channels.

cated in the signal(s) exchange between the remote organ and the heart. Two complementary hypotheses were offered, first implying the involvement of neurogenic pathway. In particular, Gho *et al.* [31] found that the cardioprotective effect of intestinal ischemia-reperfusion can be blocked by hexamethonium (Fig. **2**). Furthermore, the effect of remote PreCon can be reproduced by the electrical stimulation of stellated ganglion and abolished by capsaicin [37]. Taken together, these data can be taken as an indirect evidence that transient intestinal ischemia can cause activation of visceral afferent nerves by local accumulation of adenosine and bradykinin [31]. In turn, it may result in the activation of viscerovisceral reflex with the involvement of the sympathetic efferents. If so, then stimulation of the α_1-adrenoreceptors on the surface of cardiac myocytes might eventually lead to heart protection. At first sight, it seems to be at variance with a study by Weinbrenner *et al.* because the cardioprotective effect caused by occlusion-reperfusion of abdominal aorta could not be abolished by the ganglion blockers [38]. That is why the authors suggested that the factor(s) responsible for the protective effect of remote PreCon may be transferred from the skeletal muscle or kidney to the heart either wholly or predominantly in a humoral way. It is known that reperfusion of these two organs is associated with the increased release of adenosine, bradykinin, and opioid peptides into the systemic circulation [38-41]. The effect of remote PreCon due to ischemia-reperfusion of the two can be abolished by the antagonists of A_1-adenosine [41], δ_1-opioid [40], and AT_1-angiotensin [42] receptors. As to the organs with ample sensory innervation (e. g., small intestine) they seem to «talk» to the heart mainly through the neurogenic pathway while the skeletal muscle and kidney do so by virtue of humoral factors.

Little is known about the mechanisms of intracellular signal transduction evoked by remote PreCon. Some data are available on the abolition of the remote PreCon protective effect with the blockers of protein kinase C (PKC) and ATP-sensitive potassium channels (K_{ATP} channels) [43]. Besides, Weinbrenner *et al.* [40] demonstrated that the reactive oxygen species (ROS) can be involved in the mechanisms of remote PreCon since the administration of synthetic ROS scavenger N-2-mercaptopropionylglycine abrogated the protection.

It seems, therefore, that remote PreCon is a distinct version of the ischemic one that may be induced by brief ischemia-reperfusion of phenotypically different tissues. Of those, a skeletal muscle appears to be most favourable in the clinical setting.

3.3. Pharmacological Preconditioning

The idea that PreCon response could be mimicked by the administration of certain pharmacological agents has been proposed soon after the discovery of ischemic PreCon itself [44]. Experimentally, a number of pharmacological agents have been shown to elicit cardioprotective phenotype similar to that of ischemic PreCon when given in lieu of brief ischemia-reperfusion. Here we consider the main groups of chemical compounds capable of producing pharmacological PreCon with special attention to possible therapeutic applications.

The first group comprises the agonists of G-protein coupled receptors (GPCR) known as a triggers of PreCon response. These are bradykinin, adenosine and adenosine receptor agonists, acetylcholine, opioid peptides, and catecholamines. For comprehensive update on the cardioprotective effects of PreCon ligand triggers, two recent reviews can be helpful [45, 46]. One more group of the PreCon triggers have nothing to do at least with the sarcolemmal receptors such as nitric oxide (NO). Although the evidence for the involvement of endogenous NO in triggering PreCon is somewhat controversial, this agent can produce cardiac protection when given exogenously. In the isolated rat heart model, Bilinska *et al.* [47] showed that NO donor molecules can mimic PreCon effect by preventing reperfusion-induced arrhythmias. Lochner *et al.* [48] observed improved postischemic contractile recovery in the isolated rat heart model after administration of sodium nitroprusside and S-nitroso-N-acetylpenicillamine (SNAP). However, administration of NO precursor L-arginine was not associated with increase in myocardial tol-

erance to ischemia. On the other hand, Horimoto *et al.* [49] demonstrated that L-arginine mimicked infarct-limiting effect of ischemic PreCon in the isolated rabbit heart. Using precesely the same model, Nakano *et al.* [50] reported a smaller infarct size after both ischemic PreCon and exogenous administration of SNAP. These findings were later corroborated by Qin *et al.* [51] who showed that SNAP-induced cardioprotection was dependent on the activation of protein kinase G (PKG), opening of mitochondrial K_{ATP} channels, and formation of ROS. Thus, experimental data show that exogenous supplementation with NO may induce cardioprotective response.

Until recently, both mitochondrial and sarcolemmal K_{ATP} channels have been thought to be the main effectors of PreCon protection. A number of the channels activators («openers») have been tested. For example, that administration of pinacidil [52], bimakalim [53], cromakalim [54], and diazoxide [55] prior to prolonged ischemia has been experimentally demonstrated to result in marked protection against IRI. According with recent findings, PreCon with mitochondrial K_{ATP} channel opener nicorandil can result in the substantial preservation of postischemic left ventricular function because of attenuation of ischemia-reperfusion-induced loss of sarcolemma-associated cytoskeletal protein dystrophin [56].

Phosphodiesterase-5 inhibitors (vardenafil, sildenafil, etc.) are currently used for erectile dysfunction. Interestingly, the drugs have been shown to induce cardioprotection when administered before prolonged experimental ischemia [57]. The mechanism is probably associated with the inhibition of cGMP-specific phosphodiesterase (isoform 5) resultant in the intracellular accumulation of cGMP. Kukreja *et al.* [58] discuss the following mechanisms of the inhibitor-imposed protection as equally probable. First, the build-up of cGMP would definitely result in strong vasodilation. Second, at least sildenafil is known to activate transcription of inducible NO synthase gene involved feasibly in delayed PreCon. Third, increased cGMP concentration may also activate PKG that, in turn, would stimulate the opening of K_{ATP} channels. Above all, phosphodiesterase 5 inhibitors have been shown to possess moderate hypotensive effects. Taken together, these pharmacological characteristics of phosphodiesterase 5 inhibitors make them attractive as possible remedy of choice for hypertension and CHD [59].

Volatile anesthetics were extensively studied as pharmacological PreCon mimetics both in the experimental and clinical settings. In particular, halotane, enflurane, and isoflurane were shown to improve postischemic contractile performance [60] and also limit infarct size in several species [61]. Cason *et al.* [62] first raised the question of whether the mechanisms of protection induced by these drugs were similar to the mechanisms of ischemic PreCon. Recent findings have resolved this issue positively for such a protection was abolished by K_{ATP} channel blockers [63], PKC inhibitors [64], and a G_i protein inhibitor [61]. Thus, PreCon- and halogenated anesthetic-induced cardioprotection may involve similar intracellular signalling events. Besides, halogenated anesthetics have been shown to both attenuate calcium overload of cardiomyocytes during ischemia-reperfusion and to possess antioxidant-like effects [65]. In our study, the effects of some commonly used anesthetic agents (pentobarbital, isoflurane, and a mixture of midazolam, fentanyl and fluanisone) on myocardial tolerance to IRI were investigated in the isolated mouse heart [66]. None of the anesthetics tested influenced significantly on both functional recovery and infarct size after ischemia-reperfusion. Species-related differences may be of importance, however, if one considered the results of the analogous tests performed on distinct laboratory animals [61].

Recombinant human erythropoietin (rEPO) has been found to reduce hypoxia-induced apoptosis of cardiac myocytes [67] and to enhance functional recovery in the isolated rat heart subjected to ischemia-reperfusion [68]. Furthermore, Calvillo *et al.* [69] demonstrated that acute administration of rEPO resulted in infarct size limitation in the *in vivo* model of ischemia. Very recently, Joyeux-Faure *et al.* [70] addressed the mechanisms underlying rEPO-induced PreCon to show that intraperitoneal administration of rEPO to the rats 1 h prior to heart isolation resulted in better postischemic functional recovery compared to the rEPO-free controls. Of note, rEPO-induced protection was blunted by mitochondrial K_{ATP} channel blocker and NO synthase inhibitors. Further experiments are needed to clear up the mechanisms of EPO-induced PreCon taken especially into account that application of rEPO for myocardial protection is facilitated by the fact that this drug is already approved for human clinical use.

Recent studies suggest that nitric oxide is not the only gaseous molecule species which may induce cardioprotective response similar to that in ischemic PreCon. Johansen *et al.* [71] demonstrated in the isolated rat heart that addition of sodium hydrosulfide (hydrogen sulphide donor) to the perfusion fluid 10 min prior to ischemia-reperfusion resulted in a concentration-dependent limitation of infarct size. Protective effect was lost in case of co-administration of sodium hydrosulfide and K_{ATP} channel blockers glibenclamide and 5-hydroxydecanoate.

The major problems in the prophylactic use of pharmacological agents mimicking PreCon seem to be: 1) instability of the effect, 2) the presence of hemodynamic and/or other side effects, and, 3) rapid development of tachyphylaxis in case of continuous exposure to the drugs. In the rabbit, for instance, continuous infusion of A_1-adenosine receptor agonist caused tachyphylaxis during three days [72]. Moreover, tachyphylaxis to the agonist was accompanied with the loss of protection by ischemic PreCon. Dana *et al.* [73], however, showed that repetitive infusions of A_1-adenosine receptor agonist may confer prolonged cardioprotective response lasting several weeks.

Noteworthy, a number of pharmacological agents can lower the threshold of ischemic PreCon, despite they themselves do not induce PreCon. For example, Tsuchida *et al.* [74] demonstrated in the *in vivo* rabbit model that combination of acadesine with a single 2-min episode of myocardial ischemia resulted in significant infarct size reduction. At the same time, the bout of ischemia and acadesine, when em-

ployed separately, failed to produce cardioprotection. In a similar model, Miki *et al.* [75] registered potentiation of the effect of ischemic PreCon with angiotensin-converting enzyme (ACE) inhibitor captopril at a dosage of 1 mg/kg. Again, when used separately, both interventions had no effect on myocardial infarct size. Interestingly, potentiation of a subthreshold ischemic PreCon with captopril has proved to be bradykinin-dependent suggestive of the retarded bradykinin degradation that is casually related to its subsequent accumulation in the tissue. Recently, Ebrahim *et al.* [76] demonstrated that omapatrilat (inhibitor of both neutral endopeptidase and ACE) lowered the threshold of ischemic PreCon in the isolated rat heart, and this effect was dependent on bradykinin B_2-receptor activation.

We expect the list of chemical compounds with potentiality to produce pharmacological PreCon to be extended in the coming years. After further experimental testing, cardioprotective efficacy of these agents needs to be confirmed in prospective randomized clinical trials. However, the pharmaceutical companies seem to become increasingly sceptic as to the sponsorship and financial support of such trials [77]. As stated recently by Downey and Cohen [77] it might be explained as due to the fact that cardioprotectants are «single-dose» drugs, and hence their selling may not be really gainful business.

3.4. Preconditioning with Physical Factors

Certain physical factors (e. g., hyperoxia, hyperthermia, myocardial stretch) can also produce cardiac protection when applied either locally or systemically prior to prolonged myocardial ischemia. Tahepold *et al.* [78] documented hyperoxia to protect the heart from IRI. In these experiments, the rats were kept in hyperoxic (>95% O_2) environment for 60 min followed by the hearts being harvested for Langendorff perfusion and subjected to global ischemia-reperfusion. As a result, mild oxidative stress, improved postischemic contractile recovery, and reduced infarct size were noted. Subsequent studies by the same workers demonstrated that the decreased activation of nuclear factor κB during prolonged ischemia may be implicated in the hyperoxia-induced protection [79].

Another physical factor potent in the induction of PreCon response is increased temperature. Song *et al.* [80] showed that 15 min whole-body hyperthermia (42°C) and, equally, retrograde 5 min *ex vivo* hyperthermic rat heart perfusion (42°C) resulted in myocardial protection. As to the underlying mechanism, heat-induced cardioprotection is known to be related to the release of endogenous calcitonin gene-related peptide [80]. Alternatively, hyperthermic stimulus may cause the release of other neurotransmitters and autacoids implicative in heart protection [81]. More experimentation is needed, however, to resolve the issue.

Myocardial stretch by the transient increase in left ventricular end-diastolic pressure can precondition isolated perfused rat heart as well [82]. With use of a distinct model, that is, a brief pressure overload of the left ventricle by partial occlusion of the ascending aorta, increased tolerance of the rabbit myocardium to ischemia *in vivo* was shown [83]. Although the exact mechanism of this protection mode has not been explained, it may involve the opening of stretch-activated ion channels [83].

Falck *et al*. [84] showed that the isolated rat heart can be preconditioned by brief period (2 min) of perfusion with hyperosmotic solution, which was not abolished by inhibitors of PKC, Na^+/H^+ exchanger, and stretch-activated anion channels.

Kolpakova *et al.* [85] investigated the influence of low intensity helium-neon laser radiation on myocardial tolerance to ischemia-reperfusion in the isolated rat heart model. It was found that coherent light resulted in better postischemic functional recovery, but the protective effect, as after some of the physical factors analysed above, was abolished by K_{ATP} channel blocker glibenclamide.

Thermal effects of therapeutic ultrasound (US) are currently used in physical therapy for acceleration of wound healing and pain relief. We were interested in investigation of the non-thermal effects of therapeutic US on the function of isolated rat heart. It has been shown that the exposure of the heart to therapeutic US (45-298 KHz, 0,3 W/cm^2) resulted in moderate increase in left ventricular end-diastolic pressure and the emergence of both ventricular premature beats and runs of ventricular tachycardia [86, 87]. We regarded these changes as the manifestation of mild myocardial injury caused by ultrasonic cavitation targeted presumably to the vascular lining. One can hypothesize that US directly, at least with the parameters used, might cause PreCon response that has been partially validated in the isolated rat heart model. It was shown that 10-min US irradiation prior to 30-min global ischemia followed by 90-min reperfusion resulted in the reduction of infarct size and significant amelioration of both systolic and diastolic postischemic ventricular function [88]. The protective effect of US exposure was not abolished by a synthetic ROS scavenger, N-2-mercaptopropionylglycine, but it was blunted by glibenclamide [unpublished data].

Thus, evidence is beginning to accumulate that PreCon protection may be evoked by certain physical factors, although the mechanisms of these phenomena still await further clarification.

3.5. Metabolic Preconditioning

The term «chronic metabolic preconditioning» has been introduced by Hadour *et al.* [89]. The authors noted that infarct size in the rabbits with alloxan-induced type 1 diabetes mellitus (DM) was appreciably smaller in comparison with that of healthy animals. Even earlier Liu *et al.* [90] demonstrated protection from infarction of the heart in the rat model of type 2 DM. According to the concept of metabolic PreCon, the molecular and cellular changes of myocardial phenotype in experimental DM may be responsible for the protection of the heart against ischemia. However, controversy can be noted regarding the myocardial sensitivity to ischemia in the setting of experimental DM (for review, see [91], and references therein). Thus, some data indicate that

diabetic heart is more tolerant to ischemia [92], while the others demonstrate unchanged response to ischemia [93] or even decreased ischemic tolerance [94]. The discrepancy, first of all, may arise because of different models used of both experimental diabetes and ischemia-reperfusion. In particular, while interpreting the data on myocardial tolerance to ischemia in type 1 DM, one should take into account the duration of disease since two consecutive phases – acute (1-3 weeks) and chronic (6-9 weeks) are to be distinguished. Recent work suggests that infarct-limiting and antiarrhythmic effects of metabolic PreCon are more pronounced during the acute phase, being gradually lost during chronic one [95]. Whether or not the PreCon effect is dependent on the disease phase, its mechanism is poorly understood. Some evidence suggests, however, that K_{ATP} channels are chronically activated in type 1 DM [96] so that metabolic PreCon may share certain similarity with ischemic PreCon. A complementary possibility is that decreased intensity of glycolysis and, thereby, lesser intracellular acidosis might be responsible for improved tolerance of diabetic myocardium to ischemia [97]. In our study, myocardial sensitivity to ischemia was investigated in the *in vivo* rat model of type 1 DM 6 weeks after alloxan administration [98]. It has been shown that infarct size was significantly smaller in diabetic rats than in controls and, besides, in diabetic animals the incidence of ischemic arrhythmias was significantly reduced. From these data, it seems reasonable to conclude that cardioprotective response in type 1 DM is persistent for a longer period of time than it was suggested before.

Although the concept of metabolic PreCon is substantiated by numerous experimental studies, its clinical relevance can be challenged. At variance with experimental data, large-scale epidemiological studies demonstrate higher prevalence of MI and its complications in the patients with both type 1 and type 2 DM [99]. It is believed that more severe clinical course of CHD in the patients with DM may be attributed to accelerated atherosclerosis and more pronounced diastolic dysfunction [100]. Both processes may interfere with cardioprotective effects of metabolic PreCon and even worsen the prognosis. However, further elucidation of the mechanisms underlying metabolic PreCon may be helpful in the development of new cardioprotective agents.

An important question is whether the effects of ischemic and metabolic PreCon are additive. Our experiments show that the effectiveness of ischemic PreCon can be reduced significantly in rats with alloxan-induced type 1 DM [98] consistent with the data obtained by other investigators. In particular, del Valle *et al.* [101] demonstrated a lack of ischemic PreCon protection against stunning in the conscious diabetic sheep. Nieszner *et al.* [102] failed to produce infarct-delimiting effect of ischemic PreCon in the rabbit model of alloxan-induced type 1 DM. A few authors showed that ischemic PreCon can be induced in the diabetic animals but the intensity of preconditioning stimulus must be strong enough compared to the non-diabetic controls. For instance, Tsang *et al.* [103] found that diabetic rat heart can be preconditioned with multiple cycles of ischemia-reperfusion while single episode has proved to be ineffective. The protective effect of three PreCon runs was shown to be mediated by phosphatidylinositol-3-kinase (PI3K)-Akt pathway.

Clinical data on the effectiveness of ischemic PreCon in patients with DM are generally in line with the experimental findings. Ishihara *et al.* [104] found that type 2 DM abolished the benefits of preinfarction angina in the patients with acute MI. Again, recent study in biopsy specimens by Hassouna *et al.* [105] showed that ischemic PreCon failed to protect right atrial myocardial tissue from simulated hypoxia-reoxygenation injury in patients with type 1 and 2 DM. Interestingly, unlike ischemic, pharmacological PreCon with direct activators of PKC and p38 mitogen-activated protein kinase (p38MAPK) but not with phenylephrine, adenosine, or diazoxide was shown to be effective in this model. So, the authors concluded that PreCon protection is diminished in DM because of impairment of K_{ATP} channel-dependent mitochondrial ROS production which is suggested to be proximal to activation of PKC and p38MAPK. One more mechanism potentially responsible for the compromised effectiveness of ischemic PreCon in 2 DM is thought to be drug therapy with sulfonylurea derivatives and, particularly, glibenclamide. Glibenclamide, a drug lacking tissue selectivity and blocking universally K_{ATP} channels in each tissue tested may probably abolish the effects of both metabolic and ischemic PreCon in patients with type 2 DM. Noteworthy, the effect of ischemic PreCon can not be abolished by glimepiride, a novel sulfonylurea derivative with more selective that the above drug influence on pancreatic tissue [106]. Thus, the development of novel hypoglycemic drugs with the improved selectivity relative to the myocardium may facilitate the maintenance of PreCon protection in patients with type 2 DM.

In the recent years, the concept of metabolic PreCon was further extended by the demonstration of cardioprotective phenotype in hyper- and hypothyroid states Thus, Pantos *et al.* showed that subcutaneous administration of thyroxin to the rats for two weeks resulted in the improved postischemic contractile recovery in the isolated heart model [107]. It was suggested that cardioprotection in hyperthyroidism may involve increased basal myocardial expression of PKCδ [108], as well as heat shock proteins 27 [109] and 70 [110], and also the decreased activation of p38MAPK during test ischemia [111]. Propylthiouracil-induced hypothyroidism is also associated with preservation of postischemic contractile function of the rat left ventricle [112]. At the molecular level, increased myocardial expression of PKCε and decreased activation of c–jun NH_2-terminal kinase (JNK) during ischemia-reperfusion were observed in propylthiouracil-treated rats. It is of note that metabolic PreCon in type 1 DM may be at least in part explained by hypothyroidism concomitant commonly with DM. This notion is supported by the study of Zhang *et al.* [113] in which chronic administration of triiodothyronine to the rats with streptozotocin-induced T1DM abolished the antiarrhythmic effect of metabolic PreCon.

It appears, therefore, that at least such metabolic disorders as diabetes mellitus, hyper- and hypothyroidism can

induce steady cardioprotective phenotype associated with the increased myocardial tolerance to ischemia-reperfusion. Perhaps the most important conclusion from the concept of metabolic PreCon one can deduce is that the cardioprotective response may be maintained for a relatively long period of time at the acceptably high level. In fact, induction of persistent cardiac protection by virtue of non-invasive procedure (for instance, intake of an appropriate pharmacological agent) would be useful for the prevention of IRI in the clinical realm.

3.6. Other Factors

It is known that physical exercise stress can also contribute to increased myocardial tolerance to IRI. For instance, Radak *et al.* [114] showed that such a load in the rat resulted in prevention of oxidative modification of myocardial proteins caused by hydrogen peroxide. Furthermore, Venditti *et al.* [115] demonstrated improved postischemic functional recovery in the rat heart after 5 h of swimming. However, the exercise lasting for 8 h had no influence on postischemic contractile function. The mechanisms of exercise-induced PreCon remain unclear although hypothetically the following mechanisms might be implicated. First, exercise can produce mild systemic oxidative stress known to be a trigger of PreCon at large. Second, several ligand triggers of PreCon (e. g., catecholamines, opioids, adenosine) are released into the systemic circulation during exercise stress. And lastly, exercise results in severely increased pre- and afterload. Thus, the protection by PreCon with physical exercise might be triggered by several factors involved in the mechanisms of other PreCon types.

4. MECHANISMS OF PROTECTION BY PRECONDITIONING: IN SEARCH OF ELUSIVE END EFFECTOR

Ample evidence on the mechanisms of PreCon is available. Traditionally, the mechanisms of PreCon are viewed as a complex signalling cascade which includes three sequential stages: trigger, mediator, and effector one (Table **1**). Trigger stage is characterized by the accumulation of a number of chemical factors able to activate intracellular enzymes in both receptor dependent and receptor independent ways. Mediator stage involves activation of several families of intracellular kinases (PKC, tyrosine kinase (TK), MAPKs, etc.) and complex interplay between them eventually leading to activation of PreCon end effectors. Finally, effector stage is generally held to be the upregulation of hypothetical cellular targets that are responsible for increased myocardial tolerance to ischemia. It should be acknowledged that despite intensive research the most intriguing part of PreCon response, effector stage, is the most unsettled issue in the mechanism of protection induction. In the following sections, we present the overview of recent data regarding the mechanisms of PreCon.

4.1. Trigger Stage

Application of PreCon stimulus results in the elevated myocardial concentration of several substances known as PreCon triggers (Table **1**). Furthermore, some exogenously administered agents may also trigger PreCon response. The triggers under scrutiny could be divided into receptor-dependent and independent. The main receptor-dependent triggers are adenosine, opioid peptides, and bradykinin. The fact that these substances are implicated in the initiation of PreCon response is well documented by several lines of evidence. First, administration of antagonists of appropriate GPCRs completely or partially abolished the protective effect of ischemic PreCon. Second, administration of both receptor-

Table 1. Preconditioning Triggers, Mediators, and End Effectors

PreCon triggers
Receptor dependent (ligand triggers)
- adenosine
- opioid peptides
- bradykinin
- catecholamines (?)
- angiotensin (?)
- prostaglandins (?)
- endothelin (?)
Receptor independent
- reactive oxygen species
- calcium ions
- nitric oxide (?)
PreCon mediators
Protein kinase C
Cytosolic receptor tyrosine kinase
p38 mitogen-activated protein kinase*
Protein kinase A*
Protein kinase B (Akt)*
Protein kinase G*
Extracellular signal-regulated kinase*
c-Jun NHP2 terminal kinase*
Phosphatidylinositol 3 kinase*
Sphingosine kinase*
Glycogen synthase kinase-3β*
PreCon end effectors
Sarcolemmal K_{ATP} channels
K^+ channels of the inner mitochondrial membrane
Mitochondrial permeability transition pore
Attenuated generation of ROS upon reperfusion (?)
Alterations in fatty acid metabolism (?)
Mitochondrial K_{ATP} channels (?)

Note. **(*) –** not shown rigorously whether a given protein kinase is trigger or mediator.

dependent triggers and the agonists to their receptors before prolonged ischemia may mimic PreCon protection. The role of such receptor-dependent triggers as prostaglandins, catecholamines, angiotensin, and endothelin has been shown to be controversial because of poor reproducibility [116].

Furthermore, there are three receptor-independent triggers, namely, NO, ROS and calcium. As mentioned above, the role of endogenous NO in triggering PreCon is not quite clear. Some studies show that administration of NO synthase inhibitors does not result in attenuation of PreCon. We investigated the role of endogenous NO in the mechanism of both local and remote ischemic PreCon in the *in vivo* rat model using Nω-nitro-L-arginine as an inhibitor of NO synthase [117]. It was demonstrated that NO synthase was redundant for PreCon protection so that its involvement in ischemic PreCon seems unlikely. The role of ROS in PreCon is supported by the evidence that some synthetic antioxidants and ROS scavengers prevent induction of cardioprotective phenotype if administered parallel to a PreCon stimulus. Besides, PreCon protective effect can be reproduced by the use of ROS-generating compounds [118]. However, the situation is complicated by the known dual role ROS in PreCon signalling. On one hand, generation of ROS during brief episodes of ischemia-reperfusion can trigger cardioprotection. On the other hand, small burst of ROS generation within the mitochondria is known to occur after activation of intracellular kinases and, therefore, may be regarded as a mediator of protection. Transient elevation in the extracellular calcium is one more receptor-independent PreCon trigger [119]. It should be kept in mind, however, that calcium antagonists do not abolish the effect of ischemic PreCon [120].

One very important feature of PreCon signalling is the availability of multiple pathways that can be efficient in cardioprotection. This factor is particularly apparent at the trigger stage where many synergic factors converge to potentiate the effect.

4.2. Mediator Stage

In the past decade, considerable information has been accumulated on the molecular signal transduction pathways which transmit extracellular signal initiated by the PreCon stimuli to the cellular targets of cardioprotection. These pathways comprise different protein kinases including PKC, cytosolic TK, p38MAPK, protein kinase A (PKA), protein kinase B (signalling factor Akt), PKG, extracellular signal-regulated kinase (ERK), JNK, PI3K, and glycogen synthase kinase-3β (GSK-3β). Classically, these kinase cascades are viewed as PreCon mediators. It should be noted that the terminology of triggers and mediators may differ throughout the literature. Thus, some experts define the latter as the molecules capable of securing protection by the maintenance of active/upregulated state during the prolonged ischemic event. If so, most of the kinases listed would not fulfil the criteria of PreCon mediators. On the other hand, the administration of p38MAPK, PKA, Akt, PKG, ERK, JNK, and PI3K inhibitors during PreCon stimulus have been shown to block the protection suggestive of their trigger rather than mediator function (Table **1**). Comprehensive reviews on the issue can be found elsewhere [29, 121].

4.3. Effector Stage

Until recently, mitochondrial K_{ATP} channel was believed to be a major end effector of PreCon. This view was first proposed by Garlid *et al.* [122] and Liu *et al.* [123]. Early studies showed that PreCon leads to the opening of mitochondrial K_{ATP} channels during subsequent prolonged ischemia which, in turn, provides attenuation of mitochondrial Ca^{2+} overload thus preventing the myocardium from ischemic injury [124]. Furthermore, it has been shown that the opening of mitochondrial K_{ATP} channels may result in the formation of ROS important in conveying PreCon signal to distal effectors [125]. Finally, a number of workers supposed that activation of a putative mitochondrial K_{ATP} channels can alter the volume of mitochondrial matrix resulting in optimization of ATP production [126]. It should be stressed, however, that the entire molecular structure of mitochondrial K_{ATP} channel has not been completely sequenced [127]. So, the evidence for the involvement of this particular polypeptide in the mechanism of PreCon is solely restricted by the finding that certain pharmacological inhibitors of these channels can abolish PreCon protective effect while the addition of the channel openers (e. g., diazoxide) may mimic PreCon protection by reducing necrosis and apoptosis [56, 128]. Two of these inhibitors most commonly used are glibenclamide and 5-hydroxydecanoate [129]. However, glibenclamide is a nonselective blocker with a capacity to inhibit the function of both sarcolemmal and mitochondrial K_{ATP} channels. Moreover, recent evidence suggests that both glibenclamide and 5-hydroxydecanoate may have several pleiotropic actions including inhibition of fatty acid oxidation [130]. In more detail, the alterations in myocardial metabolism of fatty acids may be involved in the mechanisms of PreCon; it follows, therefore, that the abolition of PreCon effect with glibenclamide and 5-hydroxydecanoate may actually arise from the activities of the drugs irrelevant to mitochondrial K_{ATP} channel inhibition [127]. Of note, trimetazidine, another blocker of fatty acid oxidation, attenuates PreCon-induced reduction in infarct size [131]. Therefore, the confirmation of the effector role of mitochondrial K_{ATP} channels in PreCon response requires further investigation.

On the basis of thorough analysis of current literature, Hanley and Daut [127] concluded that there may be several distinct end effectors responsible for PreCon-induced cardioprotection. One of these effectors is the activation of sarcolemmal K_{ATP} channels that has been documented following any of the PreCon stimuli tested. The activation of sarcolemmal K_{ATP} channels noted has been shown to reduce the duration of action potentials and, hence, to attenuate intracellular Ca^{2+} overload [132]. Also, ischemic PreCon was demonstrated to fail in the protection of the hearts from Kir6.2, the sK_{ATP} channel subunit, knockout mice from the subsequent IRI [133]. Of significance are probably the potassium channels of the inner mitochondrial membrane with the appreciable functional resemblance to mitochondrial K_{ATP} channels [127]. Furthermore, the limitation in ROS produc-

tion during ischemia-reperfusion can be an important PreCon mechanism on the basis of the data that the excessive ROS amounts do cause damage to the mitochondrial electron transport chain [134] and oxidative burst at early reperfusion can lead to severe myocardial injury [135]. Thus, is seems justified to suggest that PreCon may reduce massive ROS production typical of reperfusion after index ischemia while low ROS concentration is a potent PreCon trigger consistent with the recent data [136]. At last, it was suggested that mitochondrial permeability transition pore (mPTP) is of crucial importance in the attenuation of reperfusion injury by PreCon. Since mPTP opening is considered to be a stigma of early reperfusion it also, along with the above factors, can strongly contribute to the development of reperfusion injury due to burst-threatening mitochondrial matrix swelling, reduction in ATP production, and activation of apoptosis [137]. It seems, therefore, that inhibition of mPTP opening is beneficial in the setting of myocardial reperfusion although the precise mechanism itself of mPTP inhibition still remains not quite clear. It cannot be ruled out, however, that several upstream PreCon mediators (e. g., PI3K, Akt, MAPK, and/or PKC) can inhibit GSK-3β which in turn would result in the increased threshold of mPTP opening [138].

Thus, it seems that the view widely held through the last years on the existence of a single PreCon end effector may probably be misleading. One can rather suppose that the protection by PreCon results from recruitment of several mechanisms including activation of both K^+ channels of the inner mitochondrial membrane and sarcolemmal K_{ATP} channels, attenuation of ROS production during reperfusion, modulation of fatty acid metabolism, and inhibition of mPTP. As to the immediate protection mechanism, one may only speculate that it must conceptually involve reduced myocardial ATP consumption during prolonged ischemia.

5. MECHANISMS OF POSTCONDITIONING: LIMITATION OF REPERFUSION INJURY

Acute reperfusion injury develops within the first minutes after restoration of blood flow mainly because of the formation of high amounts of ROS and appearance of abnormally big transmembrane gradients in osmolarity, pH, and ion concentrations. These factors contribute to intracellular edema and uncontrolled activation of contractile apparatus termed hypercontracture, both resultant in sarcolemmal rupture and cell necrosis. Thus, prevention of cardiomyocyte edema and hypercontracture *via* stuttering reperfusion may play an important role in limiting lethal reperfusion injury. Assuming that PostCon represents a form of modified reperfusion, it seems conceivable that the mechanism of PostCon may be at least in part akin to that of gradual reperfusion. Tsang *et al.* [139] proposed that the events underlying PostCon could be divided into two groups: passive and active. Since the passive ones are similar to the effects of gradual reperfusion it is implied that brief reinstitution of ischemia during early reperfusion phase may result in attenuated ROS production and, furthermore, less abrupt change in extracellular environment.

As to the active mechanism of PostCon, it is unique for this phenomenon and can be related to the activation of specific molecular targets within the cell. Currently, three main constituents of this mechanism are recognized, that is, the activation of adenosine receptors, recruitment of RISK pathway with subsequent involvement of downstream mediators, and inhibition of mPTP. The involvement of endogenous adenosine in the infarct-limiting effect of PostCon has been investigated by Kin *et al.* [140]. The authors showed that, first, PostCon is associated with delayed washout of adenosine accumulated in the myocardium during ischemia and, second, the protective effect of PostCon is abolished in the presence of A_{2A} and A_3 adenosine receptor blockers. The fact of the blockage has been later corroborated by Yang *et al.* [141]. RISK pathway that seems to be distal to activation of adenosine receptors includes two main functionally connected modules, PI3K-Akt and mitogen activated protein p42/p44 extracellular signal regulated kinases 1 and 2 (MEK 1/2-ERK 1/2). Pharmacological activation of RISK pathway during reperfusion has been shown to result in anti-apoptotic effect and limitation of myocardial necrosis [142, 143]. Also, inhibition of both PI3K-Akt and MEK 1/2-ERK 1/2 during the early minutes of reperfusion was shown to abrogate the effect of PostCon, which provide strong evidence that RISK pathway may operate in a capacity of one of the PostCon protection factors. It can be speculated, therefore, that handling RISK pathway pharmacologically may represent a promising target for cardioprotection during revascularization procedures. In this juncture, Tsang *et al.* [139] provided a basis for the concept of «pharmacological PostCon», which implies that several agents including insulin [144], bradykinin [145], transforming growth factor β [146], atorvastatin [147], and glucagon-like peptide 1 [148] may afford protection against lethal reperfusion injury due to upregulation of RISK pathway.

Upregulation of RISK pathway has been documented to cause secondary activation of several downstream mediators of PostCon. Among them, endothelial NO synthase, p70S6 kinase [17], GSK-3β, PKG, and PKCε [149] were all shown to be implicated in the mechanism of PostCon. It is felt that these mediators may probably converge on an end effector of PostCon – mPTP, a single one identified to date. The involvement of mPTP in the mechanisms of PostCon protection was first reported by Argaud *et al.* [150] who demonstrated that either PreCon, PostCon, or administration of mPTP inhibitor NIM811 were able to reduce roughly equally infarct size in the anesthetized rabbits. Besides, each of the intervention used resulted in a lesser mitochondrial susceptibility to Ca^{2+} overload. Recently, in the isolated rat heart model Bopassa *et al.* [151] demonstrated that PostCon both increased phosphorylation of Akt and reduced Ca^{2+}-induced mPTP opening. This inhibition simultaneously with reperfusion can provide several beneficial effects. Thus, pharmacological inhibition of mPTP resulted in a lesser calcium accumulation within the mitochondria, reduced swelling of mitochondrial matrix, decreased release of proapoptotic proteins (e. g., cytochrome c) from the intermembrane mitochondrial space, and, on the whole, attenuation of myocardial IRI

[152]. Generally, one may view mPTP opening as a mitochondria-derived cell suicide which has significant impact on the entire infarction area size in the setting of ischemia-reperfusion. Thus, the development of drugs targeted to PTP function seems to be of significance for the prevention of reperfusion-induced heart injury.

Recently, Kerendi *et al.* [153] described the phenomenon of remote ischemic PostCon. In the anesthetized rats, 5-min occlusion of the renal artery released 1 min before myocardial reperfusion resulted in a significant infarct size limitation and the protective effect was abolished by the administration of 8-sulfophenyl theophylline, a nonselective adenosine receptor blocker. According to the findings of the same team, the extent of infarct size limitation observed was higher than that achieved with the routine, that is, local PostCon (50% *vs*. 23%, respectively) [15]. Surprisingly, the data on the ischemic PreCon evidence for lesser infarct-limiting effect of remote mode of protection compared to local one; so the remote PostCon can hardly be more efficient than local one in the infarct limitation. We also investigated the infarct-limiting effect of remote PostCon using the model of Kerendi *et al.* but still failed to reproduce the results of the team [unpublished observation], which needs further experimentation.

In conclusion, investigation of the molecular mechanisms of PostCon have an apparent potentiality in cardioprotective therapies as far as the prevention of reperfusion-induced myocardial damage is concerned.

6. CLINICAL APPLICATIONS OF PRECONDITIONING

In 1990s, there has been a great enthusiasm as to the perspectives of clinical use of robust protective effect of PreCon [154, 155]. However, certain factors have been lost from consideration. To begin with, since PreCon is a preventive measure, a PreCon stimulus when applied should be used before the onset of test ischemia. Besides, in the most of the cases the PreCon-induced cardioprotection is transient to disappear rapidly after the stimulus applied. It follows, therefore, that the effective clinical use of PreCon would require the registration of the ischemic event startpoint as precisely as possible, *before* the event (which is practically unrealistic). However, at least for MI high risk patients the regularly applied PreCon protocol would seem to be justified on order for the heart to be maintained at the persistently preconditioned state. PreCon might be even more helpful in cardiac surgery, where the onset of ischemia is scheduled. Furthermore, the 'ancestor' form of PreCon, that is, intraoperative local ischemic intervention, can be clinically applied exclusively in cardiac surgery.

Investigation of PreCon in patients undergoing coronary artery bypass graft (CABG) was pioneered by Yellon *et al.* [156] in 1993. Local ischemic procedure induced by 2 episodes of 3-min aortic cross-clamping prior to 10-min global ischemia. Higher tissue ATP levels were found in preconditioned hearts, and this has been the first evidence for the feasibility of the PreCon in humans. Using the protocol just mentioned, Jenkins *et al.* [157] showed lower serum troponin T levels in preconditioned patients. On the other hand, increased need in inotropic stimulation [158] and higher CK levels [159] in preconditioned GABG patients was also shown. In the recent years, in a series of papers Wu *et al.* demonstrated distinct aspects of protection by ischemic PreCon in patients underwent CABG. The protocol included two episodes of 2-min aortic cross-clamping, each followed by 3-min reperfusion performed before prolonged ischemia. The major findings of this series concerning the PreCon benefits are: the improved cardiac index [160], lowered troponin I levels, shortened postoperative mechanical ventilation, lessened use of inotropes [161], fewer ventricular tachyarrhythmias [162], and better right ventricular ejection fraction [163]. And nevertheless, the procedure of local ischemic PreCon has not become the routine practice in cardiac surgery units. The reasons were reviewed by Vaage and Valen [164]. The most important barriers that hamper widespread use of local ischemic PreCon in cardiac surgery are as follows. Firstly, many surgeons tend to avoid repetitive aortic occlusions because of increased risk of atheroembolism. Secondly, it was shown that cardiopulmonary bypass (CPB) itself may induce PreCon response [165]. Thirdly, ethical obstacles may arise in some surgeons facing the necessity of inflicting the additional, albeit ultimately beneficial injury, to the patient's heart.

Remote ischemic PreCon has several advantages in comparison to local one, especially as to its use in cardiac surgery. In particular, there is no need in intermittent aortic cross-clamping in remote PreCon. Besides, this procedure is non-invasive, safe, and relatively easy technically [166]. Its effect on CK and lactate dehydrogenase (LDH) levels in blood sampled from the coronary sinus during CPB was first studied by Gunaydin *et al.* [167]. Thus, despite CK levels were not different between the PreCon and a control group, activity of LDH was higher after remote PreCon induced by 2 episodes of 3-min inflation-deflation of the cuff around the upper limb. Although the change in LDH level is not believed to be a test reliable enough currently, the authors nevertheless suggested that it may somehow reflect activation of anaerobic glycolysis in the myocardium of preconditioned patients. Published very recently, the results of the first randomized clinical trial on remote PreCon in children who underwent repair of congenital heart defects deserve special attention [168]. In this trial, remote PreCon was induced with four 5-min episodes of lower limb ischemia-reperfusion just before the initiation of CPB. As a result, significant reduction in postoperative troponin I, lessened need in inotropic support, and lowered airway resistance were observed in PreCon group. Thus, remote PreCon has turned out to be a safe and effective cardioprotective approach in this category of patients.

Although the existence of such clinical correlates of ischemic PreCon as preinfarction angina and warm-up phenomenon is beyond doubt [169, 170] while the relevance of

both to the development of new cardioprotective strategies is questionable. Nonetheless, there exists both simple and widespread approach closely related to the beneficial effect of preinfarction angina on infarct size, that is, regular physical exercise. It particular, Paraskevaidis *et al.* [171] demonstrated that repeated treadmill exercise test in the patients with stable angina resulted in both early and delayed cardioprotection as assessed by electro- and echocardiographical criteria. Thus, by citing Rezkalla and Kloner [172], - «enhancing cardiac conditioning is yet another reason to stay physically active».

Pharmacological PreCon has the advantage over the rest as being the least invasive form of PreCon. That is why the studies on the pharmacological PreCon were carried out not only in cardiac surgery but also in non-invasive approaches, in particular for the patients with angina and those with angioplasty. Adenosine and adenosine receptor agonists were extensively studied as PreCon mimetics [173]. Mentzer *et al.* [174] demonstrated that high doses of adenosine used as an adjunct to blood cardioplegia during CABG surgery were associated with the improved left ventricular ejection fraction and lessened need for dopamine. Also, high-dose adenosine adjunctive therapy before and during cardioplegia resulted in the reduced rate of such adverse events as myocardial infarction, death, and need for intraaortic balloon counterpulsation [175]. In trial performed by Wasir *et al.* [176] adenosine pretreatment before aortic cross-clamping in CABG surgery was shown to result in the elevated cardiac output and decreased systemic vascular resistance. However, positive effects of adenosine could not be consistently reproduced by others. For instance, Teoh *et al.* [177] demonstrated lowered troponin I levels in CABG patients subjected to ischemic PreCon *vs*. unchanged troponin I levels in patients receiving adenosine or adenosine A_1 receptor agonist GR79236 prior to aortic cross clamp. These data proved to be consistent with the results obtained by Belhomme *et al.* [178] who showed that 5-min infusion of adenosine followed by 10-min washout prior to cardioplegia failed to reduce postoperative troponin I levels. Adenosine-induced PreCon was also evaluated in percutaneous transluminal coronary angioplasty (PTCA). Leesar *et al.* [179] found that adenosine administration 10 min before PTCA resulted in lessened systolic and diastolic dysfunction and decreased lactate production. In contrast, Kopecky *et al.* [180] did not show infarct size limitation with adenosine PreCon in PTCA patients. Despite the controversy, pharmacological PreCon with adenosine is safely used both in cardiac surgery and during revascularization procedures without documented complications.

Several clinical studies tested cardioprotective effects of pharmacological PreCon using halogen-containing volatile anesthetics. Inhalation of isoflurane prior to cardioplegia in CABG patients resulted in the decreased myocardial injury assessed by serum levels of troponin I and MB isoform of CK [181]. Anesthesia with sevoflurane but not with propofol was associated with less postoperative troponin I and higher stroke volume in patients underwent elective coronary surgery [182]. Garsia *et al.* [183] showed that the CABG patients who received sevoflurane before cardioplegia developed lesser number of late adverse cardiac events.

Among K_{ATP} channel openers, nicorandil has been used for pharmacological PreCon. In CABG patients, Kawamura *et al.* [184] observed a significant decrease in a postoperative release of troponin T and CK after nicorandil administration. On the other hand, Blanc *et al.* [185] failed to demonstrate any benefit from nicorandil in such patients. The effects of nicorandil on major cardiovascular events in patients with stable angina were investigated in the IONA trial [186]. It was shown that the incidence of endpoint events was significantly lower in the patients receiving nicorandil, and this effect could be at least in part accounted for by pharmacological PreCon.

Other pharmacological agents such as nitroglycerin and bradykinin were evaluated as potential PreCon mimetics. The benefit of intracoronary infusion of nitroglycerin prior to PTCA was first recognized by Doorey *et al.* [187], which is particularly noteworthy because it took place a year before the introduction of PreCon concept. Leesar *et al.* [188] showed that nitroglycerin may induce second window of protection in PTCA patients. This team also demonstrated that bradykinin can cause pharmacological PreCon when given to the patients just before PTCA [189]. Thus, diverse pharmacological agents including adenosine, volatile anesthetics, nicorandil, nitroglycerin, and bradykinin can confer PreCon effect when patients are exposed to the drug before index ischemic event.

At present, there is a substantial evidence for the effective preconditioning of the human heart. However, a need for cardiac protection from IRI occurs predominantly in the aged patients with such common comorbidities as DM, hypercholesterolemia, and arterial hypertension. Both experimental and clinical data indicate that these factors can blunt or even reverse PreCon response. For instance, Wu *et al.* reported diminished protective effect of ischemic PreCon in aged (>68 years) patients undergoing CABG surgery [161]. Attenuation of ischemic PreCon protection in the DM patients was tackled in the present paper before. Ghosh *et al.* demonstrated no benefit from simulated *in vitro* PreCon in the right atrial myocardial sections obtained from patients with DM or chronic heart failure [190]. Furthermore, several studies showed ineffectiveness of PreCon-induced protection in patients with hypercholesterolemia [191, 192]. These results mean that the occurrence of the pathologies frequently associated with CHD may hinder effective clinical use of PreCon. Taken together, the data considered in this section, suggest that the strong cardioprotective potential of PreCon is clinically underused as yet and deserves further attention. However, it is felt that some modes of PreCon protection (e. g., local and remote ischemic PreCon) may have limited resource in the clinical realm to be restricted wholly to myocardial protection in cardiac surgery. Some noninvasive types of PreCon (e.g., with drugs and/or physical factors) may once become common for the prevention of IRI not only in CABG patients but also during PTCA and unstable angina.

7. CLINICAL APPLICATIONS OF POSTCONDITIONING

PostCon protocol is applied during the initial stage of reperfusion after prolonged ischemia and, therefore, it might be considered a therapeutic cardioprotective approach in contrast to prophylactic cardioprotection performed before the index ischemic event (e. g., ischemic or pharmacological PreCon). This suggests that PostCon may have better prospect of clinical application to become a valuable adjunct to current reperfusion therapies. In theory, PostCon procedure could be performed in the patients undergoing PTCA for acute MI by producing sequential transient balloon inflations-deflations after the intra-arterial plaque debulking.

To date, only solitary studies aimed at investigation of PostCon in the clinical setting have been published. In a randomized, multicenter, controlled study, Staat *et al.* showed that PostCon elicited by four episodes of 1-min ischemia separated by 1-min episodes of reperfusion produced significant cardioprotective effect in the patients with MI undergoing PTCA-stent implantation [193]. The authors observed 36% reduction in the infarct size estimated as an area under curve of serum CK activity during 72 h. Besides, the effectiveness of PostCon was confirmed by both a higher blush grade and less pronounced, albeit statistically insignificant, ST segment elevation after PostCon. No adverse effects were noted during or after PostCon. This study for the first time showed that PostCon can in principle be relied upon as an effective and safe method of protection against reperfusion injury at least in the interventional suite. Recently, Loukogeorgakis *et al.* [194] demonstrated that PostCon can attenuate endothelial dysfunction caused by 20-min occlusion-20-min reperfusion of brachial artery. In this study, three cycles of 10-s ischemia-30-s reperfusion performed just after prolonged ischemia were sufficient to cause an improvement in flow-dependent vasodilation in response to reactive hyperemia.

Thus, the first experience in clinical application of PostCon seems to be encouraging. Additional studies will be required, however, to test more thoroughly all potentialities of PostCon in the clinical arena, particularly, in the cardiac surgery.

CONCLUSION

Since the discovery of PreCon in 1986, much research effort has been applied at the investigation of cellular mechanisms of this phenomenon. Although the end-effector(s) of PreCon protection is (are) still unknown, recent research has made significant strides in identifying the molecular targets of pharmacological interventions with a propensity of mimicking PreCon. The concept of pharmacological PreCon has proved to be a prerequisite of the clinical use of PreCon. A number of pharmacological agents might be used for eliciting the subthreshold ischemic PreCon protection in the patients with anginal episodes who take high risk of MI. Of interest seems to be investigation of the mechanisms underlying metabolic PreCon that may be helpful in approaching the major clinical goal, that is, the clinical implementation of the phenomenon in the sustained fashion. Besides, noninvasive experimental PreCon versions are suitable enough to be extrapolated to some clinical scenarios directly.

Much more recently introduced PostCon may also turn out to be a valuable means as to the myocardial reperfusion injury prevention. Although our knowledge about the molecular background of the PostCon is currently limited, one can anticipate that further research would be primarily targeted to the development of drug therapies, in particular, pharmacological activation of RISK pathway as a promising tool for attenuation of both ischemic and reperfusion injury.

Finally, the additional studies are necessary for the development of novel pharmacological analogues of PreCon and PostCon with stable and reliable cardioprotective action and as minimal as possible side-effects.

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ABBREVIATIONS

ACE	=	Angiotensin-converting enzyme
CABG	=	Coronary artery bypass graft
CHD	=	Coronary heart disease
CK	=	Creatine kinase
CPB	=	Cardiopulmonary bypass
DM	=	Diabetes mellitus
ERK	=	Extracellular-signal regulated kinase
GPCR	=	G-protein coupled receptors
GSK-3β	=	Glycogen synthase kinase-3β
IRI	=	Ischemia-reperfusion injury
JNK	=	c–jun NH_2-terminal kinase
K_{ATP} channel	=	ATP-sensitive potassium channel
LDH	=	Lactate dehydrogenase
MI	=	Myocardial infarction
mPTP	=	Mitochondrial permeability transition pore
NO	=	Nitric oxide
PI3K	=	Phosphatidylinositol-3-kinase
PKA	=	Protein kinase A
PKB	=	Protein kinase B
PKC	=	Protein kinase C
PKG	=	Protein kinase G
p38MAPK	=	p38 mitogen-activated protein kinase

PreCon	=	Preconditioning
PostCon	=	Postconditioning
PTCA	=	Percutaneous transluminal angioplasty
rEPO	=	Recombinant erythropoietin
RISK	=	Reperfusion injury salvage kinase
ROS	=	Reactive oxygen species
SNAP	=	S-nitroso-N-acetylpenicillamine
TK	=	Tyrosin kinase
US	=	Ultrasound

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