Current perspectives

Involvement of nitric oxide in ischemic preconditioning

Pasquale Pagliaro, Donatella Gattullo, Raffaella Rastaldo*, Gianni Losano*

*Department of Clinical and Biological Sciences, *Department of Neuroscience, Division of Physiology, University of Turin, Turin, Italy*

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In ischemic preconditioning, nitric oxide (NO) limits the extension of a subsequent infarct and protects against ischemia/reperfusion-induced endothelial dysfunction, arrhythmias and myocardial stunning. The protective activity concerns both the first and the second window of protection. The antiarrhythmic effect is attributed to microvessel dilation and to the production of cyclic guanosine monophosphate in the myocardium. The limitation of the infarct size is likely to depend on the opening of the mitochondrial adenosine triphosphate-sensitive potassium channels, to which NO participates via the activation of a protein kinase C (PKC). The endothelial protection involves an NO-mediated reduction in neutrophil adherence to the coronary endothelium and platelet aggregation and is accompanied by an enhanced response to vasodilator stimuli.

During preconditioning ischemia, NO is released from the coronary endothelium as a result of bradykinin-induced activation of B_2 endothelial receptors. In addition to the early protection, endothelium-derived NO is also responsible for a signaling cascade which leads to the activation of myocardial inducible NO synthase, which in turn is responsible for the release of NO involved in the delayed protection. The signaling cascade includes the activation of PKC-ε, tyrosine kinase and some mitogen-activated protein kinases. It has been suggested that the activation of PKC-ε is mediated by peroxynitrite produced by the combination of NO and the superoxide anion, the latter being generated during reperfusion which follows preconditioning ischemia.

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Address:

Dr. Pasquale Pagliaro

Dipartimento di Scienze Cliniche e Biologiche Università degli Studi Ospedale S. Luigi Gonzaga Regione Gonzole 10043 Orbassano (TO) E-mail: pasquale.pagliaro@unito.it

Ischemic preconditioning, i.e. the myocardial protection which follows one or more brief coronary occlusions, has a peculiar time course: after an early phase or first window of protection, which occurs immediately after the preconditioning maneuver and lasts 1-3 hours, the heart is normally sensitive to ischemic insults for about 20-24 hours. Then it becomes protected again for a period lasting approximately 72-96 hours, this period being termed the delayed phase or second window of protection[1,2].

The effect of ischemic preconditioning consists of a limitation in the size of an infarct caused by a sufficiently long coronary occlusion as well as by the limitation of ischemia/reperfusion arrhythmias and by a faster recovery from stunning, which is observed mainly during the phase of delayed protection[1,3-9]. It has also been seen that the coronary vasculature of a preconditioned heart shows a faster response to vasodilator stimuli and a reduced response to vasoconstrictor agents[10-12]. Nitric oxide (NO) participates in both windows of protection and it is also believed to play a role in the signaling pathway from the first to the second window.

Antiarrhythmic effect of nitric oxide

First window of protection. To our knowledge the first papers dealing with the possible involvement of NO in preconditioning were published in the early '90s by the group of Vegh and Parratt[3,13,14]. Initially attention was paid to the protection against the development of arrhythmias and electrocardiographic modifications in ischemia and reperfusion during the first window of protection. In fact, in the anesthetized dog it was seen that the preliminary administration of the NO synthase inhibitor L-N$^{\omega}$-arginine-methyl-ester (L-NAME) impaired the protective effect exerted by ischemic preconditioning against ischemia/reperfusion-induced ventricular tachycardia and fibrillation as well as against ST segment elevation[3]. Previously, the same group had reported that bradykinin, administered to anesthetized dogs in a dose which did not

alter coronary flow, totally prevented ventricular tachycardia and fibrillation and reduced the number of premature beats during a subsequent period of 25 min of occlusion of the left descending coronary artery[13]. Such a result suggested the possibility that bradykinin contributes to the protective effect of ischemic preconditioning acting as an "endogenous myocardial protective substance".

Later on, it was seen that the protection exerted by bradykinin against the development of arrhythmias during prolonged ischemia was prevented by L-NAME, which indicated that NO was a mediator of the antiarrhythmic effect of bradykinin[14]. However, L-NAME exerted its effect only during the period of ischemia, but did not reduce the protective effect of bradykinin against reperfusion arrhythmias. The administration of the B_2 receptor blocker icatibant (i.e. HOE-140), before prolonged ischemia after ischemic preconditioning, showed that the activation of these receptors by bradykinin was required to achieve the antiarrhythmic effect[15].

On the basis of the above reported results, the following hypothesis was proposed to explain the mechanism involved in the prevention of ischemia-induced arrhythmias by preconditioning[16,17]: the reduction in pH occurring in the coronary vessel wall during the preconditioning maneuver activates a kininogenase present in the vascular smooth muscle fibers. Kininogenase causes the production of bradykinin from a plasma kininogen. Bradykinin activates endothelial B_2 receptors which are in turn responsible for the release of NO and prostacyclin (PGI_2). Although NO and prostacyclin cannot be considered the end effectors of the antiarrhythmic protection, their protective role has been observed in isolated guinea pig and rat hearts, in cultured rat cardiomyocytes and in intact dogs[18-22]. It is noteworthy that Arad et al.[19] suggest that in the isolated rat heart PGI_2, rather than NO, is responsible for the protection against reperfusion arrhythmias.

The possibility that NO is a mediator of ischemic preconditioning against ischemia/reperfusion arrhythmias during the first window of protection has not been confirmed by the experiments of Lu et al.[23]. These investigators studied the effect of preconditioning on the development of reperfusion arrhythmias in the rat. They observed that preconditioning reduced the occurrence of reperfusion premature beats from 100% of rats to 17% only, and of ventricular tachycardia from 93 to 8%. This antiarrhythmic effect of ischemic preconditioning was not abolished by the preliminary administration of either L-NAME or of the NO synthase inhibitor L-mono-methyl-arginine (L-NMMA) and was not enhanced by pretreatment with L-arginine. This negative effect could depend on the fact that the authors focused their attention on the arrhythmias occurring during reperfusion, i.e. when the antiarrhythmic effect is mainly attributed to PGI_2. In these experiments, in fact, arrhythmias were not observed during coronary occlusion which, however, lasted only 5 min.

It is also possible that the lack of evidence for a role of NO in preventing arrhythmias in preconditioned hearts depends on the animal species under evaluation, since it has been confirmed in dogs[24] and sometimes refuted in rats[23,25,26]. Unlike what was observed in these latter studies, Bilinska et al.[27] report that NO donors mimic the effect of ischemic preconditioning on reperfusion arrhythmias also in isolated rat hearts. In particular they saw that nitroglycerin, administered for 5 min and washed out for another 5 min before ischemia, protected the heart against reperfusion-induced ventricular tachycardia and fibrillation, whereas the same compound increased the incidence of arrhythmias if administered at the time of reperfusion. The increased incidence of arrhythmias was also observed when another NO donor, 3-morpholino-synodimine-hydrochloride (SIN-1), was administered during reperfusion. However, this effect of NO donors administered during reperfusion was not observed in other investigations[28,29].

Second window of protection. In the dog it has been seen that NO can exert an antiarrhythmic effect also during the delayed protection obtained with the infusion of monophosphoryl lipid A. In fact, the ischemia/reperfusion arrhythmias are not prevented if aminoguanidine and meclofenamate, inhibitors of inducible NO synthase (iNOS) and cyclooxygenase respectively, are administered[21]. In the same animal species, even high rate pacing was seen to induce a delayed antiarrhythmic protection[24], which was attenuated by aminoguanidine administered half an hour prior to the relevant ischemia[30].

Possible pathway of the antiarrhythmic effect of nitric oxide. The final step through which NO leads to protection against ischemia/reperfusion arrhythmias has not yet been fully elucidated. The NO-induced production of cyclic guanosine monophosphate (cGMP) in the myocardium could be the mediator of such a protection. In dogs with a healed myocardial infarction, ventricular fibrillation, which may be induced by coronary occlusion during exercise, was prevented by the infusion of 8-bromo cGMP, before and during exercise[31]. In isolated rat hearts, methylene blue, a soluble guanylate cyclase inhibitor, was seen to enhance the incidence of reperfusion ventricular arrhythmias[32]. In addition to the production of cGMP in myocardial fibers, even NO-cGMP induced coronary vasodilation has been suggested to contribute to the antiarrhythmic effect of preconditioning with the participation of prostacyclin which is also released by the activity of bradykinin on B_2 endothelial receptors[21,33].

A recent study which shows that bradykinin B_2 receptors are poorly represented in adult cardiomyocytes[34], further supports the relevance of the endothelial receptors in the protection.

In addition to the activity of bradykinin on endothelial B_2 receptors[16,17], the release of NO during the phase of early protection could also be attributed to the activation of the endothelial cells by adenosine released by the myocardium during preconditioning ischemia. In fact, adenosine-induced dilation of porcine isolated subepicardial arterioles was seen to be prevented by L-NMMA[35].

Limitation of the infarct size by nitric oxide

After the initial studies of the group of Vegh and Parratt[3,13-16] on the antiarrhythmic role of NO, attention was also paid to the possible involvement of NO in the limitation of the infarct size by preconditioning. The contribution of NO to myocardial protection has mainly been studied by inhibiting its production as well as by administering NO donors. The data obtained by such techniques indicate that NO contributes to both the early and delayed protection[9,36].

First window of protection. Experiments performed in rats[37] showed that, if three preconditioning cycles of 5 min of coronary occlusion and 5 min of reperfusion preceded 20 min of occlusion and 1 hour of reperfusion, the size of the resulting infarct with respect to the risk area was significantly smaller (2.2 vs 57%) than in the absence of preconditioning. The involvement of NO in ischemic preconditioning was suggested by the fact that only in non-preconditioned animals ischemia/reperfusion reduced the endothelium-dependent coronary vasodilation by acetylcholine.

In *in vivo* rabbit hearts it has been demonstrated that a non-methylated inhibitor of NO synthase, L-N^{ω}-nitro-arginine (L-NNA) increases the size of an infarct produced by a sufficiently long coronary occlusion followed by reperfusion[33]. In these experiments, L-NNA was administered either before occlusion or during reperfusion. A preconditioning-dependent increase in endothelial NO release during the early phase of protection has been confirmed in the anesthetized goat in which some changes induced in coronary vascular reactivity by ischemic preconditioning were prevented by the preliminary administration of L-NNA[11]. Consistent with these results are those obtained in isolated and perfused rat hearts[38], in which the myocardial protection obtained with a 600 b/min pacing was abolished if NO synthesis was prevented with L-NNA. Interestingly enough, in the same investigation it was observed that the myocardial protection was also abolished if the rats had been fed with a cholesterol-enriched diet for 24 weeks before the experiments. Cholesterol, in fact, is known as a scavenger of NO. Also in isolated perfused rat hearts it was seen that the iNOS inhibitor aminoguanidine reduces the early protective effect obtained by means of 15 min of perfusion with a non-oxygenated buffer solution[39]. In this investigation it was observed that the protection was also impaired by VIP 10-28, an inhibitor of the vasoactive intestinal peptide, so that the authors concluded that a coordination in the regulation of cardioprotection should be brought about by NO and vasoactive intestinal peptide. It is remarkable that the early protective effect of NO was blunted by inhibiting the iNOS, which, due to its slow activation, is usually considered active only in the second window of protection. It is possible that the partial suppression of myocardial protection by aminoguanidine depended on the yet incomplete activation of iNOS in the presence of the still persisting effect of NO produced by constitutive endothelial NO synthase (eNOS). Furthermore, it cannot be excluded that aminoguanidine also inhibited the eNOS isoforms as it has been reported that this inhibitor is not fully selective for iNOS only[40].

Also data obtained after the administration of NO donors are in favor of a role of NO in the first window of protection[41,42]. In the cat it was observed that after myocardial ischemia/reperfusion, the release of NO from the coronary endothelium is reduced[41]. This reduction is attributed to the so-called endothelial dysfunction, which is considered responsible for the adherence of polymorphonuclear leukocytes to the endothelium with production of reactive oxygen species and the occurrence of myocardial necrosis during reperfusion. The administration of the NO donor SIN-1 just before reperfusion attenuated myocardial necrosis, suggesting that exogenous NO can replace endogenous NO, when the latter is not produced as a result of endothelial dysfunction[42]. In the anesthetized dog it was seen that supplementing blood cardioplegia with L-arginine or with the NO donor N-nitratopivaloyl-S-(N'-acetylalanyl)-cysteine ethylester reduced the infarct size and preserved endothelial function after regional or global ischemia and reperfusion[42]. The preservation of endothelial function and, consequently, of NO synthesis was also in this case revealed by an attenuation of the accumulation of neutrophils in the area at risk.

Second window of protection. In the heart of anesthetized rabbits, the infarct size observed when a 30 min coronary occlusion was followed by 2 hours of reperfusion was reduced by about 50% if the occlusion was preceded by preconditioning maneuvers performed 48 hours earlier[43]. The reduction in infarct size did not take place if the selective iNOS inhibitors aminoguanidine or dexamethasone were given before ischemic preconditioning[43]. These results show the involvement of NO produced by iNOS in the second window of protection against the extension of the infarct size.

Also data obtained after the administration of NO donors are in favor of a role of NO in the second window of protection[9,36,44]. In particular, Takano et al.[36] demonstrated that in conscious rabbits, the administration of two structurally unrelated NO donors [diethyl-

enetriamine/NO and S-nitroso-N-acetylpenicillamine (SNAP)] induced protection 24 hours later against the extension of the infarct size in a magnitude similar to that observed during the late phase of preconditioning obtained with short episodes of ischemia. They also showed that the limitation of the infarct size observed during the late phase of ischemic preconditioning is mediated by the production of NO which derives from the increased activity of iNOS 24 hours after the preconditioning maneuvers[9].

Possible mechanisms of the limitation of the infarct size. The activation of mitochondrial adenosine triphosphate (ATP)-sensitive potassium (mito-K_{ATP}) channels is considered to be one of the compulsory final steps leading to myocardial protection[45-50]. It is accepted that protein kinase C (PKC) can activate these channels[47-51]. In particular, early mito-K_{ATP} channel activation has been attributed to PKC-α and/or PKC-δ[49]. Although it is not yet clear whether NO can open mito-K_{ATP} channels directly or only through the activation of PKC, it is noteworthy that in a number of investigations the inhibitor of PKC, chelerythrine, has been seen to suppress the protection induced not only by brief ischemia or adenosine, but also by the administration of exogenous NO[44,45,49,52].

A shortening of the action potential duration observed in guinea pig hearts during endotoxic shock provides reasonably convincing evidence that NO can also activate sarcolemmal ATP-sensitive potassium channels[53]. The activation of these channels is believed to occur via cGMP rather than via PKC. The opening of sarco K_{ATP} channels by NO can contribute to the reduction in the intracellular Ca^{2+} concentration and in myocardial metabolism brought about by ischemic preconditioning. Cardioprotection by NO has also been attributed to microvascular dilation[54], induction of heat stress proteins[55], and to the antiarrhythmic effect in which myocardial cGMP seems to play a relevant role[32,56]. In particular, recent studies performed on cultured rat ventricular cardiomyocytes showed that the preconditioning obtained with simulated ischemia followed by reoxygenation was abolished by NO synthase inhibition[56]. It was also abolished by the blocker of guanylate cyclase 1H-[1,2,4]oxadiazolo [4,3-a]quinoxalin-1-one (i.e. ODQ), but not by chelerythrine, so that the authors concluded that NO-induced cardioprotection was mediated by cGMP and was independent of PKC. However, in spite of the results in favor of a role of cGMP, the exclusion of PKC from the signaling cascade induced by ischemic preconditioning has not yet been sufficiently supported.

As seen above, the protective role of NO includes the inhibition of the adherence of neutrophils to the coronary endothelium during a sufficiently long period of ischemia and the release of reactive oxygen species during reperfusion[41]. Even this activity of NO is likely to be responsible for the reduction of an infarct size[42].

In spite of all the above reported findings, some controversy still exists about the role of NO in myocardial protection. In fact, a number of investigators[57-59] report that in *in situ* and isolated rabbit hearts, it is the inhibition, and not the stimulation, of NO release which limits the ischemia/reperfusion injuries. Moreover, in isolated rabbit hearts it has recently been seen that early preconditioning can be mimicked by SNAP, while L-NAME cannot block the protective effect of a preconditioning ischemia against the extension of the infarct size[52]. It has then been suggested that exogenous, but not endogenous, NO could trigger early myocardial protection.

Since, from the studies on the first window of protection, opposite results were obtained in the same animal species, the difference in the results cannot be attributed to the difference in the species. It is possible that the discrepancy depends on whether the NO synthase inhibitors were methylated, as was the case for L-NAME and L-NMMA which sometimes induced myocardial protection against the extension of an infarct, or non-methylated, as was the case for L-NNA which always prevented ischemic preconditioning from causing protection. It is possible that the protective effect of methylated inhibitors is due to their antimuscarinic activity which contributes to coronary vasoconstriction, myocardial ischemia and to the release of adenosine[57-59]. The explanation may also reside in the fact that during prolonged ischemia NO synthase produces more nitroxyl anion than NO, especially when the antioxidant defenses of the myocardium are reduced[29]. Since the nitroxyl anion has deleterious effects in such a condition, NO synthase inhibition may result in a beneficial effect.

Limitation of myocardial stunning by nitric oxide

The contribution of ischemic preconditioning to the faster recovery from post-ischemic stunning has been mainly described as an aspect of the delayed period of protection, in which NO produced by iNOS plays a pivotal role[8,9].

In conscious rabbits, the group of Bolli[44] studied the effect of six cycles, each lasting 4 min, of coronary occlusion followed by 4 min of reperfusion for 3 consecutive days. The authors studied the occurrence and the severity of myocardial stunning by evaluating the deficit in systolic wall thickening after the last cycle of each day. They observed that the deficit decreased from day to day, demonstrating that the previous occlusions had caused a delayed preconditioning against stunning. A protection against stunning was already observed after the sixth 4 min reperfusion of the first day if the animals had been treated with nitroglycerin 24 hours before. Such a delayed preconditioning against myocardial stunning was prevented if chelerythrine was administered together with nitroglycerin. This finding

suggested that the protection was obtained via a PKC-dependent pathway. Using a protocol similar to that of the previous set of experiments (6 occlusion per day for 3 days), the same group[60] observed that the administration of nitroglycerin or of the NO donor SNAP during ischemia/reperfusion sequences attenuated myocardial stunning on the first day to an extent similar to that observed in late preconditioning. It was also observed that, after the sixth occlusion of the first day, the systolic wall thickening was improved to the same extent as after the last occlusion of the third day in the absence of NO donors. These results strongly suggest that NO is responsible for the prevention of myocardial stunning. The results of this study also showed that the combination of pharmacological and ischemic preconditioning can improve the deficit in systolic wall thickness during the first day (i.e. during the first window of protection). However, the protection against stunning was not achieved if nitroglycerin (2 μg/kg/min) was infused prior to ischemia for 1 hour followed by 30 min of washout.

The question arises why nitroglycerin was ineffective in the early phase if administered before and not during the ischemia/reperfusion sequence. It may be speculated that a synergistic action of NO donors and ischemia may trigger an early protection against stunning, which is likely to be effective because of the addition of exogenous NO with the endogenous one released as a result of the preconditioning maneuver. The authors, in fact, do not exclude that a higher dose of nitroglycerin administered before ischemia could be effective in eliciting an early protection against stunning[60].

Nitric oxide and vascular preconditioning

As mentioned above, in addition to myocardial injury, ischemia/reperfusion can also be responsible for the endothelial damage mediated by the adherence of neutrophils to the endothelium during prolonged ischemia and by the release of reactive oxygen species during reperfusion[41,42]. The prevention of endothelial dysfunction by ischemic preconditioning may be considered as an aspect of the vascular preconditioning, i.e. of the effect of ischemic preconditioning on the coronary vasculature. Vascular preconditioning also includes a reduction in platelet aggregation as well as a change in coronary reactivity[10-12].

In ischemia/reperfusion, neutrophil adhesion to the endothelial cells is mediated by the expression of adhesion molecules such as the intercellular adhesion molecule-1 (ICAM-1)[61,62]. In rat aortic endothelial cells, ICAM-1 expression was seen to be induced by episodes of anoxia and of reoxygenation and inhibited by preconditioning[62]. The incubation of rat aortic endothelial cells and neutrophils with L-NNA showed that NO synthase inhibition prevents the protective effect of preconditioning on ICAM-1 expression and neutrophil adhesion[63], suggesting that a reduced endothelial release of NO contributes to the endothelial damage by ischemia/reperfusion.

Although ischemic preconditioning prevents endothelial damage in both early and delayed protection, experiments performed in rats showed that the latter is not affected by selective inhibition of iNOS with N-(3-aminomethyl-benzyl-acetaminide)[64]. This finding seems to be in contrast with the demonstrated role of NO in delayed protection against myocardial stunning and the extension of an infarct area. It has also been suggested that the overproduction of NO by iNOS can cause injury to the endothelial cells after reacting with the superoxide anion to form peroxynitrite. However, the fact that the iNOS is not involved in the delayed protection of endothelial cells does not exclude a role of NO in this aspect of vascular preconditioning. Experiments performed on cultured rat endothelial cells showed that ischemic preconditioning can induce an increase of nuclear factor AP-1 in the nucleus of these cells. AP-1 could then determine an increased expression of eNOS RNA[65].

The change in vascular reactivity has been evidenced in goats and dogs[10-12]. In particular, in the anesthetized goat, immediately after ischemic preconditioning obtained by means of 5 min of occlusion of the left circumflex coronary artery, clearly evident changes were observed in the reactive hyperemia which followed 15 s of occlusion of the same artery[11]. The changes consisted in a reduction in the total hyperemic flow and in the acceleration of the vasodilation which occurs in the initial phases of hyperemia. While the reduction of the total hyperemic flow, which was prevented by the blockade in adenosine A_1 receptors, was attributed to a reduction in myocardial metabolism, the acceleration of the increase in flow, which was suppressed by the administration of L-NNA, was attributed to the release of NO. A further investigation in the anesthetized goat showed that diazoxide, which causes myocardial protection, was unable to accelerate the vasodilation of reactive hyperemia[66]. Since diazoxide activates mito-K_{ATP} channels directly, without the involvement of the coronary endothelium, the finding suggests that the aspect of vascular preconditioning we studied depends on the endothelial release of NO and not on one of the final steps of myocardial protection limiting the infarct size.

Nitric oxide from the first to the second window of protection

On the basis of the above description, it appears that NO participates in both the first and the second window of protection. Recently, Xuan et al.[67] have shown that, while the release of NO during the first hour after preconditioning ischemia can be attributed to the activa-

tion of eNOS, the involvement of NO in the second window mainly depends on the delayed activation of myocardial iNOS. From these data the authors suggest a role for eNOS as a trigger and for iNOS as a mediator of late preconditioning. The activation of iNOS has also been observed when late cardioprotection was initially triggered by activation of adenosine A_1 receptors[68] or by infusion of monophosphoryl lipid A[21].

Although the signaling pathways leading to the second window have not yet been fully elucidated, we think that the following points should be entertained:
1) both early and late myocardial protection are mediated by the opening of mito-K_{ATP} channels[47-50,69]. The opening of these channels by NO may involve the activation of PKC[47,49,50]. It has been suggested that, in late preconditioning, these channels may play an essential role against infarction, but not against stunning[9];
2) during the preconditioning maneuver eNOS is responsible for the release of NO[3,33,42,67];
3) since the late myocardial protection can be abolished using N-(2-mercaptopropionyl)-glycine, a scavenger of peroxynitrite and of the hydroxyl radical, a role of these two compounds has been suggested[36]. It has been proposed that NO released by the endothelium can react with the superoxide anion produced during the reperfusion which follows preconditioning ischemia[36,37]. The reaction produces peroxynitrite, which can also originate the hydroxyl radical. Peroxynitrite and the hydroxyl radical activate the ε-isoform of PKC, thus triggering a signaling cascade which includes tyrosine kinase, some mitogen-activated protein kinases (MAPK) and the nuclear factor kappa B (NF-κB), a transcription factor for genes involved in inflammatory responses[70]. Such a cascade leads to an increased synthesis of iNOS (Fig. 1). Although the role of peroxynitrite may be accepted, controversy exists whether it may play a beneficial or deleterious role during reperfusion. Indeed, a deleterious effect of this molecule has been often observed in *in vitro* studies, whereas most of the *in vivo* studies report a beneficial effect[29,41,43];
4) it has also been proposed that the pathway involving tyrosine kinase, MAPK and NF-κB is activated by oxidative stress without the participation of NO and of peroxynitrite[71];
5) it is also possible that NF-κB is directly generated by the oxidative stress occurring during reperfusion after preconditioning ischemia[72,73].

From points 3, 4 and 5 it appears that iNOS can be activated by pathways which do not necessarily require the endothelial release of NO occurring during preconditioning maneuvers. It may then be argued that some pathways link the preconditioning maneuver directly with the late protection without the intervention of the cascade leading to the early protection.

With regard to the role of peroxynitrite, it is noteworthy that the use of N-(2-mercaptopropionyl)-glycine allowed Hoshida et al.[73] to suppress not only the delayed but also the early protection, suggesting

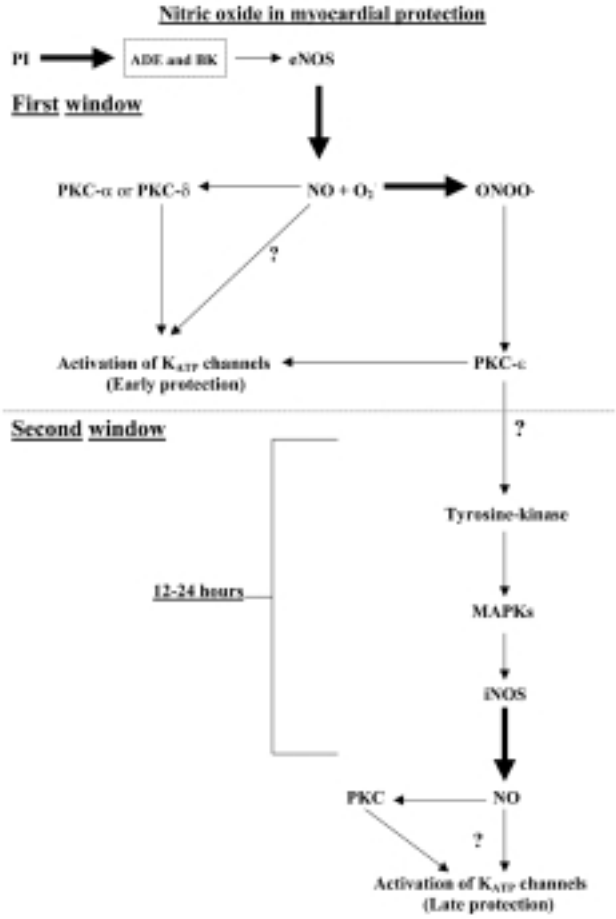

Figure 1. *Nitric oxide pathways in early and late myocardial protection by ischemic preconditioning. Both pathways cause the activation of K_{ATP} channels.* Thick arrows *indicate production,* thin arrows *indicate activation. ADE = adenosine; BK = bradykinin; eNOS = endothelial constitutive nitric oxide synthase; iNOS = inducible nitric oxide synthase; MAPKs = mitogen-activated protein kinases; NO = nitric oxide; O_2^- = superoxide anion; $ONOO^-$ = peroxynitrite; PI = preconditioning ischemia; PKC-α, PKC-δ and PKC-ε = isoforms α, δ and ε of protein kinase C.*

that even the early opening of K_{ATP} channels may require the activation of PKC by peroxynitrite. Finally, in a feline model of ischemia/reperfusion it has been observed that the infusion of 2 μM of peroxynitrite during reperfusion provided cardioprotection by reducing neutrophil adhesion to the coronary endothelial cells, thus preventing the release of reactive oxygen species during reperfusion[74]. It was then proposed that such a beneficial effect could be due to the reaction of peroxynitrite with glutathione to form S-nitrosoglutathione which acts as an NO donor.

Clinical implications

Although the endpoint protections examined in humans are different from those analyzed in studies performed in experimental animals, a protective effect of ischemic preconditioning has been suggested even in the clinical setting. A protection by preconditioning seems to provide a reliable explanation of the observa-

tion that the extension of an infarct as well as the incidence of ischemia-induced ventricular arrhythmias are usually reduced in patients who previously experienced anginal attacks[5,75]. Moreover, in studies performed during percutaneous transluminal coronary angioplasty it was seen that the occurrence of arrhythmias was higher during the first than during the second inflation[6], while the ST segment shift and the severity of cardiac pain during the second inflation were less than that during the first one[76].

In spite of numerous experimental data concerning the protective effects of NO against arrhythmias, extension of infarct size and stunning in animal models[3,27,44,60], there are so far only a few studies on the role of NO in ischemic preconditioning in humans. Recently, it has been reported that a 4-hour intravenous infusion of nitroglycerin provides protection against myocardial dysfunction during coronary angioplasty 24 hours later, as evidenced by reduced ST segment elevation, attenuation of contractile dysfunction and less pain. This study is particularly important because it shows that a clinically feasible tool, i.e., nitroglycerin, may induce protection[77].

Conclusions

Several data are in favor of a relevant role of NO in the first and in the second window of protection elicited by ischemic preconditioning. Myocardial protection includes the prevention of ischemia/reperfusion arrhythmias, the faster recovery from stunning and the limitation of an infarct size. The vascular aspect of ischemic preconditioning concerns the prevention of endothelial dysfunction and a change in coronary reactivity. NO, released by the activity of eNOS, is involved in the early protection and can trigger the cascade which leads to the delayed one. Since one of the final steps to both early and late preconditioning is represented by the opening of mito-K_{ATP} channels while the inactivation of PKC has been seen to prevent both phases of protection by NO donors, it may be concluded that it is more likely that the activity of NO on the channels occurs via the activation of PKC rather than directly. Even cGMP has been proposed as a mediator of the protective activity of NO.

In spite of the various implications of NO, it must be stressed that several other mechanisms [9,17,50] interact in the achievement of the myocardial and vascular protection which characterizes ischemic preconditioning.

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