

## Nitric oxide triggers late preconditioning against myocardial infarction in conscious rabbits

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**Qiu, Yumin, Ali Rizvi, Xian-Liang Tang, Srinivas Manchikalapudi, Hitoshi Takano, Asad K. Jadoon, Wen-Jian Wu, and Roberto Bolli.** Nitric oxide triggers late preconditioning against myocardial infarction in conscious rabbits. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 42): H2931–H2936, 1997.—We tested the hypothesis that late preconditioning (PC) against myocardial infarction is triggered by the formation of nitric oxide (NO). Conscious rabbits underwent a 30-min coronary occlusion followed by 3 days of reperfusion. In *group I* (control group,  $n = 10$ ), rabbits were not preconditioned, whereas in *group II* ( $n = 10$ ), they were preconditioned 24 h earlier with a sequence of six 4-min occlusion/4-min reperfusion cycles. Myocardial infarct size (tetrazolium staining) was reduced by 50% by PC ( $28.6 \pm 3.2\%$  of the risk region in *group II* vs.  $56.9 \pm 5.9\%$  in controls,  $P < 0.05$ ). This reduction in cell death was associated with improved recovery of myocardial function [systolic thickening fraction (by sonomicrometry) at 3 days:  $2.0 \pm 11.0\%$  of baseline in *group II* vs.  $-20.0 \pm 2.8\%$  in *group I*,  $P < 0.05$ ]. *Group III* rabbits ( $n = 11$ ) underwent the same protocol as *group II* except that the rabbits received the NO synthase inhibitor  $N^G$ -nitro-L-arginine (L-NNA, 13 mg/kg) before the PC ischemia. In these animals, infarct size did not differ significantly from that observed in control rabbits, indicating that L-NNA completely blocked the development of late PC against myocardial infarction. In *group IV* ( $n = 9$ ), rabbits received L-NNA as in *group III*, but without the six occlusion-reperfusion cycles, and were subjected to the 30-min occlusion 24 h later. In this group, infarct size did not differ from that observed in controls, demonstrating that pretreatment with L-NNA, in itself, did not affect the extent of cell death. Taken together, these results indicate that, in the conscious rabbit, the development of late PC against myocardial infarction is triggered by the generation of NO during the PC ischemia. It is proposed that NO plays a key role in the delayed myocardial adaptation to ischemic stress.

L-arginine; nitric oxide synthase; oxygen radicals; nitrogen radicals; myocardial ischemia-reperfusion

ISCHEMIC PRECONDITIONING (PC) elicits an early (1, 7, 8–10, 26, 28) and a late (2, 3, 5, 18, 22, 23, 28, 31, 32, 34, 39) phase of protection against ischemic injury. Although much research has focused on the early phase of ischemic PC, the mechanism for the late phase remains poorly understood (4). We (5) have previously found that  $N^G$ -nitro-L-arginine (L-NNA), an inhibitor of nitric oxide (NO) synthase, blocks late PC against myocardial stunning, indicating that NO triggers this form of

delayed myocardial adaptation. However, it is unknown whether NO also triggers late PC against infarction. Myocardial stunning and infarction represent two very different types of injury, and the effects of PC on one cannot be extrapolated to the other. For example, in dogs the early phase of PC confers powerful protection against myocardial infarction (1, 26) but apparently fails to protect against the stunning induced by a 10- or 15-min coronary occlusion (4, 24, 27). Conversely, in conscious pigs a sequence of ten 2-min coronary occlusions elicits a late PC effect against stunning (31, 32, 34) but not against infarction (28). These examples of a dissociation between the effects of PC on stunning and on infarction underscore the notion that, at least in certain experimental conditions, different mechanisms may be involved in the PC protection against reversible and irreversible ischemic injury (4).

Accordingly, the goal of the present study was to explore the role of NO in the development of the late phase of PC against myocardial infarction. Because the very existence of a delayed protection against cell death is somewhat controversial (16, 28, 33), we first determined whether a late PC effect against infarction does occur in our conscious rabbit model. To this end, we used the same PC protocol (six 4-min occlusion/4-min reperfusion cycles) that has previously been found to induce a potent and reproducible late PC effect against myocardial stunning in this preparation (5, 22). We then investigated whether administration of L-NNA during the PC ischemia blocks the delayed protection against infarction. The study was conducted in conscious animals to obviate the potentially confounding influence of factors associated with open-chest preparations, such as anesthesia, surgical trauma, abnormal hemodynamics, elevated catecholamine levels, fluctuations in body temperature, exaggerated formation of reactive oxygen species (ROS), release of cytokines, etc., which could interfere with NO-mediated signaling, myocardial infarction, and/or ischemic PC (7, 14, 9). The results demonstrate, for the first time, that generation of NO triggers late PC against infarction.

### MATERIALS AND METHODS

The conscious rabbit model of myocardial ischemia has been described in detail previously (5, 22) and is briefly summarized here.

**Experimental preparation.** New Zealand White male rabbits (wt  $2.4 \pm 0.1$  kg) were instrumented under sterile conditions with a balloon occluder around a major branch of the left coronary artery, a 10-MHz pulsed Doppler ultrasonic crystal in the center of the region to be rendered ischemic, bipolar pacing leads in the left atrial appendage, and bipolar electrocardiogram (ECG) leads on the chest wall. The chest wound was closed in layers, and a small tube was left in the thorax for 3 days to evacuate air and fluids postoperatively. Gentamicin was administered before surgery and on the first and second postoperative days (0.7 mg/kg im each day). Rabbits were allowed to recover for a minimum of 10 days after surgery.

**Experimental protocol.** Throughout the experiments, rabbits were kept in a cage in a quiet, dimly lit room. Left

ventricular (LV) systolic wall thickening (WTh), range gate depth, and ECG were continuously recorded on a thermal array chart recorder. Regional myocardial function was assessed as systolic thickening fraction using the pulsed Doppler probe, as previously described (5, 22). All rabbits were subjected to a 30-min coronary artery occlusion followed by 3 days of reperfusion. The performance of successful coronary occlusion was verified by observing the development of S-T segment elevation and changes in the QRS complex on the ECG and the appearance of paradoxical wall thinning on the ultrasonic crystal recordings. Diazepam was administered 20 min before the onset of ischemia (4 mg/kg iv) to relieve the stress caused by the coronary occlusion. No antiarrhythmic agents were given at any time. Rabbits were assigned to four groups (Fig. 1). *Group I* (control group) underwent the 30-min

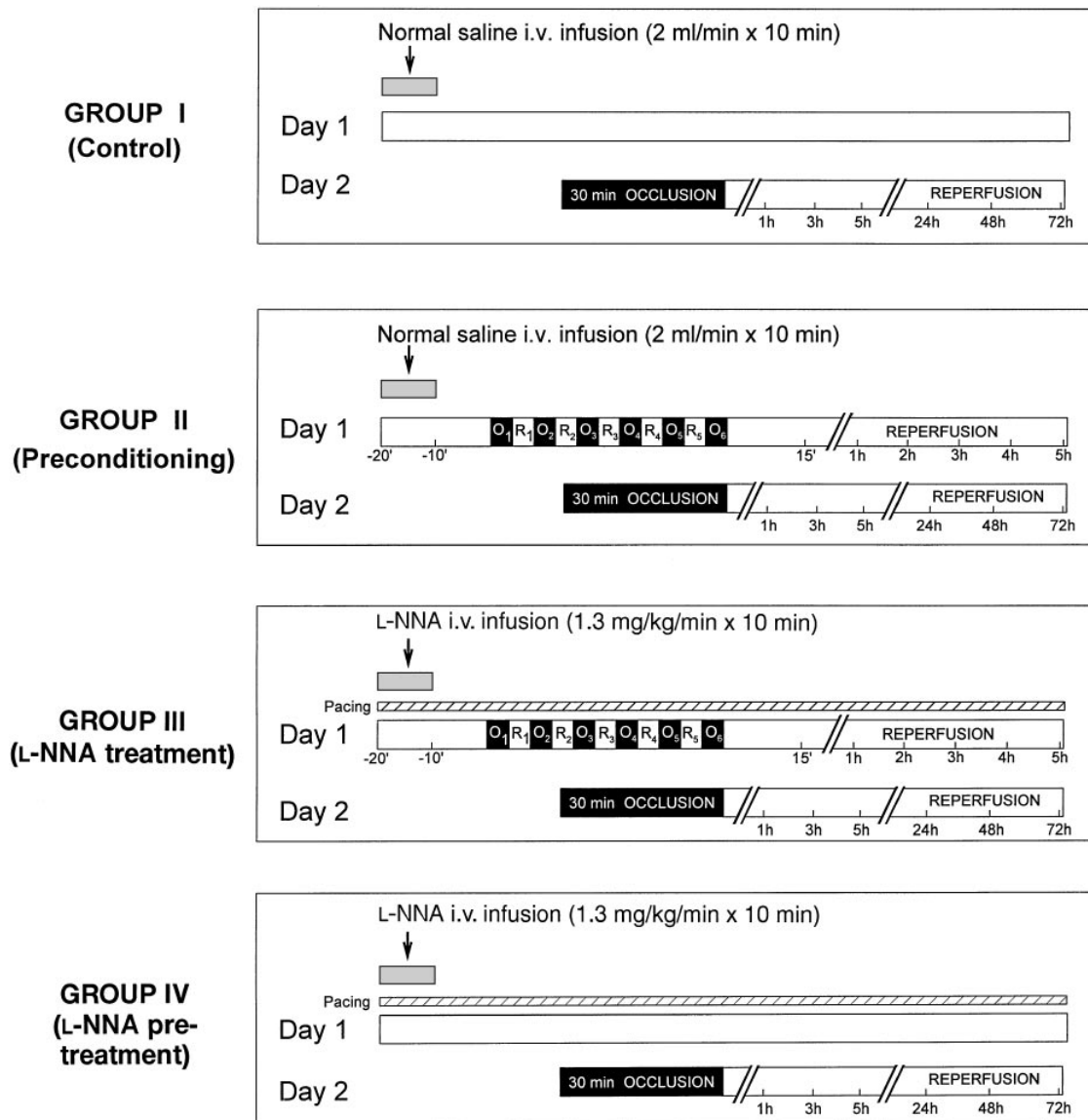


Fig. 1. Experimental protocol. Four groups of rabbits were studied. On *day 2*, all groups underwent 30-min coronary occlusion followed by 3 days of reperfusion. On *day 1*, rabbits in *group I* ( $n = 10$ , control group) received only an infusion of normal saline (20 ml). Rabbits in *group II* ( $n = 10$ , PC group) received same saline infusion on *day 1* and underwent a sequence of six 4-min coronary occlusion (O)-4-min reperfusion (R) cycles. Rabbits in *group III* [ $n = 11$ ,  $N^G$ -nitro-L-arginine (L-NNA)-treated group] underwent same sequence of O-R cycles on *day 1*; in addition, they received an intravenous infusion of L-NNA ( $1.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) starting 20 min and ending 10 min before 1st coronary occlusion (total dose  $13 \text{ mg/kg}$ ). Rabbits in *group IV* ( $n = 9$ , L-NNA-pretreated group) received same dose of L-NNA on *day 1* but did not undergo coronary occlusion.

occlusion with no PC protocol and no drug treatment. *Group II* (PC group) underwent a sequence of six 4-min coronary occlusions interspersed with 4 min of reperfusion 24 h before the 30-min coronary occlusion. *Group III* (L-NNA-treated group) underwent the same protocol as *group II* except that the rabbits received an intravenous infusion of L-NNA at a rate of  $1.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 10 min starting 20 min before and ending 10 min before the first coronary occlusion (total dose 13 mg/kg). To maintain constant heart rate, atrial pacing was initiated after the administration of L-NNA and continued until 5 h after reperfusion at rates similar to the baseline (pretreatment) values. L-NNA (Sigma Chemical, St. Louis, MO) was dissolved in normal saline (total volume infused 20 ml), and the solution was filtered through a  $0.2\text{-}\mu\text{m}$  Millipore filter to ensure sterility. In *group IV* (L-NNA-pretreated group), rabbits received the same dose of L-NNA but did not undergo the sequence of six occlusion-reperfusion cycles; these animals were subjected to the 30-min coronary occlusion 24 h later. Atrial pacing at rates similar to pretreatment heart rates was started after administration of L-NNA and continued for 6 h.

**Postmortem tissue analysis.** At the conclusion of the study, the rabbits were given heparin (1,000 U iv), after which they were anesthetized with pentobarbital sodium (50 mg/kg iv) and euthanized with KCl. The heart was excised, and the size of the occluded-reperfused coronary vascular bed was determined by tying the coronary artery at the site of the previous occlusion and by perfusing the aortic root for 2 min with a 5% solution of phthalo blue dye in normal saline at a pressure of 70 mmHg using a Langendorff apparatus. The heart was then cut into six or seven transverse slices, which were incubated for 10 min at  $37^\circ\text{C}$  in a 1% solution of triphenyltetrazolium chloride in phosphate buffer (pH = 7.4). All atrial and right ventricular tissues were excised, after which the slices were weighed, fixed in a 10% formaldehyde solution, and photographed (Nikon AF N6006). Transparencies were projected onto a paper screen at a 10-fold magnification, and the borders of the infarcted, ischemic-reperfused, and nonischemic regions were traced. The corresponding areas were measured by computerized planimetry (Adobe Photoshop, version 4.0), and from these measurements infarct size was calculated as a percentage of the region at risk.

**Statistical analysis.** Data are reported as means  $\pm$  SE. Heart rate and thickening fraction were analyzed by a two-way repeated-measures analysis of variance (ANOVA; time and group). Infarct sizes and risk region sizes were analyzed with a one-way ANOVA followed by Student's *t*-tests for unpaired data with the Bonferroni correction.

## RESULTS

**Exclusions.** Of the 49 rabbits instrumented for this study, 13 were assigned to the control group (*group I*), 12 to the PC group (*group II*), 13 to the L-NNA-treated group (*group III*), and 11 to the L-NNA-pretreated group (*group IV*). Of the 13 rabbits assigned to the control group, 2 died of ventricular fibrillation during coronary occlusion and 1 was excluded because of failure of the balloon occluder. Of the 12 rabbits assigned to the PC group, 2 died of ventricular fibrillation during the 30-min coronary occlusion. Of the 13 rabbits assigned to the L-NNA-treated group, 1 was excluded because of ventricular fibrillation during the 30-min occlusion and 1 because of a technical problem with the postmortem perfusion. Of the 11 rabbits assigned to the L-NNA-pretreated group, 2 were excluded because of

malfunction of the occluder. Therefore, a total of 10 rabbits completed the protocol in the control group, 10 in the PC group, 11 in the L-NNA-treated group, and 9 in the L-NNA-pretreated group.

**Hemodynamic variables.** It has previously been shown that the dose of L-NNA used in this study does not alter systemic arterial pressure or systolic thickening fraction in conscious rabbits (5). In that investigation, however, L-NNA induced a sustained decrease in heart rate. Consequently, in this study we elected to pace the heart at a rate similar to the baseline heart rate throughout the six coronary occlusion-reperfusion cycles and the first 5 h of reperfusion in *group III* and for equivalent periods of time in *group IV* (Fig. 1). As a result, there were no appreciable differences in heart rate between *groups II* and *III* on the first day of the protocol [occlusion 1:  $263 \pm 6$  and  $260 \pm 7$  beats/min (bpm), respectively; occlusion 6:  $260 \pm 5$  and  $259 \pm 6$  bpm, respectively]. On the second day of the protocol, the heart rate did not differ among the four groups before, during, or after the 30-min coronary occlusion (preocclusion:  $243 \pm 8$ ,  $253 \pm 6$ ,  $245 \pm 9$ , and  $232 \pm 8$  bpm, respectively, in *groups I-IV*; 15 min of occlusion:  $246 \pm 5$ ,  $252 \pm 8$ ,  $248 \pm 9$ , and  $238 \pm 7$  bpm, respectively; 1 h of reperfusion:  $251 \pm 8$ ,  $258 \pm 7$ ,  $232 \pm 11$ , and  $236 \pm 7$  bpm, respectively). As expected (see Ref. 5), in *groups III* and *IV* systolic thickening fraction was not altered by L-NNA (data not shown).

**Region at risk and infarct size.** There were no significant differences among *groups I-IV* with respect to LV weight ( $4.7 \pm 0.4$ ,  $4.3 \pm 0.2$ ,  $4.7 \pm 0.3$ , and  $3.8 \pm 0.1$  g, respectively) or weight of the region at risk [ $0.8 \pm 0.1$  g ( $17.0 \pm 1.7\%$  of LV wt),  $0.7 \pm 0.1$  g ( $15.8 \pm 1.2\%$  of LV wt),  $0.7 \pm 0.1$  g ( $14.8 \pm 1.2\%$  of LV wt), and  $0.6 \pm 0.1$  g ( $15.4 \pm 1.5\%$  of LV wt), respectively]. The average infarct size was 50% smaller in *group II* compared with control animals (*group I*) ( $28.6 \pm 3.2$  and  $56.9 \pm 5.9\%$  of region at risk, respectively;  $P < 0.05$ ; Fig. 2), indicating a late PC effect against myocardial infarction. In *group III*, however, infarct size ( $48.6 \pm 3.8\%$  of the region at risk) was significantly greater than in *group II* ( $P <$

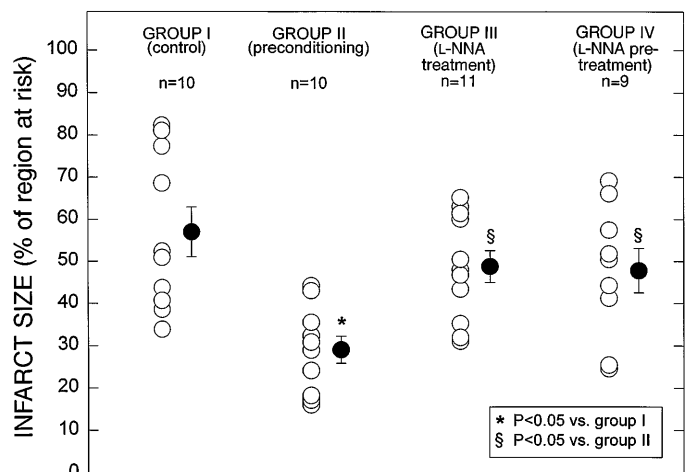


Fig. 2. Myocardial infarct size in *groups I-IV*. Infarct size is expressed as percentage of region at risk of infarction. O, Individual rabbits; ●, means  $\pm$  SE; n, number of rabbits.

0.05) and not significantly smaller than in controls (Fig. 2), indicating that L-NNA abrogated the late PC effect against infarction. In *group IV*, infarct size ( $47.8 \pm 5.3\%$  of region at risk) did not differ significantly from that in controls (Fig. 2), indicating that pretreatment with L-NNA, in itself, did not affect the extent of necrosis. In all four groups, the size of the infarction was positively and linearly related to the size of the region at risk ( $r = 0.83, 0.85, 0.86,$  and  $0.91$  in *groups I-IV*, respectively). The regression line, however, was shifted to the right in *group II* ( $y = 0.43x - 0.087$ ) compared with *group I* ( $y = 0.54x - 0.016$ ), indicating that, for any given size of the region at risk, the resulting infarction was smaller in preconditioned than in control rabbits. In *groups III* and *IV*, the regression lines were similar to that of *group I* (data not shown).

**Regional myocardial function.** Because of Doppler probe malfunction, complete measurements of WTh for 3 days after reperfusion could be obtained only in 5 of 10 rabbits in *group I* and 9 of 10 rabbits in *group II*. On the second day of the protocol, baseline systolic thickening fraction averaged  $39.7 \pm 2.5\%$  in *group I* and  $35.7 \pm 5.1\%$  in *group II* [ $P =$  nonsignificant (NS)]. After release of the 30-min occlusion, control rabbits (*group I*) exhibited essentially no recovery of WTh, even at 3 days (Fig. 3). In preconditioned rabbits (*group II*), the recovery of WTh was significantly ( $P < 0.05$ ) improved compared with controls at 5 h and 1, 2, and 3 days after reperfusion (Fig. 3). The total deficit of WTh over the 3-day reperfusion period (an integrative assessment of the overall severity of contractile dysfunction in this time interval; see Refs. 5, 22) was decreased by 18% in *group II* vs. *group I* ( $P < 0.05$ ; Fig. 3). However, the effect of late PC on WTh was highly heterogeneous, with some rabbits in *group II* exhibiting no recovery (persistent dyskinesia) for 3 days and others resuming active WTh as early as 1 h after reperfusion. This

variability is manifested in the large standard errors bracketing the means in Fig. 3. In *groups III* and *IV*, the recovery of WTh was indistinguishable from that in the control group (data not shown).

## DISCUSSION

Although NO has been shown to elicit a late PC effect against myocardial stunning (5), no previous study has examined the role of NO as a possible trigger of the late phase of ischemic PC against infarction. With regard to the role of NO in the early phase of ischemic PC, the available data are conflicting. Some studies have concluded that NO production induces early PC against arrhythmias (35, 36) and infarction (15), whereas others (6, 12, 21, 37, 38) found no evidence to support this concept. The reason(s) for these discrepant results is unknown.

The present study demonstrates that the NO synthase inhibitor L-NNA blocks the development of late PC against myocardial infarction in conscious rabbits, indicating that formation of NO during the PC ischemia is necessary to trigger the development of the cardioprotection observed 24 h later when the heart is subjected to a lethal ischemic insult. The abrogation of protection against infarction cannot be attributed to an inherent deleterious effect of L-NNA pretreatment, because administration of L-NNA 24 h before the 30-min coronary occlusion in *group IV* had no influence on infarct size. These results show, for the first time, that NO plays a key role in eliciting the late phase of ischemic PC against myocardial infarction. This is also the first study to examine the role of NO in ischemic PC against myocardial infarction (early or late) in a conscious animal model.

We used the same dose of L-NNA that was previously found to prevent late PC against stunning in conscious rabbits (5). This dose of L-NNA blunts acetylcholine-induced vasodilation without raising arterial pressure (i.e., without causing systemic vasoconstriction) (5), suggesting that it is sufficient to block increased synthesis of NO (such as that which occurs in response to ischemia-reperfusion) without affecting basal endothelial release of NO. Previous studies have shown that administration of L-NNA causes a decrease in heart rate without changes in arterial pressure in conscious rabbits (5, 20) and in conscious dogs (29), an effect that is thought to reflect the central regulatory function of NO on sympathetic and parasympathetic tone (20). To avoid any potential interference caused by decreases in heart rate, in the present investigation all rabbits treated with L-NNA were paced at rates similar to their baseline (pretreatment) heart rates.

In this study, the same ischemic PC protocol previously found to induce late PC against stunning (5, 22) effected a 50% reduction in infarct size 24 h later. Although this reduction in infarct size is less than that generally observed during the early phase of PC (1, 7-10, 26, 28), it is still substantial. Thus, in the conscious rabbit, the late phase of ischemic PC confers powerful protection not only against stunning, but also against cell death. These results are consonant with

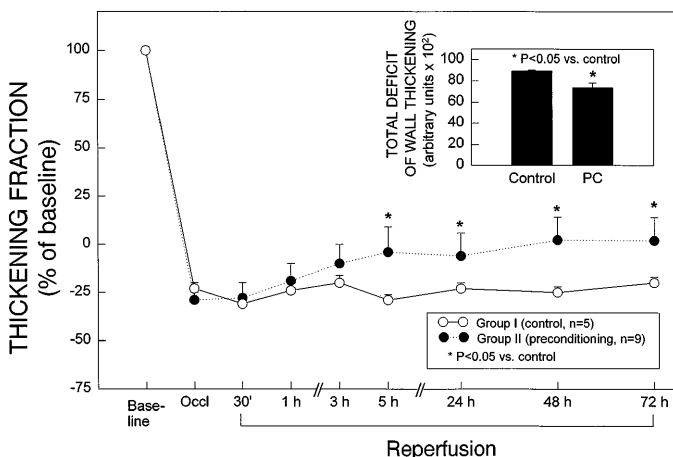


Fig. 3. Systolic thickening fraction in ischemic-reperfused region in *groups I* and *II*. Measurements were obtained at baseline, 15 min into 30-min occlusion (Occl), and 30 min and 1, 3, 5, 24, 48, and 72 h after reperfusion. Thickening fraction is expressed as percentage of baseline values. Total deficit of wall thickening (WTh; inset) was calculated by measuring area between systolic WTh vs. time line and baseline (100% line) during 3-day reperfusion period after 30-min occlusion (see Refs. 5, 22). PC, preconditioning. Data are means  $\pm$  SE.

those previously reported by Yang et al. (39) in conscious rabbits preconditioned 24 h earlier with four 5-min coronary occlusions, although the reduction in infarct size observed in that study (32%) was less than that noted in the present investigation. In open-chest rabbits preconditioned 24 h earlier with four 5-min occlusions, reductions in infarct size ranging between 35 and 45% have been reported (2, 3, 23), although in one study (33) no protection was observed. We (28) have previously failed to detect an infarct-limiting effect during the late phase of ischemic PC in conscious pigs subjected to ten 2-min coronary occlusions followed 24 h later by a 40-min occlusion. The reason(s) for the apparent discrepancy between those previous results and our present observations probably relates to the differences in species and PC protocols. Further studies will be necessary to determine whether late PC against infarction is dependent on the species used.

The measurements of WTh demonstrate that the reduction in cell death afforded by late PC in *group II* was associated with an enhanced recovery of contractile function (Fig. 3). Although the average improvement observed in the first 3 days of reperfusion was modest (because of a marked variability among rabbits), it is plausible that a greater benefit would have been detected over the subsequent days and weeks, because it has been shown that full recovery of surviving, stunned myocardium after a lethal ischemic insult requires up to 2 wk (19). To our knowledge, this is the first study to demonstrate that the beneficial effects of ischemic PC (early or late) on myocardial infarction are translated into a functional improvement.

The source of increased NO formation during the PC stimulus is likely to be the constitutively expressed endothelial (type III) isoform of NO synthase, which has been identified not only in endothelial cells but also in cardiac myocytes (13, 17). Ischemic PC could stimulate synthesis of NO by this enzyme by at least three mechanisms: ischemia-induced increases in intracellular calcium and NADPH, increased shear stress during the repeated reactive hyperemias, and/or release of bradykinin with activation of endothelial B<sub>2</sub> receptors (25). Regardless of the source of NO, we propose that formation of this radical during the PC ischemia leads to the synthesis of cardioprotective proteins that subsequently render the heart more resistant to infarction. NO exerts a plethora of actions that could culminate in upregulation of gene transcription; for example, several transcription factors (such as nuclear factor- $\kappa$ B, activator protein-1, adenosine 3',5'-cyclic monophosphate response element binding protein), enzymes, receptors, G proteins, protein kinases, protein phosphatases, and ion channels are known to be affected by NO (reviewed in Ref. 13). NO could also modulate gene expression through the formation of ONOO<sup>-</sup> and/or secondary ROS, which in turn could act via activation of protein kinase C (PKC; Ref. 11) or a *cis*-acting regulatory element (antioxidant responsive element) that mediates cellular responses to oxidative stress (30). Further studies will be necessary to elucidate the mechanism whereby NO induces late PC against infarction

and also to determine whether this phenomenon is triggered by NO itself or by one of its reactive byproducts (such as ONOO<sup>-</sup>; Ref. 13).

Previous studies have implicated adenosine receptors (3) and PKC (2) in late PC against infarction. These prior results and our present data are not mutually exclusive. Because NO can cause formation of ROS (13), and because both adenosine (8) and ROS (11) may activate PKC, it is possible that PKC serves as a common transduction element in the signaling pathways that lead to late PC. At present, it is unclear whether adenosine acts independently of NO and whether both of these stimuli are needed to achieve the threshold necessary to induce late PC against infarction.

In conclusion, this study expands our understanding of the complex cellular functions of NO by demonstrating that inhibition of NO production prevents the development of late PC against myocardial infarction in conscious rabbits. We propose that NO serves as a key signal in the cellular events responsible for the delayed myocardial adaptation to brief ischemic stresses.

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## REFERENCES

1. **Auchampach, J. A., and G. J. Gross.** Adenosine A<sub>1</sub> receptors, K<sub>ATP</sub> channels, and ischemic preconditioning in dogs. *Am. J. Physiol.* 264 (*Heart Circ. Physiol.* 33): H1327–H1336, 1993.
2. **Baxter, G. F., F. M. Goma, and D. M. Yellon.** Involvement of protein kinase C in the delayed cytoprotection following sublethal ischaemia in rabbit myocardium. *Br. J. Pharmacol.* 115: 222–224, 1995.
3. **Baxter, G. F., M. S. Marber, V. C. Patel, and D. M. Yellon.** Adenosine receptor involvement in a delayed phase of myocardial protection 24 hours after ischemic preconditioning. *Circulation* 90: 2993–3000, 1994.
4. **Bolli, R.** The early and late phases of preconditioning against myocardial stunning and the essential role of oxyradicals in the late phase: an overview. *Basic Res. Cardiol.* 91: 57–63, 1996.
5. **Bolli, R., Z. A. Bhatti, X. L. Tang, Y. Qiu, Q. Zhang, Y. Guo, and A. K. Jadoon.** Evidence that late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide. *Circ. Res.* 81: 42–52, 1997.
6. **Bugge, E., and K. Ytrehus.** Bradykinin can protect against infarction but does not mediate ischemic preconditioning in the rat heart (Abstract). *Circulation* 92: 1-456, 1995.
7. **Burckhardt, B., X. M. Yang, A. Tsuchida, K. M. Mullane, J. M. Downey, and M. V. Cohen.** Adenosine extends the window of protection afforded by ischaemic preconditioning in conscious rabbits. *Cardiovasc. Res.* 29: 653–657, 1995.
8. **Cohen, M. V., and J. M. Downey.** Preconditioning during ischemia. *Cardiovasc. Res.* 3: 137–149, 1995.
9. **Cohen, M. V., X. M. Yang, and J. M. Downey.** Conscious rabbits become tolerant to multiple episodes of ischemic preconditioning. *Circ. Res.* 74: 998–1004, 1994.
10. **Downey, J. M.** Ischemic preconditioning: nature's own cardioprotective intervention. *Trends Cardiovasc. Med.* 2: 170–176, 1992.

11. **Gopalakrishna, R., and W. B. Anderson.** Ca<sup>2+</sup>- and phospholipid-independent activation of protein kinase C by selective oxidative modification of the regulatory domain. *Proc. Natl. Acad. Sci. USA* 86: 6758–6762, 1989.
12. **Goto, M., Y. Liu, X. M. Yang, J. L. Ardell, M. V. Cohen, and J. M. Downey.** Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. *Circ. Res.* 77: 611–621, 1995.
13. **Gross, S. S., and M. S. Wolin.** Nitric oxide: pathophysiological mechanisms. *Annu. Rev. Physiol.* 57: 737–769, 1995.
14. **Haessler, R., K. Kuzume, G. L. Chien, R. A. Wolff, R. F. Davis, and D. M. Van Winkle.** Anaesthetics alter the magnitude of infarct limitation by ischaemic preconditioning. *Cardiovasc. Res.* 28: 1574–1580, 1994.
15. **Hartman, J. C., H. Houshyar, S. C. Leva, and T. M. Wall.** A role for nitric oxide in myocardial ischemic preconditioning (Abstract). *Circulation* 92: I-716, 1995.
16. **Jagasia, D., J. M. Whiting, and P. H. McNulty.** Ischemic preconditioning fails to produce a second window of protection 24 hrs later in the rat (Abstract). *Circulation* 94: I184–I185, 1996.
17. **Kelly, R. A., J. L. Balligand, and T. W. Smith.** Nitric oxide and cardiac function. *Circ. Res.* 79: 363–380, 1996.
18. **Kuzuza, T., S. Hoshida, N. Yamashita, H. Fuji, H. Oe, M. Hori, T. Kamada, and M. Tada.** Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. *Circ. Res.* 72: 1293–1299, 1993.
19. **Lavallee, M., D. Cox, T. A. Patrick, and S. F. Vatner.** Salvage of myocardial function by coronary artery reperfusion 1, 2, and 3 hours after occlusion in conscious dogs. *Circ. Res.* 53: 235–247, 1983.
20. **Liu, J.-L., H. Murakami, and I. H. Zucker.** Effects of NO on baroreflex control of heart rate and renal nerve activity in conscious rabbits. *Am. J. Physiol.* 270 (Regulatory Integrative Comp. Physiol. 39): R1361–R1370, 1996.
21. **Lu, H. R., P. Remeysen, and F. De Clerck.** Does the antiarrhythmic effect of ischemic preconditioning in rats involve the L-arginine-nitric oxide pathway? *J. Cardiovasc. Pharmacol.* 25: 524–530, 1995.
22. **Maldonado, C., Y. Qiu, X.-L. Tang, M. V. Cohen, J. Auchampach, and R. Bolli.** Role of adenosine receptors in late preconditioning against myocardial stunning in conscious rabbits. *Am. J. Physiol.* 273 (Heart Circ. Physiol. 42): H1324–H1332, 1997.
23. **Marber, M. S., D. S. Latchman, J. M. Walker, and D. M. Yellon.** Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation* 88: 1264–1272, 1993.
24. **Miyamae, M., H. Fujiwara, M. Kida, R. Yokota, M. Tanaka, M. Katsuragawa, K. Hasegawa, M. Ohura, K. Koga, Y. Yabuuchi, and S. Sasayama.** Preconditioning improves energy metabolism during reperfusion but does not attenuate myocardial stunning in porcine hearts. *Circulation* 88: 223–234, 1993.
25. **Moncada, S., and A. Higgs.** The L-arginine-nitric oxide pathway. *N. Engl. J. Med.* 329: 2002–2012, 1993.
26. **Murry, C. E., R. B. Jennings, and K. A. Reimer.** Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124–1136, 1986.
27. **Ovize, M., K. Przyklenk, S. L. Hale, and R. A. Kloner.** Preconditioning does not attenuate myocardial stunning. *Circulation* 85: 2247–2254, 1992.
28. **Qiu, Y., X. L. Tang, S. W. Park, J. Z. Sun, A. Kalya, and R. Bolli.** The early and late phases of ischemic preconditioning: a comparative analysis of their effects on infarct size, myocardial stunning, and arrhythmias in conscious pigs undergoing a 40-minute coronary occlusion. *Circ. Res.* 80: 730–742, 1997.
29. **Reinhart, G. A., T. E. Lohmeier, and H. L. Mizelle.** Temporal influence of the renal nerves on renal excretory function during chronic inhibition of nitric oxide synthesis. *Hypertension* 29: 199–204, 1997.
30. **Rushmore, T. H., M. R. Morton, C. B. Pickett.** The antioxidant responsive element: activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *J. Biol. Chem.* 266: 11632–11639, 1991.
31. **Sun, J. Z., X. L. Tang, A. A. Knowlton, S. W. Park, Y. Qiu, and R. Bolli.** Late preconditioning against myocardial stunning. An endogenous protective mechanism that confers resistance to posts ischemic dysfunction 24 h after brief ischemia in conscious pigs. *J. Clin. Invest.* 95: 388–403, 1995.
32. **Sun, J. Z., X. L. Tang, S. W. Park, Y. Qiu, J. F. Turrens, and R. Bolli.** Evidence for an essential role of reactive oxygen species in the genesis of late preconditioning against myocardial stunning in conscious pigs. *J. Clin. Invest.* 97: 562–576, 1996.
33. **Tanaka, M., H. Fujiwara, K. Yamasaki, M. Miyamae, R. Yokota, K. Hasegawa, T. Fujiwara, and S. Sasayama.** Ischemic preconditioning elevates cardiac stress protein but does not limit infarct size 24 or 48 h later in rabbits. *Am. J. Physiol.* 267 (Heart Circ. Physiol. 36): H1476–H1482, 1994.
34. **Tang, X. L., Y. Qiu, S. W. Park, J. Z. Sun, A. Kalya, and R. Bolli.** Time course of late preconditioning against myocardial stunning in conscious pigs. *Circ. Res.* 79: 424–34, 1996.
35. **Vegh, A., J. G. Papp, L. Szekeres, and J. R. Parratt.** Prevention by an inhibitor of the L-arginine-nitric oxide pathway of the antiarrhythmic effects of bradykinin in anaesthetized dogs. *Br. J. Pharmacol.* 110: 18–19, 1993.
36. **Vegh, A., L. Szekeres, and J. Parratt.** Preconditioning of the ischaemic myocardium; involvement of the L-arginine nitric oxide pathway. *Br. J. Pharmacol.* 107: 648–652, 1992.
37. **Weselcouch, E. O., A. J. Baird, P. Slep, and G. J. Grover.** Inhibition of nitric oxide synthesis does not affect ischemic preconditioning in isolated perfused rat hearts. *Am. J. Physiol.* 268 (Heart Circ. Physiol. 37): H242–H249, 1995.
38. **Woolfson, R. G., V. C. Patel, G. H. Neild, and D. M. Yellon.** Inhibition of nitric oxide synthesis reduces infarct size by an adenosine-dependent mechanism. *Circulation* 91: 1545–1551, 1995.
39. **Yang, X. M., G. F. Baxter, R. J. Heads, D. M. Yellon, J. M. Downey, and M. V. Cohen.** Infarct limitation of the second window of protection in a conscious rabbit model. *Cardiovasc. Res.* 31: 777–783, 1996.