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Nicorandil induces late preconditioning against myocardial infarction in conscious rabbits

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Abstract

Nicorandil has been shown to induce an infarct-limiting effect similar to that induced by the early phase of ischemic preconditioning (PC). The goals of this study were to determine whether nicorandil induces a delayed cardioprotection that is analogous to the late phase of ischemic PC and, if so, whether nicorandil-induced late PC is associated with upregulation of cardio-protective proteins. Chronically instrumented, conscious rabbits received vehicle (intravenous normal saline; control group, $n = 10$), nicorandil ($100 \mu\text{g/kg}$ bolus + $30 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ iv for 60 min; nicorandil group, $n = 10$), or ischemic PC (6 cycles of 4-min coronary occlusion/4-min reperfusion; PC group, $n = 8$). Twenty-four hours later, rabbits underwent a 30-min coronary occlusion, followed by 3 days of reperfusion. Myocardial infarct size was significantly reduced in rabbits pretreated with nicorandil ($27.5 \pm 5.3\%$ of the risk region) or with ischemia ($30.3 \pm 4.2\%$) versus controls ($59.1 \pm 4.7\%$, $P < 0.05$ vs. both). Furthermore, the expression of cyclooxygenase-2 (COX-2) and Bcl-2 was significantly elevated (+38% and +126%, respectively; $P < 0.05$) in myocardium of rabbits given nicorandil 24 h earlier versus controls. We conclude that nicorandil induces delayed cardioprotection against myocardial infarction similar to that afforded by the late phase of ischemic PC, possibly by upregulating COX-2 and Bcl-2.

Keywords

Bcl-2; cyclooxygenase-2; infarct size; ischemia-reperfusion

Nicorandil is a hybrid agent with ATP-sensitive K^+ (K_{ATP}) channel opener-like and nitrate-like properties (40, 61) and has been shown to have a protective effect in several experimental animal models of myocardial ischemia-reperfusion (1, 15, 16, 21, 24, 30, 31, 36, 37, 45, 46, 57, 69). Clinical studies (23, 34, 43, 54, 60) have shown that nicorandil attenuates ECG changes during coronary occlusion and that it improves the functional recovery of the reperfused myocardium in patients with acute myocardial infarction. More recently, the Impact of Nicorandil in Angina study (a double-blind placebo-controlled trial of >5,000 patients) demonstrated that nicorandil improves the prognosis in stable angina pectoris (22). In addition to its direct beneficial effect on hemodynamics, nicorandil has also been shown to induce an early preconditioning (PC)-like cardioprotective effect (34, 35, 53), to lower the threshold of ischemic PC (38), and to potentiate the infarct size-limiting effect of the early phase of ischemic PC (39). Whether nicorandil also induces a delayed cardioprotection similar to the late phase of ischemic PC, however, remains unknown. Because of the sustained duration of late PC (4), elucidation of this issue may have

considerable importance to explain the salubrious actions of nicorandil in the clinical arena (22).

In addition, because the late phase of ischemic PC provides sustained cardioprotection (4), exploitation of late PC is of clinical significance. Recent studies in various experimental animal preparations have demonstrated that a delayed cardio-protective effect indistinguishable from the late phase of ischemic PC can be elicited by a variety of pharmacological manipulations including nitric oxide donors (2, 20, 47, 65, 67), adenosine receptor agonists (3, 62), opioid receptor agonists (11, 12, 27, 44), reactive oxygen species (68), endotoxin derivatives (70, 72), and the K_{ATP} channel opener diazoxide (41, 66). However, many of these interventions are not clinically applicable or have significant side effects. Because nicorandil is already used in patients with coronary artery disease and is well tolerated, determining whether it induces a late PC-like cardioprotection is clinically important. Because previous studies have shown that late PC is mediated by upregulation of cyclooxygenase-2 (COX-2) (27, 58, 59) and Bcl-2 (51), we also tested whether these cardioprotective proteins are involved in the late PC-like effect of nicorandil.

Accordingly, the goals of the present study were to determine whether pretreatment with nicorandil, in the absence of ischemia, can reproduce the protective effect of the late phase of ischemic PC against myocardial infarction and to determine whether nicorandil-induced delayed cardioprotection is associated with expression of COX-2 and Bcl-2, which has previously been shown to increase 24 h after ischemic PC (51, 59). All studies were performed in our well-established conscious rabbit model (51, 59). The choice of a conscious model was dictated by the preclinical nature of this study and by our motivation to rigorously test the cardioprotective actions of nicorandil under conditions that are as physiological as possible. Openchest animal preparations are associated with several factors (such as anesthesia, surgical trauma, fluctuations in temperature, elevated catecholamine levels, abnormal hemodynamics, and exaggerated formation of reactive oxygen species) that may interfere with myocardial infarct size (9, 56) and/or with ischemic PC (18, 55).

MATERIALS AND METHODS

The experimental procedures and protocols used in this study were reviewed and approved by the Animal Care and Use Committee of the University of Louisville, School of Medicine, and conformed to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86–23, Revised 1996).

Experimental Protocols

Infarct size studies—Chronically instrumented New Zealand White male rabbits (2.0–2.5 kg wt; age 3 to 4 mo) were prepared as described previously (5, 6, 25, 26, 47, 49, 50, 63–65, 71). Briefly, rabbits 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, and bipolar 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 aspirate air and fluid postoperatively. Gentamicin was administered before surgery and on the first and second postoperative days (5 mg/kg im each day).

After a minimum of 10 days postsurgical recovery, rabbits were studied in the conscious state. An intravenous infusion line was established by cannulating the marginal ear vein with a 24-gauge angiocatheter. Rabbits were divided into three groups and given one of the following treatments: vehicle (intravenous normal saline; control group), nicorandil (100 μ g/kg bolus injection, followed by 30 μ g·kg⁻¹·min⁻¹ infusion for 60 min iv; nicorandil group), or ischemic PC (a sequence of six 4-min coronary occlusion/4-min reperfusion

cycles; PC group) (Fig. 1). Nicorandil (Chugai Pharmaceutical; Tokyo, Japan) was dissolved in normal saline; the total volume infused over 60 min was 6 ml/kg. To confirm the absence of hemodynamic changes, arterial pressure and heart rate were monitored in the nicorandil group. Arterial pressure was measured by cannulating the ear dorsal artery with a 22-gauge angiocatheter, as previously described (5). Ischemia-reperfusion in the PC group was produced by inflating and deflating the balloon occluder (5). The performance of successful coronary occlusions was verified by observing the development of ST-segment elevation and the changes in the QRS complex on the ECG and the appearance of paradoxical systolic wall thinning on the ultrasonic crystal recordings. No sedative or antiarrhythmic agent was given at this time. Twenty-four hours later, all rabbits were subjected to a 30-min coronary artery occlusion, followed by 3 days of reperfusion. Diazepam was administered 20 min before the 30 min occlusion (4 mg/kg ip) to relieve the stress caused by ischemia.

At the conclusion of the study, the occluded/reperfused vascular bed and the infarct were identified by postmortem perfusion of the heart with triphenyl tetrazolium chloride and phthalo blue dye (26, 50, 63, 65). Infarct size was calculated by using computerized planimetry (26, 47, 50, 59, 63, 65, 71).

Western Blot Analysis

Two additional groups of rabbits were studied to determine whether nicorandil induces upregulation of COX-2 and Bcl-2. Rabbits received vehicle or nicorandil (same dose) (Fig. 1). Twenty-four hours later, rabbits were euthanized and myocardial samples were rapidly removed from the left ventricular (LV) wall and frozen in liquid N₂. Tissue samples were homogenized in *buffer A* composed of 25 mM Tris-HCl (pH 7.4), 0.5 mM EDTA, 0.5 mM EGTA, 1 mM PMSF, 25 µg/ml leupeptin, 1 mM DTT, 25 mM NaF, and 1 mM Na₃VO₄ and centrifuged at 14,000 *g* for 12 min at 4°C, and the resulting supernatants were collected as cytosolic fractions (48). The pellets were incubated in a lysis buffer (*buffer A* + 1% Triton X-100) for 2 h and centrifuged 14,000 *g* for 15 min at 4°C, and the resulting supernatants were collected as membranous fractions (48). Protein expression was assessed by standard SDS-PAGE immunoblotting techniques (48, 49, 71). Gel transfer efficiency was recorded carefully by making photocopies of membranes dyed with reversible Ponceau staining (48, 49); gel retention was determined by Coomassie blue staining (48, 49). Mouse monoclonal (C-33) anti-rat COX-2 antibodies (Transduction Laboratories; Lexington, KY) were used for the assay of COX-2 protein, and mouse monoclonal (C-2) anti-human/rat Bcl-2 antibodies (Santa Cruz Biotechnology; Santa Cruz, CA) were used for the assays of Bcl-2 protein. Protein signals and the corresponding records of Ponceau stains of nitrocellulose membranes were quantitated by an image scanning densitometer, and each protein signal was normalized to its corresponding Ponceau stain signal (48, 49). In all samples, the content of each protein was expressed as a percentage of the corresponding protein in vehicle control.

Statistical Analysis

Data are reported as means ± SE. Hemodynamic variables were analyzed by a one-way ANOVA, followed by Student's *t*-tests with Bonferroni's correction. Infarct size and protein expression were analyzed by a one-way ANOVA, followed by unpaired Student's *t*-tests with Bonferroni's correction. The relationship between infarct size and risk region size was compared among groups with an analysis of covariance (ANCOVA) using the size of the risk region as the covariate. The correlation between infarct size and risk region size was assessed by linear regression analysis using the least-squares method. All statistical analyses were performed using SPSS for Windows version 8.0 and SigmaStat for Windows version 2.0. A *P* value < 0.05 was considered significant.

RESULTS

A total of 55 rabbits was used in this study (5 for the pilot studies, 30 for the studies of myocardial infarction, and 20 for the studies of protein expression). Two rabbits in the PC group were excluded due to failure of the occluder balloon.

Pilot Studies

Pilot studies were conducted in five rabbits to determine the dose of nicorandil that would not cause significant hemodynamic alterations. The concern was that hemodynamic perturbations caused by this agent (e.g., a drop in blood pressure or an increase in heart rate) could contribute nonspecifically to induce a late PC effect unrelated to the direct actions of nicorandil on the heart. Three rabbits received a 100 $\mu\text{g}/\text{kg}$ iv bolus injection of nicorandil, followed by a 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ infusion for 60 min. This dose of nicorandil caused a significant increase (+25% at the end of the infusion) in heart rate and a mild drop (–11% at 40 min into the infusion) of arterial pressure (Fig. 2). Because the bolus dose did not cause significant changes in heart rate and arterial pressure, in two additional rabbits we reduced only the infusion rate to 30 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for 60 min; we found no significant changes in heart rate and arterial pressure with this lower dose (data not shown). On the basis of these pilot studies, we selected this lower infusion rate of 30 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of nicorandil for the present studies.

Hemodynamic Variables

Heart rate and mean arterial pressure remained stable in rabbits receiving nicorandil (Fig. 3). There were no significant differences in heart rate among the three groups at baseline, after diazepam injection (immediately before occlusion), during the 30-min coronary occlusion, or during the 72-h reperfusion period (data not shown).

Region at Risk and Infarct Size

There were no significant differences among the three groups with respect to the weight of the region at risk: 0.80 ± 0.12 g ($17.4 \pm 2.3\%$ of LV weight), 0.90 ± 0.11 g ($20.6 \pm 1.9\%$ of LV weight), and 0.81 ± 0.07 g ($18.03 \pm 1.4\%$ of LV weight) in the control, nicorandil, and PC groups, respectively. The average infarct size was 54% smaller in the nicorandil-pretreated rabbits compared with control ($27.5 \pm 5.3\%$ vs. $59.1 \pm 4.7\%$ of the region at risk, $P < 0.05$) (Fig. 4), indicating that nicorandil elicited delayed cardioprotection 24 h later. The infarct size in the nicorandil group was similar to that observed in the rabbits preconditioned with ischemia (PC group, $30.3 \pm 4.2\%$ of the region at risk) (Fig. 4), indicating that the protection induced by nicorandil was equivalent to that induced by ischemic PC.

In all three groups, the size of the infarction was positively and linearly related to the size of the region at risk ($r = 0.93$, 0.63 , and 0.78 , respectively, in control, nicorandil, and PC group). The regression line was shifted downward in the nicorandil and PC groups compared with the control group ($P < 0.05$ by ANCOVA) (Fig. 5), indicating that for any given size of the region at risk, the resulting infarct size was reduced either by pretreatment with nicorandil or by ischemic PC.

Western Blot Analysis

Expression of COX-2 protein—A representative Western blot from four control and four nicorandil-pretreated rabbits is illustrated in Fig. 6A. Similar to our previous findings (27, 59), a detectable COX-2 signal was noted in the myocardium of control rabbits. Densitometric measurements of COX-2 immunoreactivity showed that the expression of

COX-2 protein increased by $38 \pm 6\%$ versus control, $P < 0.05$ (Fig. 6B), in myocardium of rabbits given nicorandil 24 h earlier.

Expression of Bcl-2 protein—A representative Western blot from four control and four nicorandil-pretreated rabbits is illustrated in Fig. 7A. A weak Bcl-2 signal was detected in the myocardium of control rabbits. When rabbits were given nicorandil 24 h earlier, Bcl-2 expression increased markedly ($+126 \pm 7\%$ vs. control, $P < 0.05$) (Fig. 7B).

DISCUSSION

The ultimate goal of studying PC is to exploit this phenomenon for the protection of the ischemic myocardium in patients with coronary artery disease. Although a variety of pharmacological interventions, including nitric oxide donors (2, 20, 47, 65, 67), adenosine receptor agonists (3, 62), opioid receptor agonists (11, 12, 27, 44), reactive oxygen species (68), endotoxin derivatives (70, 72), and the K_{ATP} channel opener diazoxide (41, 66) have been demonstrated to induce a late PC-like cardioprotection in experimental models, many of these agents are not clinically applicable or have significant side effects. Therefore, it is important to develop PC-mimetic therapies that are clinically relevant (20). In this connection, nitroglycerin has been shown to induce a late PC effect in conscious rabbits (2, 20) and in patients undergoing percutaneous transluminal coronary angioplasty (PTCA) (19, 32). In the present study, we demonstrate in conscious rabbits that administration of nicorandil, another clinically relevant agent, elicits a delayed cardioprotective effect that is manifested by a reduction in infarct size 24 h later and that is equivalent to that observed after ischemic PC. The ability of nicorandil to induce late PC cannot be ascribed to myocardial ischemia secondary to a decrease in arterial pressure or an increase in heart rate, because the dose of nicorandil used did not produce appreciable hemodynamic changes; accordingly, the late PC effect must have been the result of a direct action of nicorandil on the heart. Furthermore, this study demonstrates that nicorandil-induced late PC is associated with upregulation of COX-2 and Bcl-2.

Nicorandil is a hybrid compound that consists of an *N*-(2-hydroxyethyl)nicotinamide vitamin group and an organic nitrate moiety and therefore is thought to have dual actions, a nitrate-like action and opening of K_{ATP} channels (33). Like nitrates, nicorandil activates cytoplasmic guanylate cyclase leading to an increase in cellular levels of cGMP and a reduction in cytosolic calcium and thus to a relaxation of vascular smooth muscle (10). As a K_{ATP} channel opener, nicorandil increases efflux of potassium ions from the cell, leading to a more negative resting membrane potential (hyper-polarization), and also shortens the action potential duration. This inhibits calcium influx, causing a reduction in intracellular calcium leading to relaxation of vascular smooth muscle and vasodilatation (indirect calcium channel blocking effect) (28, 29). Through its dual actions, nicorandil reduces both preload (nitrate-like effect) and afterload (K_{ATP} channel opener effect), and improves coronary blood flow (14).

Clinical studies (22, 23, 54, 60) have shown that nicorandil improves functional and clinical outcomes in patients with acute myocardial infarction, but the mechanism for these salutary actions is unclear. Nicorandil reduces myocardial infarct size in various animal models when given before or during coronary occlusion (7, 8, 13, 21, 36, 42, 69); this infarct size-limiting effect seems independent of hemodynamic alterations (8, 36). Besides its direct action in alleviating ischemia-reperfusion injury, nicorandil has been suggested to induce an early PC-like cardioprotection in patients (34, 35, 53). In these studies, pretreatment of patients undergoing PTCA with a 1-min intravenous infusion of nicorandil 5 min before the initial balloon inflation significantly reduced ST elevation (34, 35, 53) and troponin T release (53) compared with control patients. However, because in these studies nicorandil

was given just 5 min before ischemia (34, 35, 53), it is difficult to make a distinction between the cardioprotection resulting from its direct actions and that afforded by initiation of PC. In contrast, the infarct-sparing effect observed 24 h after nicorandil pretreatment in our study must be the result of late PC and cannot be ascribed to a direct drug action because a single dose of nicorandil is eliminated from plasma almost entirely within 8 h (33). The mechanism whereby nicorandil triggers the development of late PC is unclear. Our previous studies (2, 17, 20) have demonstrated that late PC can be elicited by NO donors such as nitrates, whereas others (41, 66) have shown that it can be elicited by the K_{ATP} channel opener diazoxide. Thus, in principle, either the nitrate-like or the K_{ATP} channel activating properties of nicorandil, or both, could be responsible for the development of late PC against myocardial infarction.

The finding that nicorandil pretreatment upregulates the expression of COX-2 (Fig. 6) and Bcl-2 (Fig. 7) in the myocardium has potentially important implications for our understanding of the mechanism responsible for mediating nicorandil-induced delayed cardioprotection. In previous studies in rabbits, expression of COX-2 was shown to be upregulated 24 h after ischemic PC (59) and after pretreatment with the opioid receptor agonist BW-373U86 (27). Inhibition of COX-2 activity abrogated the cardioprotective effect of late PC induced by ischemia (59) and by the opioid receptor agonist (27). In addition, expression of Bcl-2 has been shown to be enhanced 24 h after ischemic PC in rat hearts (51). In view of these studies (27, 51, 59), it is plausible that nicorandil-induced delayed cardioprotection may be mediated by upregulation of COX-2 and/or Bcl-2. Establishing a cause-and-effect relationship between the induction of these proteins and nicorandil-induced cardioprotection will require further investigation.

In summary, the present study demonstrates the PC-mimetic properties of another clinically relevant agent (besides nitroglycerin), i.e., nicorandil. Specifically, using a conscious animal model devoid of the problems associated with anesthetized models (27, 51, 59), we found that administration of nicorandil reduced myocardial infarct size 24 h later. This delayed cardioprotection was associated with enhanced expression of myocardial COX-2 and Bcl-2. The ability of nicorandil to elicit a sustained protected phenotype may explain, at least in part, the salubrious effects of the drug in clinical trials (22, 23, 33, 34, 43, 52, 54, 73). Because nicorandil has been used clinically to treat patients with coronary artery disease and is well tolerated (43), the present results have potential clinical implications. The notion that in addition to its immediate anti-is-chemic effects, nicorandil can also trigger a sustained phenotypic change that renders the heart resistant to infarction at a distance of 24 h suggests novel applications of this drug for protecting the ischemic myocardium in patients. If nicorandil exerts late PC-mimetic actions in humans, a protracted or even chronic PC state could be implemented by administering nicorandil on a regular basis. Thus the present results provide a rationale for studies aimed at investigating the usefulness of nicorandil as a prophylactic treatment against infarction in patients with coronary artery disease.

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References

1. Auchampach JA, Caverio I, Gross GJ. Nicorandil attenuates myocardial dysfunction associated with transient ischemia by opening ATP-dependent potassium channels. *J Cardiovasc Pharmacol.* 1992; 20:765–771. [PubMed: 1280739]
2. Banerjee S, Tang XL, Qiu Y, Takano H, Manchikalapudi S, Dawn B, Shirk G, Bolli R. Nitroglycerin induces late preconditioning against myocardial stunning via a PKC-dependent pathway. *Am J Physiol Heart Circ Physiol.* 1999; 277:H2488–H2494.
3. Baxter GF, Marber MS, Patel VC, Yellon DM. Adenosine receptor involvement in a delayed phase of myocardial protection 24 hours after ischemic preconditioning. *Circulation.* 1994; 90:2993–3000. [PubMed: 7994847]
4. Bolli R. The late phase of preconditioning. *Circ Res.* 2000; 87:972–983. [PubMed: 11090541]
5. Bolli R, Bhatti ZA, Tang XL, Qiu Y, Zhang Q, Guo Y, Jadoon AK. Evidence that late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide. *Circ Res.* 1997; 81:42–52. [PubMed: 9201026]
6. Bolli R, Manchikalapudi S, Tang XL, Takano H, Qiu Y, Guo Y, Zhang Q, Jadoon AK. The protective effect of late preconditioning against myocardial stunning in conscious rabbits is mediated by nitric oxide synthase. Evidence that nitric oxide acts both as a trigger and as a mediator of the late phase of ischemic preconditioning. *Circ Res.* 1997; 81:1094–1107. [PubMed: 9400391]
7. Das B, Sarkar C. Mitochondrial K_{ATP} channel activation is important in the antiarrhythmic and cardioprotective effects of non-hypotensive doses of nicorandil and cromakalim during ischemia/reperfusion: a study in an intact anesthetized rabbit model. *Pharmacol Res.* 2003; 47:447–461. [PubMed: 12741997]
8. Das B, Sarkar C, Karanth KS. Effects of administration of nicorandil or bimakalim prior to and during ischemia or reperfusion on survival rate, ischemia/reperfusion-induced arrhythmias and infarct size in anesthetized rabbits. *Naunyn Schmiedeberg's Arch Pharmacol.* 2001; 364:383–396. [PubMed: 11692221]
9. Duncker DJ, Klassen CL, Ishibashi Y, Herrlinger SH, Pavek TJ, Bache RJ. Effect of temperature on myocardial infarction in swine. *Am J Physiol Heart Circ Physiol.* 1996; 270:H1189–H1199.
10. Flaherty JT. Nitrate tolerance. A review of the evidence. *Drugs.* 1989; 37:523–550. [PubMed: 2661197]
11. Fryer RM, Hsu AK, Eells JT, Nagase H, Gross GJ. Opioid-induced second window of cardioprotection: potential role of mitochondrial KATP channels. *Circ Res.* 1999; 84:846–851. [PubMed: 10205153]
12. Fryer RM, Hsu AK, Nagase H, Gross GJ. Opioid-induced cardio-protection against myocardial infarction and arrhythmias: mitochondrial versus sarcolemmal ATP-sensitive potassium channels. *J Pharmacol Exp Ther.* 2000; 294:451–457. [PubMed: 10900218]
13. Galie N, Guarnieri C, Ussia GP, Zimarino M, Traini AM, Parlangeli R, Vaona I, Branzi A, Magnani B. Limitation of myocardial infarct size by nicorandil after sustained ischemia in pigs. *J Cardiovasc Pharmacol.* 1995; 26:477–484. [PubMed: 8583791]
14. Gomma AH, Purcell HJ, Fox KM. Potassium channel openers in myocardial ischaemia: therapeutic potential of nicorandil. *Drugs.* 2001; 61:1705–1710. [PubMed: 11693460]
15. Gross GJ, Auchampach JA, Maruyama M, Warltier DC, Pieper GM. Cardioprotective effects of nicorandil. *J Cardiovasc Pharmacol.* 1992; 20(Suppl 3):S22–28. [PubMed: 1282172]
16. Gross GJ, Warltier DC, Hardman HF. Comparative effects of nicorandil, a nicotinamide nitrate derivative, and nifedipine on myocardial reperfusion injury in dogs. *J Cardiovasc Pharmacol.* 1987; 10:535–542. [PubMed: 2447402]
17. Guo Y, Bao W, Tang XL, Wu WJ, Bolli R. Nitric oxide donors induce late preconditioning against myocardial infarction in mice (Abstract). *J Mol Cell Cardiol.* 1999; 31:A-9.
18. Haessler R, Kuzume K, Chien GL, Wolff RA, Davis RF, Van Winkle DM. Anaesthetics alter the magnitude of infarct limitation by ischaemic preconditioning. *Cardiovasc Res.* 1994; 28:1574–1580. [PubMed: 8001048]
19. Heusch G. Nitroglycerin and delayed preconditioning in humans: yet another new mechanism for an old drug? *Circulation.* 2001; 103:2876–2878. [PubMed: 11413072]

20. Hill M, Takano H, Tang XL, Kodani E, Shirk G, Bolli R. Nitroglycerin induces late preconditioning against myocardial infarction in conscious rabbits despite development of nitrate tolerance. *Circulation*. 2001; 104:694–649. [PubMed: 11489777]
21. Imagawa J, Baxter GF, Yellon DM. Myocardial protection afforded by nicorandil and ischaemic preconditioning in a rabbit infarct model in vivo. *J Cardiovasc Pharmacol*. 1998; 31:74–79. [PubMed: 9456280]
22. IONA Study Group. Effect of nicorandil on coronary events in patients with stable angina: the Impact Of Nicorandil in Angina (IONA) randomised trial. *Lancet*. 2002; 359:1269–1275. [PubMed: 11965271]
23. Ito H, Taniyama Y, Iwakura K, Nishikawa N, Masuyama T, Kuzuya T, Hori M, Higashino Y, Fujii K, Minamino T. Intravenous nicorandil can preserve microvascular integrity and myocardial viability in patients with reperfused anterior wall myocardial infarction. *J Am Coll Cardiol*. 1999; 33:654–660. [PubMed: 10080465]
24. Iwamoto T, Miura T, Urabe K, Itoya M, Shimamoto K, Iimura O. Effect of nicorandil on post-ischaemic contractile dysfunction in the heart: roles of its ATP-sensitive K^+ channel opening property and nitrate property. *Clin Exp Pharmacol Physiol*. 1993; 20:595–602. [PubMed: 8222340]
25. Kodani E, Bolli R, Tang XL, Auchampach JA. Protection of IB-MECA against myocardial stunning in conscious rabbits is not mediated by the A1 adenosine receptor. *Basic Res Cardiol*. 2001; 96:487–496. [PubMed: 11605996]
26. Kodani E, Shinmura K, Xuan YT, Takano H, Auchampach JA, Tang XL, Bolli R. Cyclooxygenase-2 does not mediate late preconditioning induced by activation of adenosine A1 or A3 receptors. *Am J Physiol Heart Circ Physiol*. 2001; 281:H959–H968. [PubMed: 11454603]
27. Kodani E, Xuan YT, Shinmura K, Takano H, Tang XL, Bolli R. δ -Opioid receptor-induced late preconditioning is mediated by cyclooxygenase-2 in conscious rabbits. *Am J Physiol Heart Circ Physiol*. 2002; 283:H1943–H1957. [PubMed: 12384473]
28. Kukovetz WR, Holzmann S, Braida C, Poch G. Dual mechanism of the relaxing effect of nicorandil by stimulation of cyclic GMP formation and by hyperpolarization. *J Cardiovasc Pharmacol*. 1991; 17:627–633. [PubMed: 1711631]
29. Kukovetz WR, Holzmann S, Poch G. Molecular mechanism of action of nicorandil. *J Cardiovasc Pharmacol*. 1992; 20(Suppl 3):S1–7. [PubMed: 1282168]
30. Lamping KA, Christensen CW, Pelc LR, Warltier DC, Gross GJ. Effects of nicorandil and nifedipine on protection of ischemic myocardium. *J Cardiovasc Pharmacol*. 1984; 6:536–542. [PubMed: 6202983]
31. Lamping KA, Gross GJ. Improved recovery of myocardial segment function following a short coronary occlusion in dogs by nicorandil, a potential new antianginal agent, and nifedipine. *J Cardiovasc Pharmacol*. 1985; 7:158–166. [PubMed: 2580137]
32. Leeser MA, Stoddard MF, Dawn B, Jasti VG, Masden R, Bolli R. Delayed preconditioning-mimetic action of nitroglycerin in patients undergoing coronary angioplasty. *Circulation*. 2001; 103:2935–2941. [PubMed: 11413083]
33. Markham A, Plosker GL, Goa KL. Nicorandil. An updated review of its use in ischaemic heart disease with emphasis on its cardioprotective effects. *Drugs*. 2000; 60:955–974. [PubMed: 11085202]
34. Matsubara T, Minatoguchi S, Matsuo H, Hayakawa K, Segawa T, Matsuno Y, Watanabe S, Arai M, Uno Y, Kawasaki M, Noda T, Takemura G, Nishigaki K, Fujiwara H. Three minute, but not one minute, ischemia and nicorandil have a preconditioning effect in patients with coronary artery disease. *J Am Coll Cardiol*. 2000; 35:345–351. [PubMed: 10676679]
35. Matsuo H, Watanabe S, Segawa T, Yasuda S, Hirose T, Iwama M, Tanaka S, Yamaki T, Matsuno Y, Tomita M, Minatoguchi S, Fujiwara H. Evidence of pharmacologic preconditioning during PTCA by intravenous pretreatment with ATP-sensitive K^+ channel opener nicorandil. *Eur Heart J*. 2003; 24:1296–1303. [PubMed: 12871686]
36. Mizumura T, Nithipatikom K, Gross GJ. Effects of nicorandil and glyceryl trinitrate on infarct size, adenosine release, and neutrophil infiltration in the dog. *Cardiovasc Res*. 1995; 29:482–489. [PubMed: 7796441]

37. Mizumura T, Nithipatikom K, Gross GJ. Infarct size-reducing effect of nicorandil is mediated by the K_{ATP} channel but not by its nitrate-like properties in dogs. *Cardiovasc Res*. 1996; 32:274–285. [PubMed: 8796114]
38. Mizumura T, Saito S, Ozawa Y, Kanmatsuse K, Gross GJ. An ATP-sensitive potassium (K_{ATP}) channel opener, nicorandil, lowers the threshold for ischemic preconditioning in barbitol-anesthetized dogs. *Heart Vessels Suppl*. 1997; 12:175–177.
39. Nakae I, Takaoka A, Mitsunami K, Yabe T, Ito M, Takahashi M, Matsumoto T, Omura T, Yokohama H, Kinoshita M. Cardioprotective effects of nicorandil in rabbits anaesthetized with halothane: potentiation of ischaemic preconditioning via K_{ATP} channels. *Clin Exp Pharmacol Physiol*. 2000; 27:810–817. [PubMed: 11022974]
40. Nakayama K, Fan Z, Marumo F, Sawanobori T, Hiraoka M. Action of nicorandil on ATP-sensitive K^+ channel in guinea-pig ventricular myocytes. *Br J Pharmacol*. 1991; 103:1641–1648. [PubMed: 1834294]
41. Ockaili R, Emani VR, Okubo S, Brown M, Krottapalli K, Kukreja RC. Opening of mitochondrial K_{ATP} channel induces early and delayed cardioprotective effect: role of nitric oxide. *Am J Physiol Heart Circ Physiol*. 1999; 277:H2425–H2434.
42. Ohno Y, Minatoguchi S, Uno Y, Kariya T, Arai M, Yamashita K, Fujiwara T, Fujiwara H. Nicorandil reduces myocardial infarct size by opening the K_{ATP} channel in rabbits. *Int J Cardiol*. 1997; 62:181–190. [PubMed: 9476676]
43. Patel DJ, Purcell HJ, Fox KM. Cardioprotection by opening of the K_{ATP} channel in unstable angina. Is this a clinical manifestation of myocardial preconditioning? Results of a randomized study with nicorandil CESAR 2 investigation. *Clinical European studies in angina and revascularization*. *Eur Heart J*. 1999; 20:51–57. [PubMed: 10075141]
44. Patel HH, Hsu A, Moore J, Gross GJ. BW373U86, a delta opioid agonist, partially mediates delayed cardioprotection via a free radical mechanism that is independent of opioid receptor stimulation. *J Mol Cell Cardiol*. 2001; 33:1455–1465. [PubMed: 11448134]
45. Pieper GM, Gross GJ. Protective effect of nicorandil on postischemic function and tissue adenine nucleotides following a brief period of low-flow global ischemia in the isolated perfused rat heart. *Pharmacology*. 1989; 38:205–213. [PubMed: 2526951]
46. Pieper GM, Gross GJ. Salutary action of nicorandil, a new antianginal drug, on myocardial metabolism during ischemia and on postischemic function in a canine preparation of brief, repetitive coronary artery occlusions: comparison with isosorbide dinitrate. *Circulation*. 1987; 76:916–928. [PubMed: 2958176]
47. Ping P, Takano H, Zhang J, Tang XL, Qiu Y, Li RC, Banerjee S, Dawn B, Balafonova Z, Bolli R. Isoform-selective activation of protein kinase C by nitric oxide in the heart of conscious rabbits: a signaling mechanism for both nitric oxide-induced and ischemia-induced preconditioning. *Circ Res*. 1999; 84:587–604. [PubMed: 10082480]
48. Ping P, Zhang J, Qiu Y, Tang XL, Manchikalapudi S, Cao X, Bolli R. Ischemic preconditioning induces selective translocation of protein kinase C isoforms epsilon and eta in the heart of conscious rabbits without subcellular redistribution of total protein kinase C activity. *Circ Res*. 1997; 81:404–414. [PubMed: 9285643]
49. Qiu Y, Ping P, Tang XL, Manchikalapudi S, Rizvi A, Zhang J, Takano H, Wu WJ, Teschner S, Bolli R. Direct evidence that protein kinase C plays an essential role in the development of late preconditioning against myocardial stunning in conscious rabbits and that epsilon is the isoform involved. *J Clin Invest*. 1998; 101:2182–2198. [PubMed: 9593774]
50. Qiu Y, Rizvi A, Tang XL, Manchikalapudi S, Takano H, Jadoon AK, Wu WJ, Bolli R. Nitric oxide triggers late preconditioning against myocardial infarction in conscious rabbits. *Am J Physiol Heart Circ Physiol*. 1997; 273:H2931–H2936.
51. Rajesh KG, Sasaguri S, Zhitian Z, Suzuki R, Asakai R, Maeda H. Second window of ischemic preconditioning regulates mitochondrial permeability transition pore by enhancing Bcl-2 expression. *Cardiovasc Res*. 2003; 59:297–307. [PubMed: 12909313]
52. Saito S, Mizumura T, Takayama T, Honye J, Fukui T, Kamata T, Moriuchi M, Hibiya K, Tamura Y, Ozawa Y, Kanmatsue K, Osawa K, Ishihata F, Nakakimura H, Sakai K. Antiischemic effects of nicorandil during coronary angioplasty in humans. *Cardiovasc Drugs Ther*. 1995; 9(Suppl 2):257–263. [PubMed: 7647030]

53. Sakai K, Yamagata T, Teragawa H, Matsuura H, Chayama K. Nicorandil-induced preconditioning as evidenced by troponin T measurements after coronary angioplasty in patients with stable angina pectoris. *Jpn Heart J.* 2002; 43:443–453. [PubMed: 12452302]
54. Sakata Y, Kodama K, Komamura K, Lim YJ, Ishikura F, Hirayama A, Kitakaze M, Masuyama T, Hori M. Salutary effect of adjunctive intracoronary nicorandil administration on restoration of myocardial blood flow and functional improvement in patients with acute myocardial infarction. *Am Heart J.* 1997; 133:616–621. [PubMed: 9200388]
55. Schwartz LM, Jennings RB, Reimer KA. Premedication with the opioid analgesic butorphanol raises the threshold for ischemic preconditioning in dogs. *Basic Res Cardiol.* 1997; 92:106–114. [PubMed: 9166990]
56. Schwartz LM, Verbinski SG, Vander Heide RS, Reimer KA. Epicardial temperature is a major predictor of myocardial infarct size in dogs. *J Mol Cell Cardiol.* 1997; 29:1577–1583. [PubMed: 9220343]
57. Shimshak TM, Preuss KC, Gross GJ, Brooks HL, Warltier DC. Recovery of contractile function in post-ischaemic reperfused myocardium of conscious dogs: influence of nicorandil, a new antianginal agent. *Cardiovasc Res.* 1986; 20:621–626. [PubMed: 2947685]
58. Shinmura K, Nagai M, Tamaki K, Tani M, Bolli R. COX-2-derived prostacyclin mediates opioid-induced late phase of preconditioning in isolated rat hearts. *Am J Physiol Heart Circ Physiol.* 2002; 283:H2534–H2543. [PubMed: 12388283]
59. Shinmura K, Tang XL, Wang Y, Xuan YT, Liu SQ, Takano H, Bhatnagar A, Bolli R. Cyclooxygenase-2 mediates the cardioprotective effects of the late phase of ischemic preconditioning in conscious rabbits. *Proc Natl Acad Sci USA.* 2000; 97:10197–10202. [PubMed: 10963682]
60. Sugimoto K, Ito H, Iwakura K, Ikushima M, Kato A, Kimura R, Tanaka K, Masuyama T, Ogihara T, Kawano S, Fujii K. Intravenous nicorandil in conjunction with coronary reperfusion therapy is associated with better clinical and functional outcomes in patients with acute myocardial infarction. *Circ J.* 2003; 67:295–300. [PubMed: 12655158]
61. Taira N. Similarity and dissimilarity in the mode and mechanism of action between nicorandil and classical nitrates: an overview. *J Cardiovasc Pharmacol.* 1987; 10(Suppl 8):S1–9. [PubMed: 2447414]
62. Takano H, Bolli R, Black RG Jr, Kodani E, Tang XL, Yang Z, Bhattacharya S, Auchampach JA. A1 or A3 adenosine receptors induce late preconditioning against infarction in conscious rabbits by different mechanisms. *Circ Res.* 2001; 88:520–528. [PubMed: 11249876]
63. Takano H, Manchikalapudi S, Tang XL, Qiu Y, Rizvi A, Jadoon AK, Zhang Q, Bolli R. Nitric oxide synthase is the mediator of late preconditioning against myocardial infarction in conscious rabbits. *Circulation.* 1998; 98:441–449. [PubMed: 9714095]
64. Takano H, Tang XL, Bolli R. Differential role of K_{ATP} channels in late preconditioning against myocardial stunning and infarction in rabbits. *Am J Physiol Heart Circ Physiol.* 2000; 279:H2350–H2359. [PubMed: 11045972]
65. Takano H, Tang XL, Qiu Y, Guo Y, French BA, Bolli R. Nitric oxide donors induce late preconditioning against myocardial stunning and infarction in conscious rabbits via an antioxidant-sensitive mechanism. *Circ Res.* 1998; 83:73–84. [PubMed: 9670920]
66. Takashi E, Wang Y, Ashraf M. Activation of mitochondrial K_{ATP} channel elicits late preconditioning against myocardial infarction via protein kinase C signaling pathway. *Circ Res.* 1999; 85:1146–1153. [PubMed: 10590241]
67. Tang XL, Kodani E, Takano H, Hill M, Shinmura K, Vondriska TM, Ping P, Bolli R. Protein tyrosine kinase signaling is necessary for NO donor-induced late preconditioning against myocardial stunning. *Am J Physiol Heart Circ Physiol.* 2003; 284:H1441–H1448. [PubMed: 12531734]
68. Tang XL, Takano H, Rizvi A, Turrens JF, Qiu Y, Wu WJ, Zhang Q, Bolli R. Oxidant species trigger late preconditioning against myocardial stunning in conscious rabbits. *Am J Physiol Heart Circ Physiol.* 2002; 282:H281–H291. [PubMed: 11748073]
69. Tsuchida A, Miura T, Tanno M, Sakamoto J, Miki T, Kuno A, Matsumoto T, Ohnuma Y, Ichikawa Y, Shimamoto K. Infarct size limitation by nicorandil: roles of mitochondrial K_{ATP} channels,

- sarcolemmal K_{ATP} channels, and protein kinase C. *J Am Coll Cardiol.* 2002; 40:1523–1530. [PubMed: 12392845]
70. Xi L, Jarrett NC, Hess ML, Kukreja RC. Essential role of inducible nitric oxide synthase in monophosphoryl lipid A-induced late cardioprotection: evidence from pharmacological inhibition and gene knockout mice. *Circulation.* 1999; 99:2157–2163. [PubMed: 10217657]
71. Xuan YT, Tang XL, Banerjee S, Takano H, Li RC, Han H, Qiu Y, Li JJ, Bolli R. Nuclear factor- κ B plays an essential role in the late phase of ischemic preconditioning in conscious rabbits. *Circ Res.* 1999; 84:1095–1109. [PubMed: 10325247]
72. Yao Z, Auchampach JA, Pieper GM, Gross GJ. Cardioprotective effects of monophosphoryl lipid A, a novel endotoxin analogue, in the dog. *Cardiovasc Res.* 1993; 27:832–838. [PubMed: 8394211]
73. Yasuda T, Hashimura K, Matsuura Y, Kato Y, Ueda T, Mori I, Kijima Y. Nicorandil, a hybrid between nitrate and ATP-sensitive potassium channel opener, preconditions human heart to ischemia during percutaneous transluminal coronary angioplasty. *Jpn Circ J.* 2001; 65:526–530. [PubMed: 11407735]

Fig. 1. Experimental protocol for the studies of myocardial infarction (A) and Western blot analysis of cyclooxygenase-2 (COX-2) and Bcl-2 (B). PC, ischemic preconditioning; O, coronary occlusion; R, reperfusion.

Fig. 2. Pilot studies of arterial pressure (A) and heart rate (B) in three rabbits receiving an intravenous nicorandil infusion at 100 µg/kg bolus plus 50 µg·kg⁻¹·min⁻¹ for 60 min. Values are means ± SE.

Fig. 3. Arterial pressure (A) and heart rate (B) in 10 rabbits in the nicorandil group given intravenous nicorandil at 100 $\mu\text{g/kg}$ bolus plus 30 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for 60 min. Data are means $\pm$ SE.

Fig. 4.

Myocardial infarct size in the control, nicorandil, and ischemic PC groups. Infarct size is expressed as a percentage of the region at risk of infarction. Open circles represent individual rabbits, whereas solid circles represent means $\pm$ SE.

Fig. 5.

Relationship between size of the region at risk and size of myocardial infarction. The figure illustrates both individual values and regression lines obtained by linear regression analysis for the control group ($n = 10$), the nicorandil group ($n = 10$), and the ischemic PC group ($n = 8$). In all groups, infarct size was positively and linearly related to risk region size. The linear regression equations were as follows: control group, $y = 0.72x - 0.08$ ($r = 0.93$); nicorandil group, $y = 0.38x - 0.09$ ($r = 0.63$); and ischemic PC group, $y = 0.54x - 0.18$ ($r = 0.78$). The regression line was shifted downward in the nicorandil and PC groups compared with the control group (both $P < 0.05$ by ANCOVA), indicating that for any given risk region size, infarct size was smaller in rabbits pretreated with nicorandil or ischemic PC compared with control rabbits.

Fig. 6.

Effect of nicorandil on the expression of COX-2 protein in rabbit myocardium. Tissue samples were obtained from the left ventricular wall of rabbits that were euthanized 24 h after receiving vehicle (normal saline, control) or nicorandil. *A*: representative Western blots of COX-2 from four control and four nicorandil-pretreated rabbits. *B*: densitometric analysis of COX-2 signals in the two groups. The densitometric measurements of COX-2 immunoreactivity were expressed as a percentage of the average value measured in the control rabbits. Data are means $\pm$ SE.

Fig. 7.

Effect of nicorandil on the expression of Bcl-2 protein in rabbit myocardium. Tissue samples were obtained from the left ventricular wall of rabbits that were euthanized 24 h after receiving vehicle (normal saline, control) or nicorandil. *A*: representative Western blots of Bcl-2 from four control and four nicorandil-pretreated rabbits. *B*: densitometric analysis of Bcl-2 signals in the two groups. The densitometric measurements of Bcl-2 immunoreactivity were expressed as a percentage of the average value measured in the control rabbits. Data are means $\pm$ SE.