

Nitroglycerin induces late preconditioning against myocardial stunning via a PKC-dependent pathway

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Banerjee, Supratim, Xian-Liang Tang, Yumin Qiu, Hitoshi Takano, Srinivas Manchikalapudi, Buddhadeb Dawn, Gregg Shirk, and Roberto Bolli. Nitroglycerin induces late preconditioning against myocardial stunning via a PKC-dependent pathway. *Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H2488–H2494, 1999.*—Previous studies have shown that administration of nitric oxide (NO) donors induces a delayed cardioprotective effect indistinguishable from the late phase of ischemic preconditioning (PC). However, the ability of clinically relevant NO donors to elicit this phenomenon has not been evaluated. In this study we tested whether an NO-releasing agent that is nitroglycerin (NTG), which is widely used clinically, can mimic the late phase of ischemic PC. Four groups of conscious rabbits underwent six cycles of 4-min occlusion (O)/4-min reperfusion (R) for 3 consecutive days (*days 1, 2, and 3*). The severity of myocardial stunning was assessed as the total deficit of systolic wall thickening (WTh) after the last O/R cycle. In the control group (*group I, n = 6*), the total deficit of WTh was reduced by 50% and 51% on *days 2 and 3* vs. *day 1*, respectively, indicating late PC against stunning. Pretreatment with NTG ($2 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ iv over 1 h) on *day 0* (*group II, n = 6*) was as effective as ischemic PC in mitigating myocardial stunning 24 h later (*day 1*); on *days 2 and 3*, no further reduction of stunning was seen. Coadministration of the PKC inhibitor chelerythrine (5 mg/kg) with NTG (*group III, n = 6*) completely abrogated the NTG-induced protection. Pretreatment with chelerythrine alone (*group IV, n = 5*) did not alter stunning. These results demonstrate that a relatively brief infusion of NTG induces a robust protective effect against stunning 24 h later via a protein kinase C (PKC)-dependent signaling mechanism. The magnitude of NTG-induced protection is equivalent to that observed during the late phase of ischemic PC. Late PC induced by brief treatment with NTG could be a useful therapeutic strategy for myocardial protection in patients with ischemic heart disease.

nitric oxide; myocardial ischemia-reperfusion; nitrates; protein kinase C

IN RECENT YEARS, the phenomenon of ischemic preconditioning (PC) has become the focus of increasing attention among experimental and clinical investigators because of the potentially vast implications of exploiting this cardioprotective mechanism to alleviate the consequences of acute myocardial ischemia in patients (3, 5, 7, 12, 13, 17, 20, 27). The late phase of ischemic PC provides sustained cardioprotection, lasting for 3–4 days (2, 20, 29), and protects against both myocardial

stunning (6, 8, 22, 23, 27–29) and infarction (3, 19, 22, 24, 28, 31). Because of these features, clinical exploitation of late PC is potentially attractive. Recent studies have demonstrated that a delayed cardioprotective effect indistinguishable from the late phase of ischemic PC can be induced by the administration of nitric oxide (NO) donors in the absence of ischemia (22, 28), which is consistent with the concept that NO plays a major role in initiating this cardioprotective adaptation (6, 8, 24). The ability of exogenous NO to trigger a late PC-like phenomenon has important therapeutic implications. However, the NO donors that were tested in those previous studies (22, 28) [diethylenetriamine/NO (DETA/NO) and *S*-nitroso-*N*-acetylpenicillamine (SNAP)] are not clinically available. To date, no clinically relevant NO donor has been shown to induce late PC. In addition, many pharmacological manipulations that have been reported to elicit late PC in experimental models (3, 10, 11, 20, 21) are not clinically applicable. The identification of a clinically relevant treatment that can harness the protective effects of the late phase of ischemic PC would clearly have significant therapeutic implications.

The NO-releasing agent nitroglycerin (NTG) has been used for the treatment of coronary artery disease for over 100 years. This drug is generally well tolerated and has relatively few side effects. We therefore tested whether pretreatment with NTG, in the absence of ischemia, can reproduce the protective actions of the late phase of ischemic PC. We further sought to determine whether the PC-like effects of NTG are mediated by a protein kinase C (PKC)-dependent signaling pathway.

MATERIALS AND METHODS

Experimental Preparation

The experimental preparation has been described in detail previously (6, 8, 22–24, 28). Briefly, New Zealand White male rabbits (weight, 2.0–2.5 kg, age, 3–4 mo) 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 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 aspirate air and fluid postoperatively. Gentamicin was administered before surgery and on the first and second postoperative days (0.7 mg/kg im each day). The animals were allowed to recover for a minimum of 10 days after surgery. Throughout the experiments, rabbits were kept in a cage in a quiet, dimly lit room. Left ventricular systolic wall thickening (WTh), range gate depth, and the ECG were recorded throughout the experi-

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ments on a thermal array chart recorder (Gould TA6000, Valley View, OH).

Experimental Protocol

The experimental protocol consisted of 3 consecutive days of coronary artery occlusions (*days 1, 2, and 3, respectively*). On each day, the rabbits were subjected to a sequence of six 4-min coronary occlusion and 4-min reperfusion cycles (Fig. 1). The performance of successful coronary occlusions 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 systolic wall thinning on the ultrasonic crystal recordings. No sedative or antiarrhythmic agents were given at any time.

Rabbits were assigned to four groups (Fig. 1). *Group I* (control group) underwent the coronary artery occlusion-reperfusion protocol on *days 1, 2, and 3* without any treatment. In *group II* (NTG group), rabbits received an intravenous infusion of NTG ($2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ over 1 h) 24 h before the first sequence of coronary occlusion-reperfusion cycles. This dose was selected on the basis of the pilot studies

reported under RESULTS. In *group III* (NTG-chelerythrine group), rabbits received a bolus of chelerythrine (5 mg/kg) 5 min before the intravenous infusion of NTG ($2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ over 1 h) and 24 h before the first sequence of coronary occlusion-reperfusion cycles. This dose of chelerythrine was chosen because it has previously been shown to be effective in abrogating the late phase of ischemic PC against stunning in conscious rabbits (23). In *group IV* (chelerythrine group), rabbits received only an intravenous bolus of chelerythrine (5 mg/kg) 24 h before the first sequence of coronary occlusion-reperfusion. NTG (Abbot, N. Chicago, IL) was diluted in normal saline to achieve a concentration of 50 $\mu\text{g}/\text{ml}$ and was infused by an infusion pump (total volume infused, ~6 ml). Chelerythrine chloride (Research Biochemicals International, Natick, MA) was dissolved in 2 ml of dimethyl sulfoxide plus 2 ml of normal saline (total volume infused, 4 ml)

Measurement of regional myocardial function. Regional myocardial function was assessed as systolic thickening fraction using the pulsed Doppler probe, as previously described (9). The total deficit of systolic WTh (an integrative

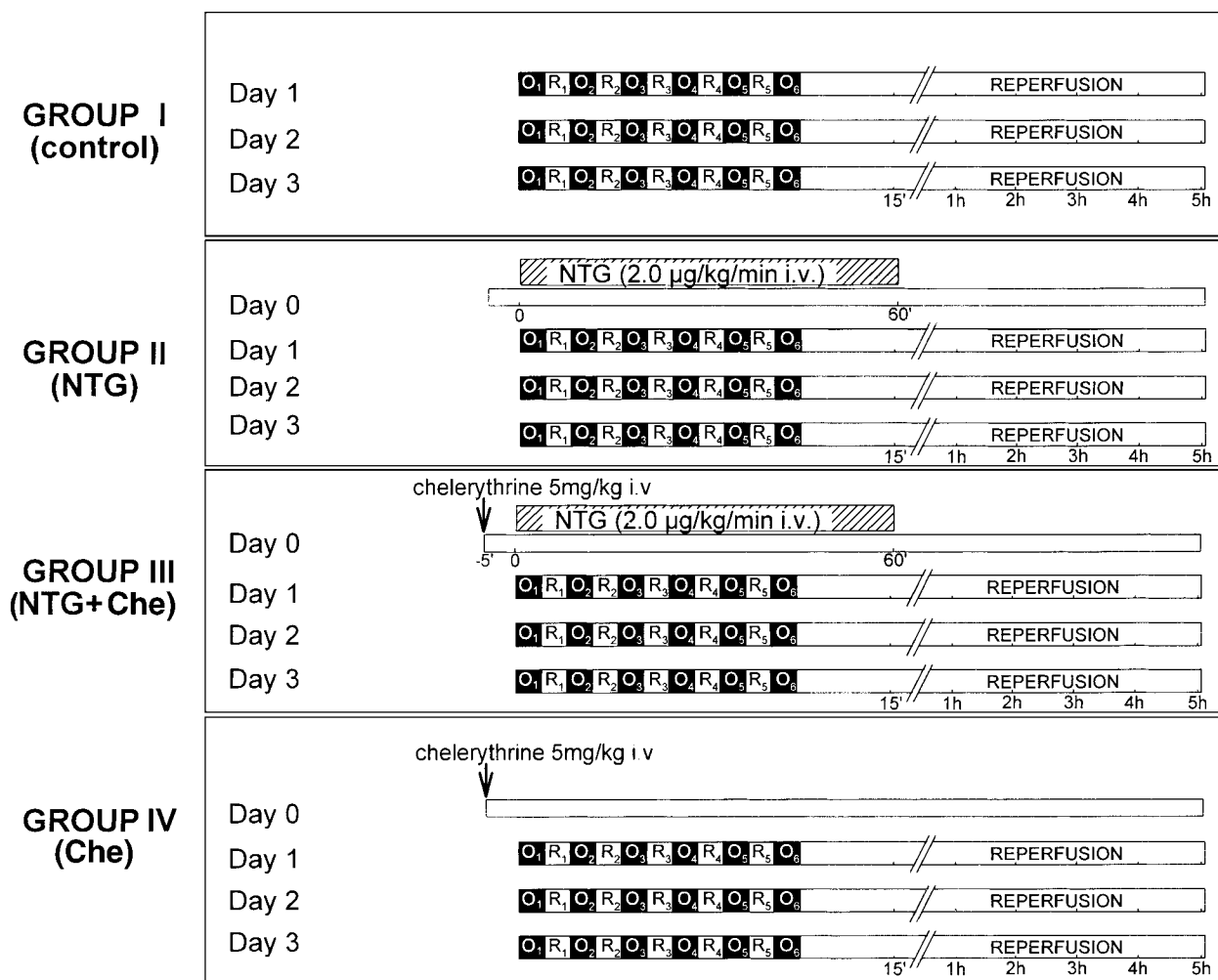


Fig. 1. Experimental protocol for studies of myocardial stunning. Four groups of rabbits underwent a sequence of six 4-min coronary occlusion and 4-min reperfusion cycles followed by a 5-h observation period for 3 consecutive days (*days 1, 2, and 3*). Twenty-four hours before first coronary occlusion (*day 0*), rabbits in *group I* ($n = 6$, control group) received no pretreatment; rabbits in *group II* ($n = 6$, NTG group) received nitroglycerin (NTG, $2 \mu\text{g} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ iv over 1 h); rabbits in *group III* ($n = 6$, NTG-Che group) received chelerythrine (Che, 5 mg/kg iv bolus) 5 min before the same dose of NTG as in *group II*; rabbits in *group IV* ($n = 5$, Che group) received only Che (5 mg/kg iv bolus).

assessment of the overall severity of myocardial stunning) was calculated by measuring the area comprised between the systolic WTh-vs.-time line and the baseline (100% line) during the 5-h recovery phase after the sixth reperfusion (6, 8, 22, 23, 28). In all animals, measurements from at least 10 beats were averaged at baseline and from at least 5 beats at all subsequent time points.

Measurement of region at risk. 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 a bolus of KCl. The heart was excised, and the size of the ischemic-reperfused region (region at risk) 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 to seven transverse slices. All atrial and right ventricular tissues were excised. The region at risk (which was identified by the absence of blue dye) was separated from the rest of the left ventricle, and both components were weighed to determine the occluded bed size as a percentage of total left ventricular (LV) weight (6, 8, 22, 23, 28).

Statistical Analysis

Data are reported as means \pm SE. For intragroup comparisons, hemodynamic variables and WTh were analyzed by a two-way repeated-measures ANOVA (time and day) followed by Student's *t*-tests for paired data with the Bonferroni correction (30). For intergroup comparisons, data were analyzed by either a one-way or a two-way repeated-measures (time and group) ANOVA, as appropriate, followed by unpaired Student's *t*-tests with the Bonferroni correction (30). All statistical analyses were performed using SigmaStat for Windows Version 2.0.

RESULTS

A total of 33 rabbits were used in this study (10 for the pilot studies and 23 for the studies of late PC).

Pilot Studies

Pilot studies were conducted in six rabbits to identify the lowest dose of NTG that would induce a late PC effect. The purpose of these studies was twofold. First, reducing the dose of NTG would help minimize the side effects associated with higher doses. Second, the dose-response relationship for NTG with respect to the induction of late PC is unknown. We found that infusion of NTG at $0.5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ iv for 1 h did not produce a late PC effect 24 h later (total deficit of WTh same as control). Increasing the infusion rate to $1 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for 1 h did not bring a late PC effect. However, when the dose of NTG was held constant at $0.5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and the duration of infusion was lengthened from 1 h to 4 h, a late PC effect was observed. When the duration of the infusion was held at 4 h and the rate of infusion was lowered from 0.5 to $0.25 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, only a partial late PC effect was observed (total deficit of WTh 24 h later: -31% vs. control rabbits). When the duration of the infusion was shortened from 4 to 1 h and the infusion rate was increased from 0.5 to $2.0 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (so that the total amount NTG infused remained constant), a robust late

PC effect was observed. For the present study, we selected a dose of $2 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ iv over 1 h, which was more convenient to administer compared with a dose of $0.5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ over 4 h and was equally effective.

Additional pilot studies were conducted in four rabbits to confirm that infusion of NTG at $2 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ had no significant hemodynamic effects. The concern was that hemodynamic perturbations caused by NTG (e.g., a fall in blood pressure or an increase in heart rate) could contribute nonspecifically to induce a late PC effect unrelated to the direct actions of NO on the heart. Arterial pressure was measured by cannulating the ear dorsal artery with a 22-gauge angiocatheter under local anesthesia (benzocaine), as previously described (6). The results showed that infusion of NTG at $2 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ did not cause any appreciable changes in arterial blood pressure, heart rate, or WTh (data not shown).

Exclusions and Postmortem Analysis

Of the 23 rabbits instrumented for the studies of myocardial stunning, 6 were assigned to *group I* (control group), 6 to *group II* (NTG group), 6 to *group III* (NTG-chelerythrine group), and 5 to *group IV* (chelerythrine group). All of the animals completed the protocol satisfactorily and were included in the data analysis. Postmortem analysis showed that the size of the occluded-reperfused vascular bed was similar in the four groups: 0.84 ± 0.08 g ($19.1 \pm 1.8\%$ of LV weight) in *group I*, 0.76 ± 0.08 g ($18.7 \pm 2.0\%$ of LV weight) in *group II*, 0.77 ± 0.04 g ($18.2 \pm 1.4\%$ of LV weight) in *group III*, and 0.90 ± 0.10 g ($18.8 \pm 1.2\%$ of LV weight) in *group IV*. Tissue staining with triphenyltetrazolium chloride confirmed the absence of infarction in all animals. In all rabbits, the ultrasonic crystal was found to be at least 3 mm from the boundaries of the ischemic-reperfused region.

Hemodynamic Variables

As shown in Table 1, on *day 0* there were no significant changes in heart rate, arterial blood pressure, or systolic WTh at any time during or after the administration of NTG, NTG-chelerythrine, or chelerythrine in *groups II, III, and IV*, respectively, except for a transient decline in heart rate at 1 h after chelerythrine in *group IV*, which resolved by 3 h. On *days 1, 2, and 3*, there were no appreciable differences in heart rate among the four groups, either during the sequence of coronary occlusion-reperfusion cycles or during the 5-h reperfusion period (Table 2).

Regional Myocardial Function

Baseline systolic thickening fraction on *days 1, 2, and 3* averaged $39.2 \pm 2.7\%$, $40.0 \pm 2.8\%$, and $39.6 \pm 2.6\%$, respectively, in *group I*; $36.0 \pm 3.5\%$, $35.8 \pm 3.4\%$, and $35.5 \pm 3.4\%$, respectively, in *group II*; $34.7 \pm 1.5\%$, $33.8 \pm 1.9\%$, and $34.3 \pm 1.8\%$, respectively, in *group III*; and $32.6 \pm 2.6\%$, $32.3 \pm 3.1\%$ and $31.9 \pm 2.6\%$, respectively, in *group IV* (Figs. 2–5). There were no

Table 1. Hemodynamic variables during and after administration of nitroglycerin, nitroglycerin + chelerythrine, or chelerythrine

	Baseline	End of Drug Administration*	1 h After End of Drug Administration
Heart rate, beats/min			
Group II	267 ± 10	247 ± 13	250 ± 13
Group III	251 ± 9	233 ± 12	248 ± 12
Group IV	251 ± 7	238 ± 11	204 ± 9†
Mean arterial pressure, mmHg			
Group II	86 ± 4	88 ± 5	80 ± 5
Group III	77 ± 4	81 ± 3	76 ± 3
Group IV	82 ± 4	84 ± 4	85 ± 4
Systolic wall thickening fraction, %			
Group II	36.0 ± 3.8	35.2 ± 3.5	32.5 ± 3.5
Group III	31.5 ± 1.0	31.1 ± 1.0	30.6 ± 1.0
Group IV	33.2 ± 3.0	33.1 ± 2.7	32.6 ± 2.6

Data are means ± SE. Group II received nitroglycerin 2 µg·kg⁻¹·min⁻¹ iv over 1 h. Group III received nitroglycerin according to the same protocol used in group II; in addition, the rabbits were given a bolus of 5 mg/kg chelerythrine 5 min before the infusion. Group IV received a bolus of 5 mg/kg of chelerythrine alone. *In groups II and III, these measurements were taken at the end of 1 h of nitroglycerin infusion. In group IV, these measurements were taken immediately after the bolus of chelerythrine. †P < 0.05 vs. baseline.

significant differences among groups I, II, III, and IV on the same day or among different days within the same group. Furthermore, within the same group, there were no significant differences among days 1, 2, and 3 with respect to the extent of paradoxical systolic wall thinning during the six occlusions (Figs. 2–5).

Table 2. Heart rate during coronary occlusion and reperfusion

	Baseline	Third Occlusion	Reperfusion		
			1 h	3 h	5 h
<i>Group I (control)</i>					
Day 1	237 ± 9	257 ± 11	232 ± 13	220 ± 8	228 ± 7
Day 2	254 ± 10	253 ± 5	237 ± 5	236 ± 13	239 ± 9
Day 3	253 ± 7	253 ± 15	235 ± 7	237 ± 15	229 ± 9
<i>Group II (NTG)</i>					
Day 1	259 ± 7	250 ± 10	245 ± 10	240 ± 7	249 ± 12
Day 2	262 ± 5	262 ± 7	242 ± 8	228 ± 5	232 ± 10
Day 3	272 ± 7	258 ± 8	244 ± 9	241 ± 9	239 ± 8
<i>Group III (NTG + Che)</i>					
Day 1	254 ± 8	251 ± 7	232 ± 8	239 ± 4	244 ± 5
Day 2	249 ± 12	253 ± 8	252 ± 12	244 ± 13	267 ± 7
Day 3	255 ± 9	245 ± 10	235 ± 12	226 ± 11	242 ± 10
<i>Group IV (Che)</i>					
Day 1	258 ± 12	267 ± 9	272 ± 12	255 ± 13	264 ± 10
Day 2	270 ± 6	267 ± 9	255 ± 12	254 ± 7	251 ± 6
Day 3	268 ± 6	277 ± 8	261 ± 14	250 ± 11	244 ± 7

Data are means ± SE. All rabbits underwent a sequence of six cycles of 4-min coronary occlusion/4-min reperfusion followed by a 5-h observation period on days 1, 2, and 3. Heart rate (beats/min) was measured 5 min before occlusion (baseline), 3 min into the 3rd occlusion, and at 1, 3, and 5 h after the 6th reperfusion. NTG, nitroglycerin; Che, chelerythrine.

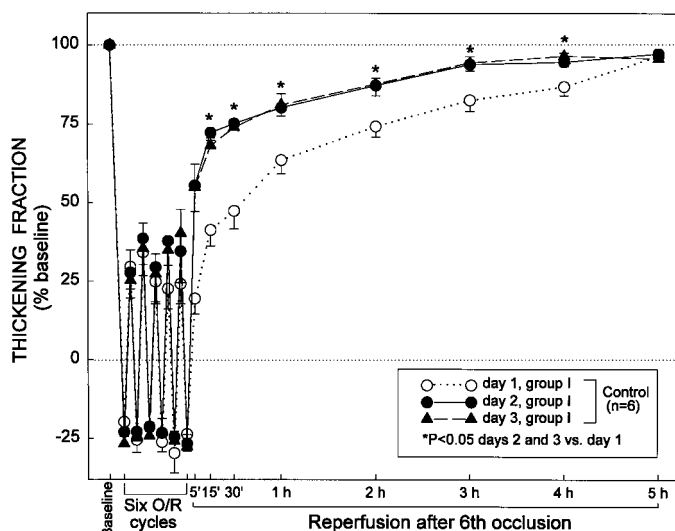


Fig. 2. Systolic thickening fraction in the ischemic-reperfused region in group I (control group) 5 min before first occlusion (Baseline), 3 min into each coronary occlusion (O), 3 min into each reperfusion (R), and at selected times during the 5-h reperfusion interval following the sixth occlusion. Thickening fraction is expressed as a percentage of baseline values. Data are means ± SE.

Group I (control group). On day 1, the sequence of six 4-min occlusion and 4-min reperfusion cycles resulted in severe myocardial stunning, as demonstrated by the fact that thickening fraction remained significantly depressed for 4 h after the sixth reperfusion and recovered to >90% of baseline only at 5 h (Fig. 2). On days 2 and 3, however, the recovery of WTh after the six occlusion-reperfusion cycles was markedly improved compared with day 1 (Fig. 2). The total deficit of WTh after the sixth reperfusion was 50% less on day 2 and 51% less on day 3 compared with day 1 (P < 0.01) (Fig.

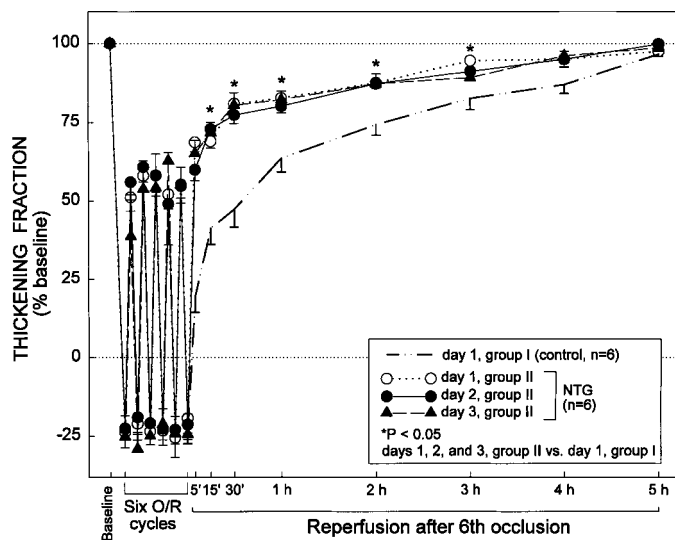


Fig. 3. Systolic thickening fraction in ischemic-reperfused region in group II (NTG group) 5 min before first occlusion (Baseline), 3 min into each coronary occlusion (O), 3 min into each reperfusion (R), and at selected times during the 5-h reperfusion interval after sixth occlusion. To facilitate comparisons, data pertaining to day 1 of group I (control) are also shown. Thickening fraction is expressed as a percentage of baseline values. Data are means ± SE.

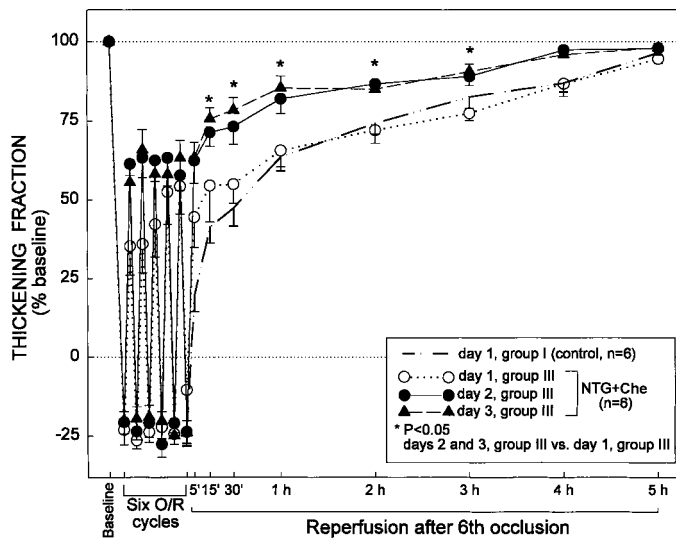


Fig. 4. Systolic thickening fraction in ischemic-reperfused region in *group III* (NTG-Che group) 5 min before first occlusion (Baseline), 3 min into each coronary occlusion (O), 3 min into each reperfusion (R), and at selected times during the 5-h reperfusion interval after sixth occlusion. To facilitate comparisons, data pertaining to *day 1* of *group I* (control) are also shown. Thickening fraction is expressed as a percentage of baseline values. Data are means \pm SE.

6). Thus, as expected (6, 8, 22, 23, 28), myocardial stunning was attenuated on *days 2* and *3* compared with *day 1*, indicating a late PC effect.

Group II (NTG group). On *day 1*, despite similar degrees of dyskinesia during coronary occlusion, the recovery of WTh after the six occlusion-reperfusion cycles was markedly improved in NTG-pretreated rabbits compared with those of control rabbits (Fig. 3). The improvement was statistically significant ($P < 0.05$ vs. control) immediately after the sixth reperfusion and

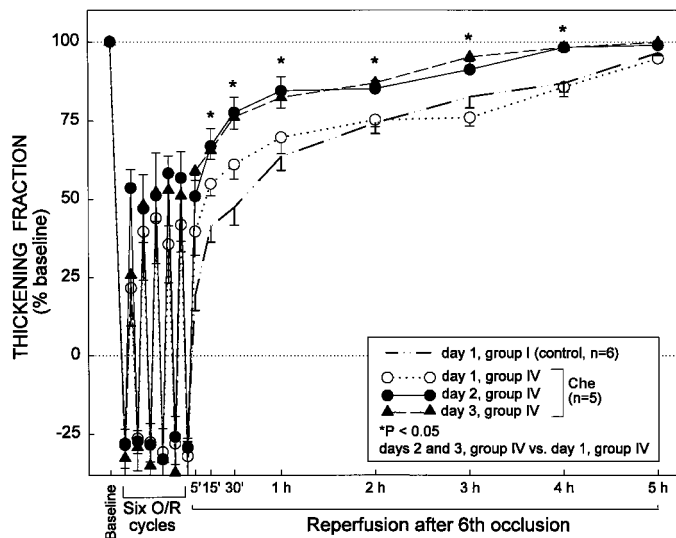


Fig. 5. Systolic thickening fraction in ischemic-reperfused region in *group IV* (Che group) 5 min before first occlusion (Baseline), 3 min into each coronary occlusion (O), 3 min into each reperfusion (R), and at selected times during the 5-h reperfusion interval after sixth occlusion. To facilitate comparisons, data pertaining to *day 1* of *group I* (control) are also shown. Thickening fraction is expressed as a percentage of baseline values. Data are means \pm SE.

persisted throughout the ensuing 3 h (Fig. 3). The total deficit of WTh on *day 1* was 54% less in NTG-pretreated rabbits compared with control rabbits and was virtually indistinguishable from that observed on *day 2* in control rabbits (Fig. 6). On *days 2* and *3*, NTG-pretreated rabbits exhibited no further improvement in either the recovery of WTh (Fig. 3) or the total deficit of WTh (Fig. 6) compared with *day 1*. Thus administration of NTG 24 h before the sequence of six 4-min occlusion-reperfusion cycles resulted in an attenuation of myocardial stunning on *day 1*, which was essentially equivalent to that afforded by ischemic PC.

Group III (NTG-chelerythrine group). The combination of NTG and chelerythrine was studied to elucidate the mechanism of the PC effect induced by NTG and, specifically, to determine whether it was mediated by a PKC-dependent pathway. On *day 1*, both the recovery of WTh (Fig. 4) and the total deficit of WTh (Fig. 6) were virtually indistinguishable from those noted in the control group, indicating that the PC effect induced by NTG was completely abrogated by the concomitant administration of chelerythrine. The expected ischemic PC effect became apparent on *days 2* and *3*, as documented by the enhanced recovery of WTh (Fig. 4) and the reduced deficit of WTh (Fig. 6) compared with *day 1*.

Group IV (chelerythrine group). Pretreatment with chelerythrine alone was examined to exclude the possibility that this agent may have a delayed deleterious effect on the myocardium resulting in exacerbation of stunning. Our finding that in chelerythrine-pretreated rabbits the recovery of WTh (Fig. 5) and the total deficit of WTh (Fig. 6) were similar to those observed in the control group on *days 1, 2* and *3*, indicates that chelerythrine has no effect on myocardial stunning in nonpreconditioned hearts.

DISCUSSION

The major obstacle to the translation of the vast amount of knowledge accumulated regarding ischemic PC into clinical therapies is the identification of agents that can be safely given to patients to recruit this cardioprotective mechanism. Although animal studies have demonstrated that exogenous NO can induce a late PC-like state (22, 28), the NO donors examined in those studies (DETA/NO and SNAP) are not used in patients.

The present investigation demonstrates that administration of NTG, in the absence of ischemia, induces protection against myocardial stunning 24 h later, and that the magnitude of this protection is indistinguishable from that observed during the late phase of ischemic PC. Our pilot studies demonstrate that the ability of NTG to elicit a late PC effect is dose dependent, such that infusion rates $< 0.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 4 h or $< 2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 1 h are ineffective or inconsistently effective. The late PC effect of NTG cannot be ascribed to myocardial ischemia secondary to a decrease in arterial pressure or an increase in heart rate but must have been the result of a direct action of NO on the heart, because the drug did not produce any appreciable hemodynamic changes. Finally, NTG-

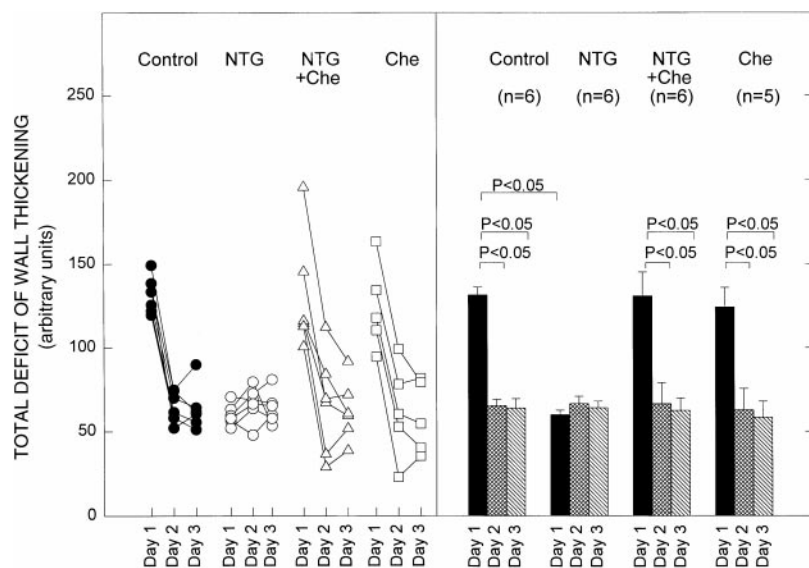


Fig. 6. Total deficit of WTh after sixth reperfusion on days 1, 2, and 3 in control ($n = 6$), NTG ($n = 6$), NTG-Che ($n = 6$), and Che ($n = 5$) groups (groups I, II, III, and IV, respectively). *Left*: values of total deficit of WTh in individual rabbits; *right*: means \pm SE values of total deficit of WTh. Total deficit of WTh was measured in arbitrary units (see text).

triggered late PC against stunning was completely abrogated by the concomitant administration of chelerythrine, indicating that the mechanism whereby NTG induces delayed protection involves a PKC-dependent pathway. To our knowledge, this is the first demonstration that a therapy widely used in patients is able to elicit a late PC effect. Taken together with our previous finding that DETA/NO and SNAP elicit late PC (22, 28), these data further support the hypothesis that NO plays a pivotal role in triggering delayed myocardial adaptations to ischemic stress.

The choice of a conscious animal model was dictated by the preclinical nature of this study. In an effort to rigorously test the cardioprotective actions of NTG under conditions that are as physiological as possible, we felt it was important that all studies be performed in a preparation devoid of the confounding factors inherent in open-chest models or isolated heart preparations, which could affect myocardial stunning (4, 18) and/or ischemic PC (15, 26). The conscious rabbit model of late PC has been extensively characterized and yields reproducible baseline levels of wall thickening as well as reproducible degrees of myocardial stunning (6, 8, 22–24, 28).

The ability of chelerythrine to block NTG-induced late PC is consistent with our previous finding that chelerythrine blocks DETA/NO- and SNAP-induced late PC in the same conscious rabbit model (22). Together with those previous observations, the present finding underscores the critical role of PKC in the NO-initiated signaling pathway that underlies the development of delayed myocardial adaptations to stress. The notion that NTG can mobilize PKC in the heart is novel and has potentially important implications for our understanding of the biological actions of this widely used nitrovasodilator.

Translation of basic concepts regarding delayed cardioprotection into clinical benefit is conceptually attractive because of the long-lasting (3–4 days) beneficial effects afforded by the late phase of ischemic PC (2, 20, 29). NTG has been used in clinical practice for over 100

years and is generally well tolerated. Intravenous infusion of NTG is usually given to treat acute myocardial ischemia or congestive heart failure (1, 14, 25). The present study supports a novel potential application of this drug in patients with coronary artery disease. The results reported herein suggest that in addition to its antianginal and vasodilator properties, NTG could be used to precondition the heart against subsequent ischemia-reperfusion injury occurring hours or days after the infusion of the drug has been discontinued. In view of the recent finding that infusion of NTG also elicits late PC against myocardial infarction (16), it would appear that this agent could be an effective pharmacological tool for conferring delayed cardioprotection against both reversible (stunning) and irreversible (infarction) ischemia-reperfusion injury. The intravenous administration of NTG examined in this investigation is particularly feasible in patients who are hospitalized for acute coronary syndromes; our data, however, imply that other forms of NTG delivery may also be able to elicit a late PC effect, which would make this therapy clinically feasible in an even larger spectrum of patients. Thus the present findings provide a rationale for clinical studies aimed at investigating the potential usefulness of NTG as a PC-mimetic therapy. In addition, this study suggests that other nitrovasodilators (e.g., sodium nitroprusside) might be exploited to recruit a late PC effect in patients. An important issue that remains to be addressed is whether the tolerance that develops after prolonged administration of NTG also applies to the PC-mimetic actions of this drug.

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