

Nitric oxide donors attenuate myocardial stunning in conscious rabbits

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Shinmura, Ken, Xian-Liang Tang, Hitoshi Takano, Michael Hill, and Roberto Bolli. Nitric oxide donors attenuate myocardial stunning in conscious rabbits. *Am. J. Physiol.* 277 (*Heart Circ. Physiol.* 46): H2495–H2503, 1999.— Although previous studies suggested that the protection of late preconditioning (PC) against myocardial stunning is mediated by nitric oxide (NO), direct evidence that exogenous administration of NO attenuates myocardial stunning is lacking. Furthermore, although exogenous NO administration was shown to elicit a late PC phase, it is unknown whether NO donors also induce an early PC phase. Therefore, conscious rabbits underwent two experimental stages (3 days of six 4-min occlusion/4-min reperfusion cycles each) 2 wk apart. In *study I*, both stages were control stages ($n = 7$). In *studies II* and *III*, *stage I* was the control stage. On *day 1* of *stage II*, seven rabbits received infusion of nitroglycerin (NTG; $2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ iv) during the ischemia-reperfusion sequence, starting 30 min before the 1st occlusion and ending 10 min after the 6th reperfusion (*study II*). Another seven rabbits received infusion of NTG ($2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ iv) for 1 h followed by a 30-min washout interval and then underwent six 4-min occlusion/4-min reperfusion cycles (*study III*). In the control stage of all three studies, recovery of wall thickening (WTh) after occlusion/reperfusion cycles was markedly enhanced on *days 2* and *3* compared with *day 1*, indicating late PC. In *study II*, infusion of NTG during the occlusion/reperfusion cycles on *day 1* resulted in significant and sustained enhancement in WTh recovery. A similar attenuation of stunning was observed in *study IV* in six rabbits given intravenous infusion of *S*-nitroso-*N*-acetylpenicillamine (SNAP) during occlusion/reperfusion cycles. The magnitude of the protection afforded by NTG and SNAP was comparable to that afforded by the late ischemic PC phase. In contrast, in *study III* infusion of NTG before occlusion/reperfusion cycles did not enhance WTh recovery, indicating that NTG failed to induce an early PC effect against stunning. This study demonstrates that administration of hemodynamically inactive doses of two unrelated NO donors alleviates myocardial stunning in conscious rabbits, providing direct evidence for a protective action of NO in this setting.

myocardial ischemia; reperfusion injury; left ventricular function; nitroglycerin

THE DEVELOPMENT OF POWERFUL protection against myocardial stunning is one of the most consistent aspects of the late phase of ischemic preconditioning (PC) (4, 8). Our laboratory reported (5–7, 21, 31–33, 35–37, 39, 45) that PC with repetitive cycles of brief ischemia induces

robust protection against myocardial stunning 24–72 h later in conscious pigs and rabbits. In recent years, a number of pharmacological studies have indicated that the production of nitric oxide (NO) is both the trigger and the mediator of late PC against stunning (Refs. 5, 7; reviewed in Ref. 6). However, direct evidence that NO attenuates myocardial stunning is still lacking. If increased biosynthesis of NO by NO synthase is responsible for the protective effects of late PC, then exogenous administration of NO donors should reproduce the protection of late PC against myocardial stunning. At present, the ability of NO precursors (such as L-arginine) or NO-releasing agents to mitigate the severity of postischemic myocardial stunning in vivo remains controversial. Infusion of L-arginine has been reported to aggravate myocardial stunning in open-chest dogs (26). Nitroglycerin (NTG) has been found to alleviate myocardial stunning in open-chest rabbits, but the duration of the reperfusion interval (30 min) was too short to enable definitive conclusions (17). In an earlier study in open-chest dogs, Gross et al. (13) found that NTG enhanced the recovery of segmental shortening only transiently (in the first 30 min of reperfusion) and that regional myocardial function was indistinguishable between control and treated animals by 2 h of reperfusion. Thus evidence that NO donors can effect a sustained mitigation of myocardial stunning is still lacking.

In addition, although exogenous NO administration was shown to elicit a late phase of protection equivalent to the late phase of ischemic PC (37), it is unknown whether NO donors also induce an early phase of PC. We have found that intravenous administration of NTG mimics late PC against stunning 24 h later via a protein kinase C (PKC)-dependent pathway (3). The activation of PKC is also known to be a key step in the development of the early phase of PC (20, 46). However, it is unknown whether the same dosage of NTG that elicits a late PC effect against stunning 24 h later can also produce an early PC effect against stunning.

Thus there were two aims in this study. First, we sought to determine whether infusion of two unrelated NO donors [NTG and *S*-nitroso-*N*-acetylpenicillamine (SNAP)] during ischemia-reperfusion attenuates myocardial stunning in conscious rabbits. Second, we investigated whether pretreatment with the same dosage of NTG that was previously shown to induce a late PC effect (3) can also induce an early PC effect against myocardial stunning in conscious rabbits.

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METHODS

The conscious rabbit model of myocardial ischemia was described in detail previously (5, 7, 21, 31, 32, 37, 45) and is briefly summarized here.

Experimental Preparation

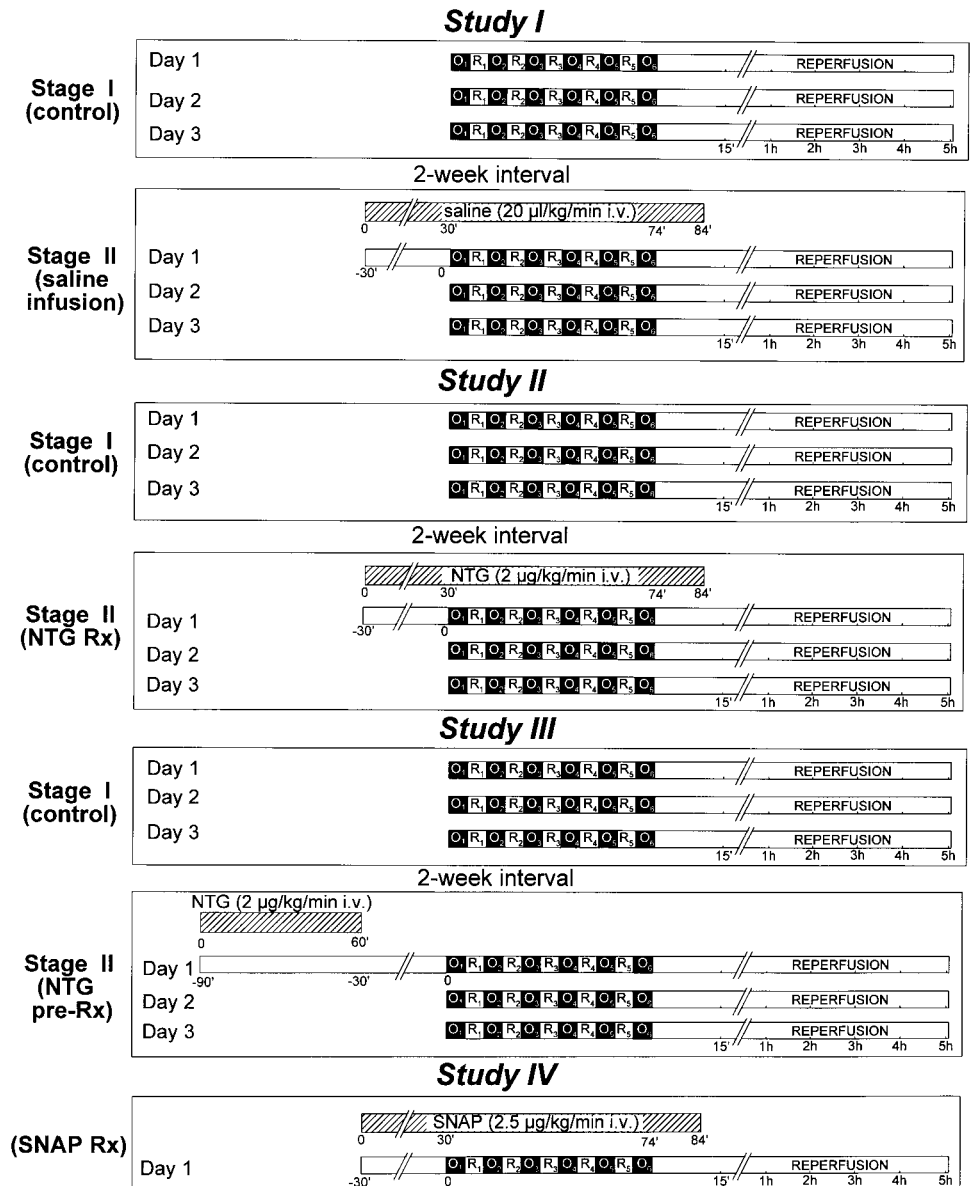
New Zealand White male rabbits (wt 2.4 ± 0.1 kg) were instrumented under sterile conditions with a balloon occluder around a major branch of the left coronary artery, a 10-MHz pulsed Doppler ultrasonic crystal in the center of the region to be rendered ischemic, and bipolar electrocardiogram (ECG) leads 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 14 days after surgery.

Experimental Protocols

Throughout the experiments, rabbits were kept in a cage in a quiet room. Left ventricular (LV) systolic wall thickening (WTh), range gate depth, and the 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 (9). No sedative or antiarrhythmic agents were given on any day. A total of 33 rabbits were assigned to four studies (Fig. 1).

Study I (control study). In this study, as well as in *studies II and III*, all rabbits underwent two stages of experiments, each consisting of three consecutive days of six 4-min coronary occlusion/4-min reperfusion cycles. 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. In *stage I* (control stage), rabbits underwent three consecutive days of six 4-min coronary occlusion/4-min reperfusion cycles with-

Fig. 1. Experimental protocol. Four groups of rabbits were studied. In *studies I, II, and III*, rabbits underwent 2 stages of experiments consisting of 3 consecutive days of six 4-min coronary occlusion (O)/4-min reperfusion (R) cycles, followed by 5 h of observation. The stages were performed 2 wk apart. *Stage I* of each study was the control stage. On *day 1* of *stage II*, rabbits received an iv infusion of saline (*study I*) or nitroglycerin (NTG, *studies II and III*). In *study I*, rabbits received an iv infusion of saline ($20 \mu\text{l} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), starting 30 min before 1st occlusion and ending 10 min after 6th reperfusion for 84 min ($n = 7$). In *study II*, rabbits received an iv infusion of NTG ($2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), starting 30 min before 1st occlusion and ending 10 min after 6th reperfusion (NTG Rx; $n = 7$). In *study III*, rabbits received an iv infusion of NTG ($2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) for 1 h followed by a 30-min washout interval and then underwent six 4-min occlusion/4-min reperfusion cycles (NTG pre-Rx; $n = 7$). In *study IV*, rabbits received an iv infusion of *S*-nitroso-*N*-acetylpenicillamine (SNAP, $2.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), starting 30 min before 1st occlusion and ending 10 min after 6th reperfusion (SNAP Rx; $n = 6$).



out any treatment. Two weeks later, they again underwent three consecutive days of six 4-min coronary occlusion/4-min reperfusion cycles. On *day 1 of stage II*, rabbits received an intravenous infusion of saline ($20 \mu\text{l} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), starting 30 min before the first occlusion and ending 10 min after the sixth reperfusion (Fig. 1).

Study II (NTG treatment). In *stage I* (control stage), rabbits underwent three consecutive days of six 4-min coronary occlusion/4-min reperfusion cycles without any treatment. Two weeks later, they again underwent three consecutive days of six 4-min coronary occlusion/4-min reperfusion cycles. On *day 1 of stage II*, rabbits received an intravenous infusion of NTG ($2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), starting 30 min before the first occlusion and ending 10 min after the sixth reperfusion (Fig. 1). NTG was diluted in normal saline and infused at a rate of $20 \mu\text{l} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 84 min. This rate of NTG infusion was selected because it is effective in inducing late PC against myocardial stunning in conscious rabbits (3) and also because it was found in pilot studies to be the highest dosage that could be given to conscious rabbits without causing hemodynamic changes.

Study III (NTG pretreatment). In *stage I* (control stage), rabbits underwent three consecutive days of six 4-min coronary occlusion/4-min reperfusion cycles without any treatment. Two weeks later, they again underwent three consecutive days of six 4-min coronary occlusion/4-min reperfusion cycles. On *day 1 of stage II*, rabbits received an intravenous infusion of NTG ($2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) for 1 h followed by a 30-min washout interval and then underwent six 4-min occlusion/4-min reperfusion cycles (Fig. 1). NTG was diluted in normal saline; the total volume of the solution that was administered over 60 min was 1.68 ml/kg. This rate and duration of NTG infusion were selected because they have previously been shown to induce late PC against myocardial stunning 24 h later in conscious rabbits (3), thereby making it possible to directly compare the early and late PC effects of NTG.

Study IV (SNAP treatment). Rabbits were subjected to six 4-min occlusion/4-min reperfusion cycles and received an intravenous infusion of SNAP ($2.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), starting 30 min before the first occlusion and ending 10 min after the sixth reperfusion (Fig. 1). This rate of SNAP infusion was selected because it is effective in inducing late PC against myocardial stunning in conscious rabbits (37).

Measurement of Regional Myocardial Function

Regional myocardial function was assessed as systolic thickening fraction using a Doppler probe, as previously described (9). Systolic thickening fraction was calculated as the ratio of net systolic WTh to end-diastolic wall thickness, multiplied by 100 (9). The total deficit of systolic WTh after the sixth reperfusion (an integrative assessment of the overall severity of postischemic dysfunction) was calculated as the area between the systolic WTh-versus-time line and the baseline (100% line) during the 5-h recovery period (5, 7, 21, 31, 32, 37, 45). In all animals, measurements from at least 10 beats were averaged at baseline and from at least 5 beats at all subsequent time points.

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 group), followed by Student's *t*-tests for paired data with the Bonferroni correction. For intergroup comparisons, data were analyzed by either a one-way or a two-way repeated-measures ANOVA (time and group), as appropriate, followed by Student's *t*-tests for paired data with the Bonferroni correction. All statistical analyses were performed using the SAS software system.

RESULTS

Hemodynamic Parameters

In four rabbits, arterial blood pressure was measured using a 22-gauge angiocatheter inserted into the middorsal ear artery as previously described (5). Infusion of $2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ of NTG for 1 h did not produce significant changes in mean arterial blood pressure, consistent with previous reports from our laboratory (85 ± 5 mmHg at baseline, 84 ± 6 mmHg at 30 min into the infusion, and 80 ± 6 mmHg at end of the infusion; Ref. 3).

Exclusions

Of the 33 rabbits instrumented for the studies of myocardial stunning, 5 were excluded because the WTh

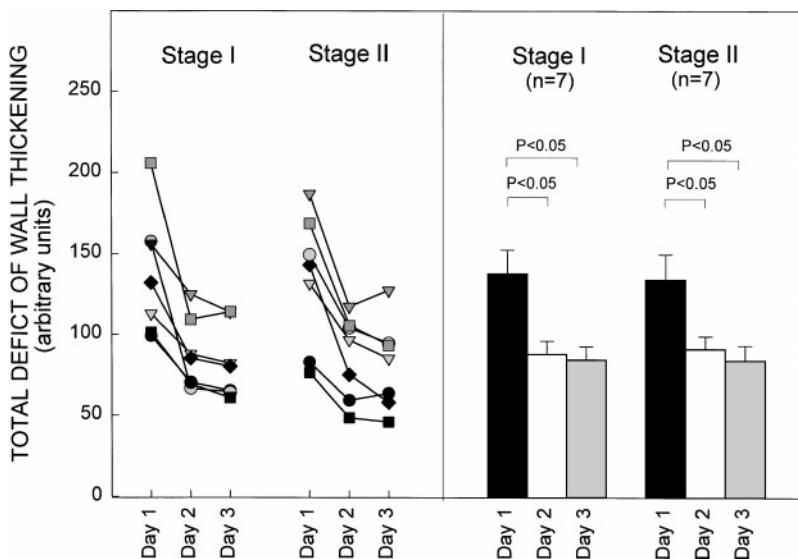


Fig. 2. Total deficit of wall thickening (WTh) after 6th reperfusion in *study I*. Values of total deficit of WTh in individual rabbits are illustrated on *left*. Total deficit of WTh was measured in arbitrary units as described in text. Data are means \pm SE.

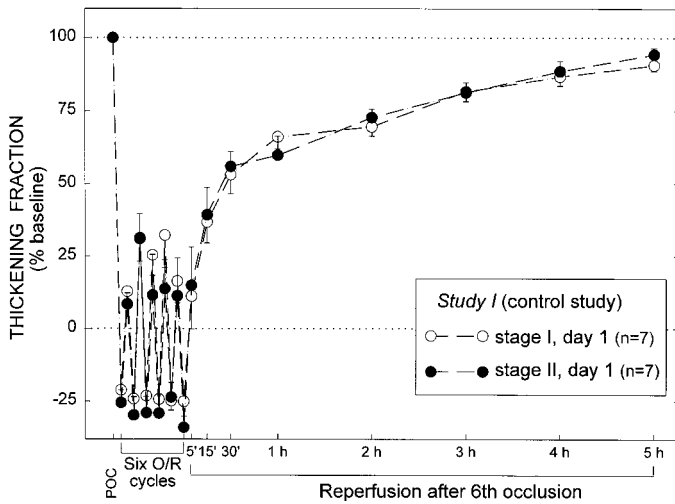


Fig. 3. Systolic thickening fraction in *study I* (*stage I* and *stage II* of *day 1*). Figure illustrates thickening fraction just before 1st occlusion (POC), 3 min into each coronary occlusion (O), 3 min into each reperfusion (R), and at selected times during 5-h reperfusion period after 6th occlusion. Thickening fraction is expressed as a percentage of baseline values. Data are means \pm SE.

signal was lost before the protocol was completed (1 in *study I*, 2 in *study II*, and 2 in *study III*). One rabbit in *study III* died of ventricular fibrillation during the fifth occlusion on *day 3* of *stage II*. Thus seven rabbits

completed the protocol in *study I*, seven in *study II*, seven in *study III*, and six in *study IV*.

Study I (control study). As expected (5, 7, 21, 32, 45), in both *stages I* and *II* the recovery of WTh during the 5-h reperfusion period was significantly improved on *days 2* and *3* compared with *day 1*, resulting in a marked decrease in the total deficit of WTh versus *day 1* (Fig. 2; late PC against stunning). There was no difference in thickening fraction between *stage I* and *stage II* of *day 1* (Fig. 3), between *stage I* and *stage II* of *day 2* (data not shown), or between *stage I* and *stage II* of *day 3* (data not shown). Similarly, the total deficit of WTh on corresponding days was similar between *stage I* and *stage II* (Fig. 2). These results demonstrate that the severity of myocardial stunning on two subsequent stages 2 wk apart is reproducible in this model and that neither the 2-wk interval nor the infusion of saline had any appreciable effect on the degree of myocardial stunning.

Study II (NTG treatment). There were no appreciable differences in heart rate throughout the experimental protocol among *stage I* of *days 1, 2, and 3* and *stage II* of *days 1, 2, and 3* (Table 1). In addition, there were no differences in thickening fraction at baseline and just before coronary occlusion (Table 2).

In *stage I* (control stage), the recovery of WTh after the sixth reperfusion on *days 2* and *3* improved signifi-

Table 1. Heart rate during and after occlusion/reperfusion on each experimental day

	Baseline	Before Occlusion	O1	O3	O6	R15'	R30'	R1h	R2h	R3h	R4h	R5h	
<i>Study I</i> (control)													
<i>Stage I</i>													
<i>Day 1</i>		256 \pm 11	267 \pm 10	252 \pm 12	254 \pm 11	237 \pm 13	230 \pm 12	238 \pm 15	233 \pm 12	238 \pm 17	216 \pm 12	217 \pm 9	
<i>Day 2</i>		234 \pm 7	247 \pm 8	244 \pm 9	230 \pm 8	227 \pm 8	221 \pm 8	231 \pm 11	226 \pm 10	223 \pm 10	217 \pm 8	211 \pm 8	
<i>Day 3</i>		244 \pm 7	251 \pm 9	251 \pm 10	229 \pm 10	223 \pm 6	229 \pm 6	220 \pm 8	212 \pm 8	215 \pm 7	218 \pm 9	206 \pm 9	
<i>Stage II</i>													
<i>Day 1</i>	239 \pm 11	230 \pm 11	249 \pm 7	239 \pm 8	235 \pm 11	217 \pm 15	218 \pm 16	216 \pm 12	215 \pm 15	216 \pm 15	211 \pm 16	218 \pm 14	
<i>Day 2</i>		238 \pm 9	244 \pm 7	233 \pm 9	229 \pm 9	213 \pm 11	214 \pm 10	213 \pm 12	216 \pm 8	221 \pm 8	214 \pm 7	207 \pm 8	
<i>Day 3</i>		222 \pm 12	230 \pm 7	231 \pm 6	221 \pm 8	217 \pm 8	218 \pm 7	210 \pm 9	213 \pm 10	209 \pm 9	212 \pm 8	203 \pm 6	
<i>Study II</i> (NTG treatment)													
<i>Stage I</i>													
<i>Day 1</i>		256 \pm 10	270 \pm 15	262 \pm 10	253 \pm 13	245 \pm 16	241 \pm 11	240 \pm 11	231 \pm 9	235 \pm 11	227 \pm 10	223 \pm 13	
<i>Day 2</i>		244 \pm 13	250 \pm 11	257 \pm 12	259 \pm 12	243 \pm 13	237 \pm 15	241 \pm 10	233 \pm 11	218 \pm 11	217 \pm 8	226 \pm 12	
<i>Day 3</i>		235 \pm 19	256 \pm 20	254 \pm 18	235 \pm 16	230 \pm 18	237 \pm 12	225 \pm 10	221 \pm 9	224 \pm 11	220 \pm 8	203 \pm 9	
<i>Stage II</i>													
<i>Day 1</i>	240 \pm 13	230 \pm 13	248 \pm 12	245 \pm 12	243 \pm 12	229 \pm 14	237 \pm 13	235 \pm 11	230 \pm 14	231 \pm 12	225 \pm 16	225 \pm 13	
<i>Day 2</i>		263 \pm 15	271 \pm 16	262 \pm 11	247 \pm 15	246 \pm 11	243 \pm 18	226 \pm 15	220 \pm 13	221 \pm 13	230 \pm 12	228 \pm 15	
<i>Day 3</i>		248 \pm 14	266 \pm 16	251 \pm 10	245 \pm 10	237 \pm 13	240 \pm 14	217 \pm 9	215 \pm 10	226 \pm 11	223 \pm 14	204 \pm 10	
<i>Study III</i> (NTG pretreatment)													
<i>Stage I</i>													
<i>Day 1</i>		252 \pm 6	257 \pm 7	253 \pm 10	253 \pm 9	243 \pm 6	242 \pm 5	234 \pm 4	229 \pm 7	229 \pm 7	226 \pm 5	217 \pm 8	
<i>Day 2</i>		254 \pm 4	257 \pm 6	257 \pm 8	244 \pm 10	234 \pm 7	243 \pm 7	233 \pm 6	234 \pm 8	225 \pm 5	231 \pm 6	231 \pm 4	
<i>Day 3</i>		237 \pm 8	235 \pm 4	249 \pm 8	227 \pm 7	229 \pm 6	218 \pm 6	215 \pm 4	215 \pm 7	208 \pm 6	217 \pm 7	220 \pm 7	
<i>Stage II</i>													
<i>Day 1</i>	244 \pm 3	241 \pm 9	248 \pm 9	247 \pm 7	236 \pm 11	231 \pm 3	239 \pm 10	233 \pm 6	227 \pm 5	233 \pm 6	220 \pm 5	206 \pm 7	
<i>Day 2</i>		235 \pm 4	251 \pm 8	250 \pm 11	234 \pm 9	232 \pm 7	226 \pm 11	226 \pm 7	209 \pm 8	221 \pm 4	220 \pm 4	218 \pm 8	
<i>Day 3</i>		229 \pm 6	243 \pm 7	227 \pm 8	227 \pm 6	214 \pm 3	216 \pm 7	215 \pm 7	208 \pm 6	210 \pm 8	206 \pm 5	208 \pm 7	
<i>Study IV</i> (SNAP treatment)													
<i>Day 1</i>		237 \pm 10	233 \pm 14	231 \pm 8	238 \pm 8	238 \pm 10	226 \pm 13	221 \pm 14	226 \pm 11	222 \pm 9	230 \pm 10	224 \pm 13	216 \pm 10

Values (in beats/min) are means \pm SE. Baseline, before infusion of saline, nitroglycerin (NTG), or *S*-nitroso-*N*-acetylpenicillamine (SNAP); before occlusion, just before coronary occlusion; O1, 1st occlusion; O3, 3rd occlusion; O6, 6th occlusion; R15', 15 min after 6th reperfusion; R30', 30 min after 6th reperfusion; R1h, 1 h after 6th reperfusion; R2h, 2 h after 6th reperfusion; R3h, 3 h after 6th reperfusion; R4h, 4 h after 6th reperfusion; R5h, 5 h after 6th reperfusion.

Table 2. Thickening fraction on each experimental day

	Baseline	Before Occlusion
<i>Study I (control)</i>		
<i>Stage I</i>		
Day 1		34.0 ± 3.4
Day 2		34.7 ± 3.6
Day 3		32.5 ± 3.2
<i>Stage II</i>		
Day 1	34.7 ± 3.8	33.5 ± 3.9
Day 2		33.3 ± 3.5
Day 3		34.3 ± 3.4
<i>Study II (NTG treatment)</i>		
<i>Stage I</i>		
Day 1		33.2 ± 1.8
Day 2		33.0 ± 1.7
Day 3		31.8 ± 2.4
<i>Stage II</i>		
Day 1	34.1 ± 3.5	32.3 ± 3.3
Day 2		33.2 ± 3.2
Day 3		33.7 ± 3.1
<i>Study III (NTG pretreatment)</i>		
<i>Stage I</i>		
Day 1		29.3 ± 0.9
Day 2		28.8 ± 0.4
Day 3		29.0 ± 0.8
<i>Stage II</i>		
Day 1	32.5 ± 2.7	33.6 ± 2.7
Day 2		32.6 ± 2.8
Day 3		33.9 ± 2.5
<i>Study IV (SNAP treatment)</i>		
Day 1	31.9 ± 3.4	32.1 ± 3.2

Values (in %) are means ± SE. Baseline, before starting infusion of saline, NTG, or SNAP; before occlusion, just before coronary occlusion.

cantly compared with *day 1* (data not shown), resulting in a marked decrease in the total deficit of WTh on *days 2 and 3* (Fig. 4) that indicated the development of late PC against stunning.

On *day 1* of *stage II* (NTG treatment stage), infusion of NTG significantly improved the recovery of WTh at 2, 3, 4, and 5 h after the sixth reperfusion, compared with *day 1* of the control stage ($P < 0.05$; Fig. 5). [A transient worsening of WTh was noted in NTG-treated rabbits during the 5th reperfusion and at 5 and 15 min after

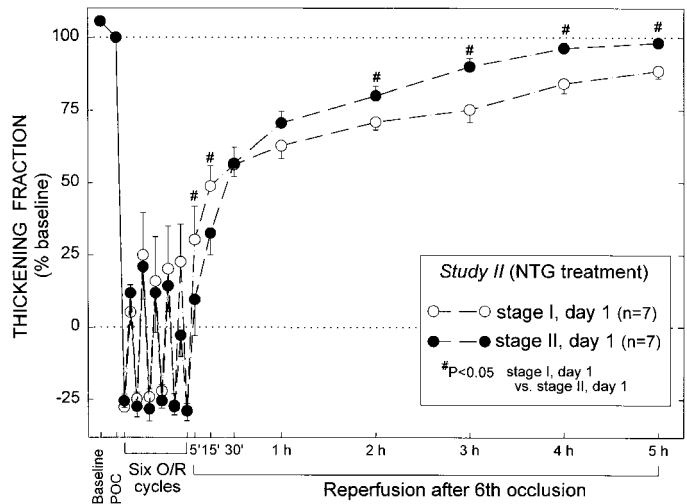


Fig. 5. Effect of NTG treatment on recovery of WTh in *study II*. Figure illustrates systolic thickening fraction at *stage I* of *day 1* (no treatment) and at *stage II* of *day 1* (NTG treatment) at baseline, just before 1st occlusion (POC), 3 min into each coronary occlusion (O), 3 min into each reperfusion (R), and at selected times during 5-h reperfusion period after 6th occlusion. Thickening fraction is expressed as a percentage of baseline values. Data are means ± SE.

the 6th reperfusion (Fig. 5)]. As a result, NTG treatment significantly decreased the total deficit of WTh (Fig. 4). Although the total deficit of WTh on *day 1* of *stage II* was attenuated, it decreased further on *days 2 and 3* (Figs. 4 and 6), indicating that late PC had an additive effect compared with NTG treatment.

Study III (NTG pretreatment). There were no appreciable differences in heart rate throughout the experimental protocol among *stage I* of *days 1, 2, and 3* and *stage II* of *days 1, 2, and 3* (Table 1). In addition, there were no differences in thickening fraction at baseline and just before coronary occlusion (Table 2).

The results of *stage I* (control stage) were similar to those obtained in *studies I and II* (Fig. 7). On *day 1* of *stage II* (NTG pretreatment stage), infusion of NTG before the six occlusion/reperfusion cycles failed to enhance the recovery of WTh (except for a transient

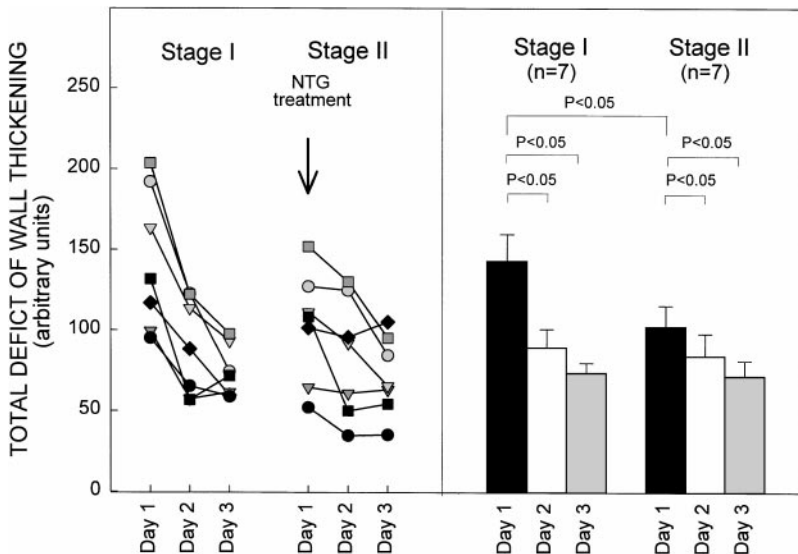


Fig. 4. Total deficit of WTh after 6th reperfusion in *study II*. Values of total deficit of WTh in individual rabbits are illustrated on *left*. Total deficit of WTh was measured in arbitrary units as described in text. Data are means ± SE.

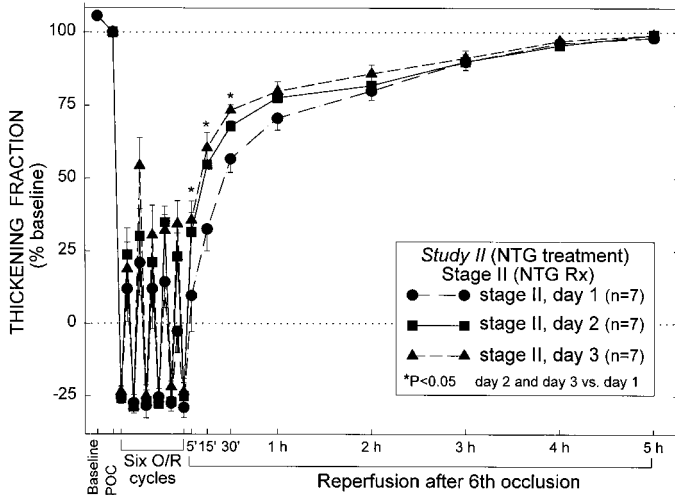


Fig. 6. Effect of NTG treatment on recovery of WTh in *study II*. Figure illustrates systolic thickening fraction on *days 1, 2, and 3 of stage II* at baseline, just before 1st occlusion (POC), 3 min into each coronary occlusion (O), 3 min into each reperfusion (R), and at selected times during 5-h reperfusion period after 6th occlusion. Thickening fraction is expressed as a percentage of baseline values. Data are means \pm SE.

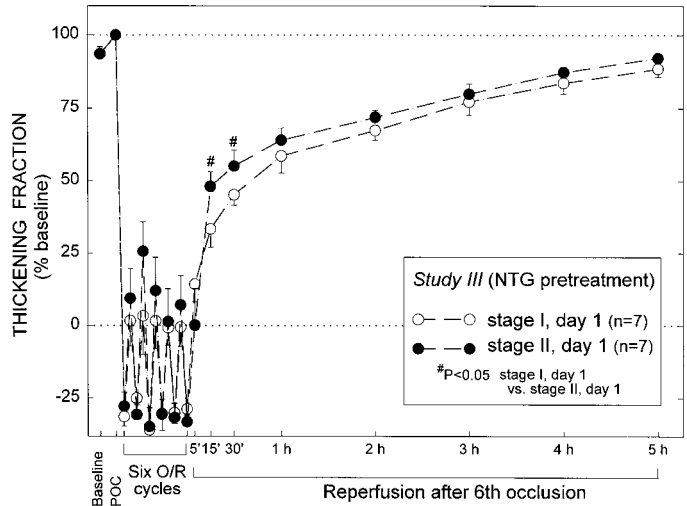


Fig. 8. Effect of NTG pretreatment on recovery of WTh in *study III*. Figure illustrates systolic thickening fraction at *stage I of day 1* (no treatment) and at *stage II of day 1* (NTG pretreatment) at baseline, just before 1st occlusion (POC), 3 min into each coronary occlusion (O), 3 min into each reperfusion (R), and at selected times during 5-h reperfusion period after 6th occlusion. Thickening fraction is expressed as a percentage of baseline values. Data are means \pm SE.

improvement at 15 and 30 min; Fig. 8) or to attenuate the total deficit of WTh (Fig. 7). On *days 2 and 3 of stage II*, the recovery of WTh (data not shown) and the total deficit of WTh (Fig. 7) exhibited changes similar to those noted in the absence of any treatment (*study I*).

Study IV (SNAP treatment). Heart rate (Table 1) and thickening fraction at baseline and before occlusion (Table 2) were similar in SNAP-treated rabbits and in control rabbits (*study I, day 1 of stage II*). In four rabbits in which arterial pressure was measured, infusion of SNAP before and during the six 4-min occlusion/reperfusion cycles had no appreciable effect (mean arterial pressure: 81 ± 4 mmHg at baseline, 78 ± 3 mmHg during 1st occlusion, 78 ± 2 mmHg during 6th occlusion, and 81 ± 4 mmHg at 30 min after 6th occlusion). Compared with control rabbits, infusion of SNAP effected a significant enhancement in the recov-

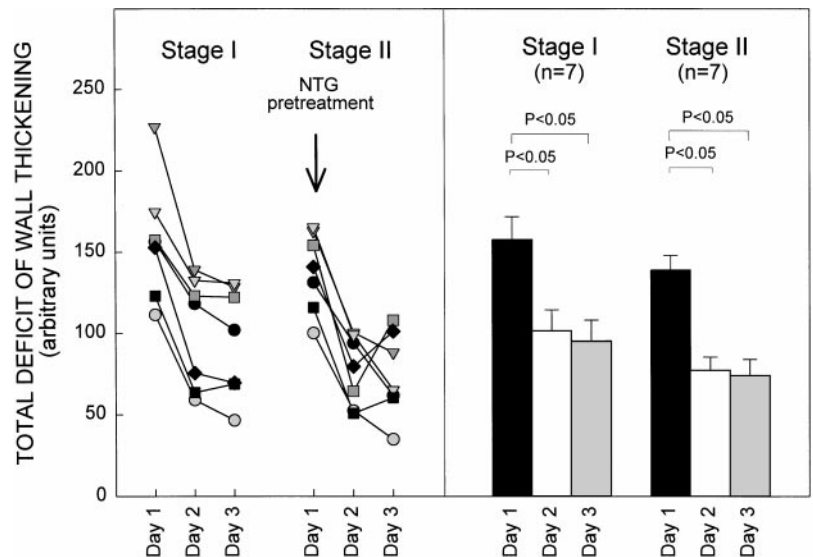
ery of WTh, which became evident immediately after the sixth reperfusion and persisted for 4 h thereafter (Fig. 9). Accordingly, the deficit of WTh was reduced by 40% in SNAP-treated rabbits compared with control rabbits (Fig. 9).

DISCUSSION

The present study demonstrates that infusion of hemodynamically inactive doses of two unrelated NO donors, NTG and SNAP, results in a significant and sustained attenuation of myocardial stunning. To our knowledge, this is the first report that exogenous administration of NO mitigates myocardial stunning *in vivo*.

Despite extensive investigation, the influence of NO on myocardial ischemia-reperfusion injury remains

Fig. 7. Effect of NTG pretreatment on total deficit of WTh after 6th reperfusion in *study III*. Values of total deficit of WTh in individual rabbits are illustrated on *left*. Total deficit of WTh was measured in arbitrary units as described in text. Data are means \pm SE.



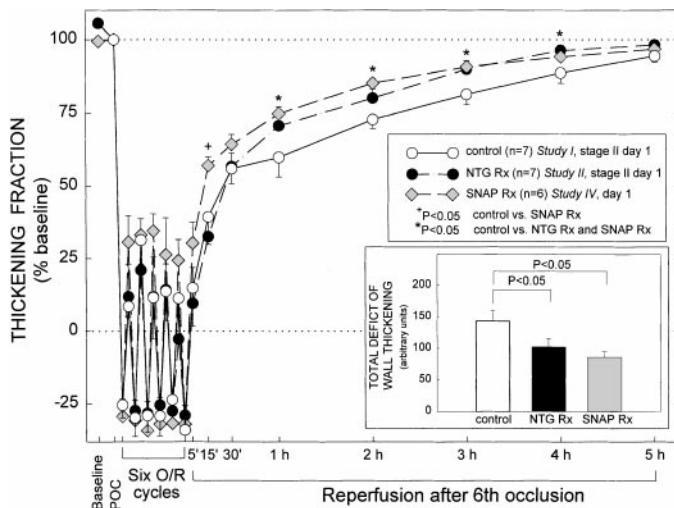


Fig. 9. Systolic thickening fraction in control rabbits (*study I, stage II, day 1*), NTG-treated rabbits (*study II, stage II, day 1*), and SNAP-treated rabbits (*study IV, day 1*). Figure illustrates thickening fraction at baseline, just before 1st occlusion (POC), 3 min into each coronary occlusion (O), 3 min into each reperfusion (R), and at selected times during 5-h reperfusion period after 6th occlusion. Thickening fraction is expressed as a percentage of baseline values. Inset, total deficit of WTh after 6th reperfusion in control, NTG-treated, and SNAP-treated rabbits. Data are means \pm SE.

controversial. Although a number of studies support the notion that endogenous production of NO is cardioprotective (12, 14, 15, 27, 42, 43), others have arrived at opposite conclusions (28, 30, 38, 44). The effect of NTG on myocardial ischemia-reperfusion injury is also controversial. For example, a nonhypotensive dose of NTG reduced infarct size in dogs (24, 25) but this beneficial effect was not observed in open-chest rabbits (16) or pigs (18). The reasons for these discrepant results remain unclear. With regard to myocardial stunning, most of these studies have examined models in which ischemia caused at least some degree of cell death, thereby making it difficult to formulate conclusions regarding the influence of NO on myocardial stunning per se. Only a few reports specifically examined the effect of NO on this latter manifestation of ischemia-reperfusion injury. Hasebe et al. (14) showed that administration of N^{ω} -nitro-L-arginine in conscious dogs subjected to a 10-min coronary occlusion aggravates myocardial stunning independent of any effects on regional perfusion, supporting the notion that NO functions as an endogenous protectant against postischemic dysfunction. On the other hand, Mori et al. (26) reported that intracoronary administration of L-arginine just before reperfusion exacerbated myocardial stunning after a 15-min coronary occlusion in dogs. These authors concluded that NO plays a detrimental role in myocardial stunning through the formation of peroxynitrite. Ehring et al. (11), however, observed no improvement in the recovery of WTh when open-chest dogs subjected to a 15-min coronary occlusion were pretreated with N^G -nitro-L-arginine methyl ester. Iwamoto et al. (17) found in open-chest rabbits that administration of NTG throughout a 10-min coronary occlusion and subsequent reperfusion resulted in increased

recovery of thickening fraction. However, because the thickening fraction was followed for only 30 min, it was not possible to determine whether this beneficial effect of NTG was sustained for the entire duration of stunning. Furthermore, NTG was infused throughout the reperfusion phase, so that it is not possible to establish whether the enhanced recovery was dependent on continuous supply of the drug. In a study in open-chest dogs, Gross et al. (13) found that infusion of NTG for 30 min before a 15-min coronary occlusion resulted in only an ephemeral augmentation of segment shortening at 5 and 15 min of reperfusion and that there were no subsequent significant differences between control and treated dogs.

Because of the numerous differences among these studies (13, 16–18, 24, 25), including species, presence or absence of anesthesia, and dose and duration of NTG infusion, it is not possible to identify the reason(s) for the apparently discrepant results. It is conceivable that the protective effects of NTG on myocardial stunning may be demonstrable only in the conscious state, because the conditions associated with open-chest preparations produce an approximately twofold increase in the severity of myocardial stunning (40). This supposition is reinforced by the finding of Hasebe et al. (14) that blockade of endogenous NO synthesis exacerbates myocardial stunning in conscious dogs. In contrast to the finding of Mori et al. (26), Engelman et al. (12) reported that pretreatment with L-arginine improved the recovery of LV function in open-chest pigs subjected to 30 min of regional ischemia followed by 90 min of reperfusion. Although this finding demonstrates a protective effect of enhanced NO biosynthesis, the duration of coronary occlusion (30 min) was associated with some degree of infarction, making it difficult to distinguish between a reduction in infarct size and an improvement in myocardial stunning per se.

The rationale for testing two different NO donors in this study was to minimize the likelihood that the protective effects observed could be caused by nonspecific actions. The choice of NTG as one of the NO donors to be tested was dictated by the preclinical nature of this investigation. NTG, a widely used nitrate, has been employed for the treatment of coronary artery disease for over 100 years. Accordingly, we reasoned that demonstrating a protective effect of NTG against myocardial stunning would have relevance for the treatment of patients with transient myocardial ischemia and subsequent postischemic dysfunction. The dosage of NTG used in *study III* (NTG pretreatment; $2 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for 60 min) was chosen because it was shown previously to induce late PC against myocardial stunning in this same conscious rabbit model (3), thereby making it possible for us to directly compare the early and late PC effects of NTG. Although it is theoretically possible that a different dosage might be effective in eliciting early PC, infusion rates of NTG $>2 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ resulted in significant decreases in arterial pressure in our pilot studies. In *study II* (NTG treatment), we used the same infusion rate ($2 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) but extended the duration of infusion

to 84 min to bracket the entire six occlusion/reperfusion cycles as well as the 30 min preceding the first occlusion and the 10 min after the sixth reperfusion. The rationale for this was to ensure that steady-state NTG plasma levels were present at the beginning of the occlusion/reperfusion cycles and in the initial minutes of reperfusion.

The precise mechanism whereby NO attenuates myocardial stunning remains to be elucidated. NO exerts a number of actions that would be expected to be beneficial during ischemia-reperfusion, including inhibition of the influx of Ca^{2+} into myocytes (19, 22), antagonism of β -adrenergic stimulation (2, 41), and opening of ATP-sensitive K^+ channels (1, 34). Interestingly, the magnitude of the beneficial effects on myocardial stunning of both NTG treatment and SNAP treatment was comparable to that observed during the late phase of ischemic PC, as demonstrated by the fact that in *study II* there were no significant differences in the deficit of WTh between *stage I* of *day 2* and *stage II* of *day 1* (Fig. 4) and that the deficit of WTh in *day 1* of *study IV* (Fig. 9) was similar to that observed on *day 2* of *stage II* in *study I* (Fig. 2). Thus the beneficial effects of the late phase of ischemic PC could be reproduced by administration of exogenous NO, further supporting the hypothesis that NO mediates this cardioprotective phenomenon.

A report by Banerjee et al. (3) showed that the same dosage of NTG used in this investigation elicits late PC against myocardial stunning 24 h later in conscious rabbits and that this effect is blocked by chelerythrine, indicating that it is mediated by PKC-dependent signaling. The failure of NTG to induce early PC in the present study suggests that PKC activation, although essential for the late PC effect of NTG, is not sufficient to induce an early protective effect against myocardial stunning. In this connection, it is important to point out that although the early phase of ischemic PC is remarkably powerful in protecting against cell death, its effects on stunning are controversial (4). Studies using a single 15-min period of coronary occlusion failed to demonstrate an early PC effect against stunning (4, 23, 29). On the other hand, when the heart is subjected to a sequence of brief coronary occlusion/reperfusion cycles, the first cycle has been found to precondition against the stunning induced by the next two cycles (10).

In conclusion, we have demonstrated that intravenous infusion of NTG or SNAP during brief ischemia-reperfusion is effective in attenuating myocardial stunning and that the magnitude of this protection is comparable to that induced by the late phase of ischemic PC. These results have obvious clinical implications for the use of NO donors in the treatment of patients with coronary artery disease. In addition, they provide direct evidence for a protective action of NO in the setting of myocardial stunning.

The authors gratefully acknowledge Gregg Shirk and Larisa Hodge for expert technical assistance and Trudy Keith for expert secretarial assistance.

This study was supported in part by National Heart, Lung, and Blood Institute Grants R01 HL-43151 and HL-55757 (R. Bolli), by an American Heart Association, Kentucky Affiliate Grant, by American

Heart Association, Ohio Valley Affiliate Grant 9951533V (X.-L. Tang), and by the Medical Research Grant Program of the Jewish Hospital Foundation, Louisville, KY.

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Received 30 July 1999; accepted in final form 24 August 1999.

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