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Late preconditioning induced by NO donors, adenosine A₁ receptor agonists, and δ_1 -opioid receptor agonists is mediated by iNOS

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Abstract

Although ischemia-induced late preconditioning (PC) is known to be mediated by inducible nitric oxide (NO) synthase (iNOS), the role of this enzyme in pharmacologically induced late PC remains unclear. We tested whether targeted disruption of the *iNOS* gene abrogates late PC elicited by three structurally different NO donors [diethylenetriamine/NO (DETA/NO), nitroglycerin (NTG), and *S*-nitroso-*N*-acetyl-penicillamine (SNAP)], an adenosine A₁ receptor agonist [2-chloro-*N*⁶-cyclopentyladenosine (CCPA)], and a δ_1 -opioid receptor agonist (TAN-670). The mice were subjected to a 30-min coronary occlusion followed by 24 h of reperfusion. In *iNOS* knockout (*iNOS*^{−/−}) mice, infarct size was similar to wild-type (WT) controls, indicating that iNOS does not modulate infarct size in the absence of PC. Pretreatment of WT mice with DETA/NO, NTG, SNAP, TAN-670, or CCPA 24 h before coronary occlusion markedly reduced infarct size. In *iNOS*^{−/−} mice, however, the late PC effect elicited by DETA/NO, NTG, SNAP, TAN-670, and CCPA was completely abrogated. Furthermore, in WT mice pretreated with TAN-670 or CCPA, the selective iNOS inhibitor 1400W also abolished the delayed PC properties of these drugs; 1400W had no effect in WT mice. These data demonstrate that iNOS plays an obligatory role in NO donor-induced, adenosine A₁ receptor agonist-induced, and δ_1 -opioid receptor agonist-induced late PC, underscoring the critical role of this enzyme as a common mediator of cardiac adaptations to stress.

Keywords

nitric oxide; inducible nitric oxide synthase; myocardial ischemia; reperfusion injury

ISCHEMIC PRECONDITIONING (PC), the phenomenon whereby brief episodes of ischemia render the heart resistant to subsequent ischemic insults, is a powerful innate cardioprotective mechanism. The late phase of PC is particularly relevant because once induced, it confers cardioprotection for 3–4 days (1, 5). Research performed in the past 10 years has identified nitric oxide (NO) as both a trigger and a mediator of the late phase of ischemic PC (6). In particular, Guo et al. (12) have demonstrated that genetic disruption of the *iNOS* gene completely abrogates the infarct-sparing effect of ischemia-induced late PC, establishing inducible NO synthase (iNOS) as an obligatory mediator.

The cardioprotection afforded by ischemia-induced late PC can be mimicked by the administration of a variety of pharmacological agents, including NO-releasing agents (16,

19, 23, 24), adenosine A₁ receptor agonists (2, 3, 22, 27), and δ_1 -opioid receptor agonists (10), in dogs, rabbits, and mice. However, the mechanism of pharmacologically induced late PC, and the role of iNOS in particular, remains unclear. Although iNOS is a necessary mediator of ischemia-induced late PC (4), its role in NO donor-induced late PC is presently unknown. A recent study (16) concluded that iNOS mediates morphine-induced late PC in mice, but the short duration of reperfusion (2 h) may not have afforded accurate assessment of final infarct size. In addition, controversy exists as to whether adenosine A₁ agonist-induced late PC is mediated by iNOS. Bell et al. (3) concluded that iNOS is not obligatorily required for the development of late PC induced by adenosine A₁ agonists, whereas Zhao et al. (27) arrived at the opposite conclusion. Both of these studies were performed with the use of in vitro models of global ischemia. To date, the role of iNOS in adenosine A₁ agonist-induced late PC has not been tested in vivo.

Accordingly, the aims of this study were 1) to determine whether three structurally different NO [diethylenetriamine/NO (DETA/NO), nitroglycerin (NTG), and *S*-nitroso-*N*-acetylpenicillamine (SNAP)], an adenosine A₁ receptor agonist (CCPA), and a δ_1 -opioid receptor agonist (TAN-670) induce delayed protection against infarction in an in vivo murine model; 2) to determine the effect of targeted disruption of the *iNOS* gene on NO donor-induced, adenosine A₁ receptor agonist-induced, and δ_1 -opioid receptor agonist-induced late PC; and 3) to verify the effects of *iNOS* gene deletion by using a selective iNOS inhibitor (1400W) to block adenosine A₁ receptor agonist-induced and δ_1 -opioid receptor agonist-induced late PC. All studies were conducted in a well-established in vivo murine model of myocardial infarction (13).

METHODS

Wild-type and iNOS knockout mice

The study was performed in male mice weighing 30.7 ± 0.4 g (age, 16.3 ± 0.5 wk). 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 (Louisville, KY) and conformed to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86–23, Revised 1996). Breeding pairs of *iNOS* knockout (*iNOS*^{−/−}) mice were purchased from Jackson Laboratory and maintained in the homozygous state. The *iNOS*^{−/−} mice were of hybrid genetic background (C57BL/6J and 129/Sv [B6129/J]). Wild-type (WT) mice with a genetic background (B6129F₂/J) as close as possible to the *iNOS*^{−/−} mice (17) were used as controls. All mice were maintained in sterile microisolator cages under pathogen-free conditions. Food and water were autoclaved, and all handling was done under a laminar flow hood according to standard procedures for maintaining pathogen-free transgenic mice. The *iNOS*^{−/−} mice were genotyped by PCR and Southern blot hybridization as previously described (17). The mice were typed as they were bred and then retyped with DNA prepared from tissue samples taken at the end of the experiments.

Experimental preparation

The experimental preparation has been described in detail previously (13). The mice were anesthetized with pentobarbital sodium (50 mg/kg iv), intubated, and ventilated with room air supplemented with oxygen at a rate of 105 strokes/min and with a tidal volume of 0.8 ml with the use of a small rodent ventilator. These respiratory settings were found to result in optimal values of arterial pH (7.39 ± 0.01) and P_{CO₂} (31 ± 2 mmHg) (12, 13). Body temperature was carefully monitored with a rectal probe and maintained as close as possible to 37.0°C. To replace blood losses, blood from a donor mouse was given intravenously at 40 ml/kg divided into three equal boluses. After the administration of antibiotics, the chest was

opened through a midline sternotomy and a nontraumatic balloon occluder was implanted around the middle left anterior descending coronary artery with the use of an 8-0 nylon suture. After the coronary occlusion/reperfusion protocol, the chest was closed in layers and the mice were allowed to recover.

Experimental protocol

Mice were assigned to 15 groups (Fig. 1). In control *groups I* (WT control) and *II* (*iNOS*^{-/-} control), the mice received no pretreatment. In the treated groups, the mice received either DETA/NO [4 consecutive bolus doses (0.5 mg/kg iv) every 20 min (total dose, 2.0 mg/kg), *groups III* (WT DETA/NO) and *IV* (*iNOS*^{-/-} DETA/NO)]; NTG [24 µg·kg⁻¹·min⁻¹ iv for 60 min, *groups V* (WT NTG) and *VI* (*iNOS*^{-/-} NTG)]; SNAP [2.5 µg·kg⁻¹·min⁻¹ iv for 60 min, *groups VII* (WT SNAP) and *VIII* (*iNOS*^{-/-} SNAP)]; TAN-670 [60 mg/kg ip, *groups IX* (WT TAN-670) and *X* (*iNOS*^{-/-} TAN-670)]; or CCPA [10 µg·kg⁻¹·min⁻¹ iv for 10 min, *groups XII* (WT CCPA) and *XIII* (*iNOS*^{-/-} CCPA)]. Twenty-four hours later (*day 2*), all mice underwent a 30-min coronary occlusion followed by 24 h of reperfusion. *Groups XI* and *XIII* consisted of WT mice treated with the same doses of TAN-670 and CCPA as *groups IX* and *XII*; on *day 2*, these mice were given 1400W (10 mg/kg ip) 30 min before the 30-min coronary occlusion. *Group XV* (WT ischemic PC) was preconditioned with a sequence of six cycles of 4-min coronary occlusion/4-min reperfusion; 24 h later (*day 2*), this group underwent a 30-min coronary occlusion followed by 24 h of reperfusion.

Postmortem tissue analysis

At the conclusion of the study, the heart was excised and perfused with Krebs-Henseleit solution through an aortic cannula. To delineate infarcted myocardium from viable myocardium, the heart was perfused with 1% triphenyltetrazolium chloride in phosphate buffer. To delineate the occluded/reperfused bed, the coronary artery was tied at the site of the previous occlusion and the aortic root was perfused with 10% Phthalo blue dye (13). As a result of this procedure, the region at risk was identified by the absence of blue dye, whereas the rest of the left ventricle was stained dark blue. The left ventricle was cut into five to seven transverse slices, which were fixed in 10% neutral buffered formaldehyde, weighed, and photographed under a microscope. The corresponding areas were measured by computerized videoplanimetry, and from these measurements the infarct size was calculated as a percentage of the region at risk (13).

Statistical analysis

Data are reported as means ± SE. Measurements were analyzed with a one-way or two-way repeated-measures ANOVA, as appropriate, followed by unpaired Student's *t*-tests with the Bonferroni correction.

RESULTS

A total of 234 mice were used. Seventy mice were excluded for the reasons specified in Table 1.

Pilot studies

Pilot studies were performed to identify doses of DETA/NO, NTG, SNAP, TAN-670, CCPA, and 1400W that would not significantly alter heart rate or blood pressure (Table 2). For each drug, heart rate and blood pressure measurements were taken carefully before, during, and after treatment. The doses were chosen on the basis of the maximum amount of drug that could be administered without significantly (≤20% from baseline) altering heart rate or mean arterial blood pressure. As shown in Table 2, at 4 consecutive bolus doses (0.5

mg/kg iv) every 20 min (total dose, 2.0 mg/kg), DETA/NO resulted in a 7% increase in heart rate and a 14% decrease in mean arterial pressure from baseline. NTG given at $24 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ iv for 60 min resulted in no significant change in heart rate or mean arterial pressure compared with baseline. SNAP at $2.5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ iv for 60 min resulted in a 10% increase in heart rate and a 6% decrease in mean arterial pressure. A single 60 mg/kg ip dose of TAN-670 decreased baseline heart rate by 14% and had no appreciable effect on mean arterial pressure. CCPA at $10 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ iv for 10 min lowered heart rate by 15% and lowered mean arterial pressure by 9%. At 10 mg/kg ip, 1400W resulted in a 6% decrease in heart rate and a 9% decrease in mean arterial pressure.

Body temperature and heart rate

By experimental design, rectal temperature remained within a narrow physiological range (36.8° to 37.3°C) in all groups (Table 3). Heart rate was measured 5 min before the 30-min occlusion, during the occlusion, and after reperfusion (Table 3). The average heart rate in groups I–XV ranged from 485 to 628 beats/min. Heart rate did not differ significantly among the 15 groups at any time during the 30-min occlusion or the ensuing reperfusion (Table 3).

Infarct size

There were no significant differences among the 15 groups with respect to body weight or weight of the region at risk (Table 4). In control WT mice (group I), infarct size averaged $58.6 \pm 1.8\%$ of the region at risk (Fig. 1). In control *iNOS*^{−/−} mice (group II), infarct size was similar ($53.4 \pm 3.5\%$ of the region at risk), indicating that iNOS does not modulate the extent of cell death in the naive (nonpreconditioned) state. In groups III (DETA/NO), V (NTG), VII (SNAP), IX (TAN-670), and XII (CCPA), infarct size was significantly ($P < 0.05$) smaller (31.0 ± 3.4 , 27.9 ± 4.1 , 22.6 ± 3.5 , 23.1 ± 2.7 , and $33.3 \pm 3.5\%$ of the region at risk, respectively) compared with the control groups (groups I and II; Fig. 1), indicating a late PC effect. To compare NO donor-induced, TAN-670-induced, and CCPA-induced late PC with the late phase of ischemic PC, the mice in group XV (late PC) were subjected to a sequence of six 4-min occlusion/4-min reperfusion cycles on day 1 followed by a 30-min coronary occlusion on day 2. In these mice, infarct size was $19.3 \pm 3.4\%$ of the risk region, similar to that measured in the pharmacologically preconditioned mice (groups III, V, VII, IX, and XII). However, when DETA/NO (group IV), NTG (group VI), SNAP (group VIII), TAN-670 (group X), or CCPA (group XIII) was given to *iNOS*^{−/−} mice, the reduction in infarct size seen in groups III, V, VII, IX, and XII was completely abrogated (53.3 ± 3.3 , 55.1 ± 2.5 , 67.5 ± 2.5 , 63.2 ± 2.8 , and $65.0 \pm 3.3\%$ of the region at risk, respectively; Fig. 1). When WT mice pretreated with TAN-670 (group XI) or CCPA (group XIV) were given the iNOS inhibitor 1400W 30 min before the 30-min coronary occlusion, infarct size (58.6 ± 3.2 and $57.6 \pm 4.1\%$, respectively) was similar to that seen in the *iNOS*^{−/−} mice pretreated with TAN-670 and CCPA and significantly ($P < 0.05$) greater than that seen in WT mice not treated with 1400W.

In all groups, infarct size was positively and linearly related to the risk region (Figs. 2 and 3). In the DETA/NO-, NTG-, SNAP-, TAN-670-, and CCPA-treated groups, the regression line for the *iNOS*^{−/−} mice (groups IV, VI, VIII, X, and XIII) was shifted to the left compared with the WT mice (groups III, V, VII, IX, and XII), indicating that for any given size of the region at risk, the resulting infarction was smaller in WT mice than in *iNOS*^{−/−} mice. Similarly, in the TAN-670- and CCPA-treated groups, the regression line for the 1400W-treated mice (groups XI and XIV) was shifted to the left compared with the untreated WT mice (groups IX and XII), confirming the absence of a late PC effect (Fig. 3).

DISCUSSION

This study demonstrates that three structurally different NO donors (DETA/NO, SNAP, and NTG), an adenosine A₁ receptor agonist (CCPA), and a δ_1 -opioid receptor agonist (TAN-670) given 24 h before ischemia-reperfusion injury attenuate myocardial infarct size in an in vivo murine model. The magnitude of the infarct size reduction was similar to that attained during the late phase of ischemic PC. The cardioprotection afforded by these drugs cannot be attributed to hemo-dynamic effects because the drugs produced no appreciable changes in heart rate or arterial pressure. The infarct-sparing properties of TAN-670 and CCPA were abolished by the iNOS selective inhibitor 1400W, suggesting that iNOS is an essential mediator of TAN-670- and CCPA-induced late PC. This was confirmed by studies with genetically targeted animals, which demonstrated that the infarct size reduction produced by NO donor-induced, adenosine A₁ receptor agonist-induced, and δ_1 -opioid receptor agonist-induced late PC was completely abrogated in *iNOS*^{-/-} mice, providing definitive evidence that iNOS is indeed an essential mediator of this cardioprotective response. Our data identify iNOS as a final common effector of pharmacological PC. In addition, to our knowledge, this is the first report that NO donor-induced late PC is iNOS dependent. This is also the first report that administration of adenosine A₁ agonists mitigates myocardial infarct size in an in vivo murine model.

Previous studies have identified iNOS as an obligatory mediator of late PC induced by ischemia (12), physical exercise (14), and endotoxin derivatives (9, 25, 26). The finding that iNOS is also a mediator of NO donor-induced, adenosine A₁ receptor agonist-induced, and a δ_1 -opioid receptor agonist-induced delayed cardioprotection further corroborates the concept that this enzyme is a central mediator of cardioprotection in response to diverse stimuli. Whether other proteins found to be essential for ischemia-induced late PC, such as cyclooxygenase-2 (11, 21), heme oxygenase-1 (7, 8), and aldose reductase (20), are also involved in pharmacologically induced late PC remains to be determined. Future studies will also be needed to further investigate the role of other NOS isoforms, namely endothelial and neuronal NOS, that have been found to play a role in ischemia-induced PC. These studies will delineate important aspects of the pathways by which these clinically relevant pharmacological agents induce cardioprotection and further clarify the roles the various NOS isoforms play in delayed preconditioning.

The rationale for the extensive investigation of pharmacological PC performed in the present study with five different PC-mimetic agents (DETA/NO, NTG, SNAP, CCPA, and TAN-670) was to determine whether the results obtained with one drug can be applied to other forms of pharmacologically induced protection. An additional reason for selecting these five agents was that the role of iNOS in mediating pharmacologically induced late PC is either unknown or unclear. Specifically, no data exist regarding the contributions of iNOS to the delayed infarct-sparing effects elicited by NO donors. With regard to δ_1 -opioid receptor-induced late PC, a recent study (16) has concluded that iNOS mediates the delayed infarct-sparing actions of morphine. However, morphine is not a specific δ_1 -opioid receptor agonist, and, in that investigation, the duration of coronary reperfusion was limited to 2 h. It is unknown whether a 2-h reperfusion period is sufficient for accurate assessment of infarct size in mice. With regard to the role of iNOS in adenosine A₁ receptor agonist-induced late PC, no data are available in in vivo murine models, and the data available in vitro are conflicting (3, 28). Consequently, we felt it was important to perform a careful analysis of NO donor-induced, δ_1 -opioid receptor-induced, and adenosine A₁ receptor-induced late PC with the use of a well-established in vivo murine model in which fundamental physiological variables (heart rate, blood pressure, body temperature, and arterial blood gases) are kept within normal limits and coronary reperfusion is extended to 24 h. We reasoned that by comparing the effects of the deletion of iNOS on three different types of pharmacological PC in the same in vivo model, we would be able to establish whether this enzyme plays a broad obligatory role or whether its involvement differs in relation to the stimulus applied.

The murine model utilized in this investigation has been extensively tested in our laboratory (13). In particular, physiological variables that affect infarct size, such as body temperature, heart rate, arterial blood pressure, and arterial blood P_{O_2} and pH, are carefully monitored and kept within normal limits (13). The measurements of infarct size are highly reproducible (13), and surgical mortality is <20%. Thus we believe that this preparation represents an excellent model for testing the role of iNOS in late PC.

In conclusion, this study expands our understanding of the significance of iNOS in the cardiovascular system by identifying this enzyme as a common mediator of cardioprotection in three different forms of pharmacological PC. From a protein previously thought to play a detrimental role in inflammatory states, iNOS has recently emerged as a powerful, ubiquitous cardioprotectant that is obligatorily required for the response of the heart to stress (4). The ultimate goal of studying PC is to utilize this powerful phenomenon for the protection of ischemic myocardium in patients with coronary artery disease. Despite our extensive understanding of the mechanisms underlying ischemic PC, no applicable therapies have been developed. PC mimetics such as NO donors (15, 18), TAN-670, and CCPA have obvious clinical potential. Combined with previous studies (6, 16, 25–27), the present data support the development of strategies aimed at selectively upregulating iNOS in cardiac myocytes to induce a chronic PC state.

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Fig. 1. Myocardial infarct size in *groups I-XV*. Infarct is expressed as percentage of region at risk of infarction. Open circles represent individual mice; solid circles represent means $\pm$ SE for respective groups.

Fig. 2.

Relationship between size of region at risk and size of infarction in diethylenetriamine/nitric oxide (DETA/NO)-, nitroglycerin (NTG)-, *S*-nitroso-*N*-acetylpenicillamine (SNAP)-, TAN-670-, and 2-chloro-*N*⁶-cyclopentyladenosine (CCPA)-treated wild-type (WT) and inducible NO synthase knockout (*iNOS*^{-/-}) mice. Individual values and regression lines obtained by linear regression analysis are shown. In all groups, infarct size was positively and linearly related to risk region size. A: DETA/NO-treated WT (group III) and *iNOS*^{-/-} mice (group IV). B: NTG-treated WT (group V) and *iNOS*^{-/-} mice (group VI). C: SNAP-treated WT (group VII) and *iNOS*^{-/-} mice (group VIII). D: TAN-670-treated WT (group IX) and *iNOS*^{-/-} mice (group X). E: CCPA-treated WT (group XII) and *iNOS*^{-/-} mice (group XIII). Linear regression equations were as follows: group III, $y = 0.63x - 3.51$, $r = 0.88$; group IV, $y = 0.46x - 6.1$, $r = 0.65$; group V, $y = 0.47x - 8.8$, $r = 0.75$; group VI, $y = 0.74x - 7.43$, $r = 0.90$; group VII, $y = 0.18x + 1.65$, $r = 0.47$; group VIII, $y = 0.72x - 1.61$, $r = 0.94$; group IX, $y = 0.07x + 5.32$, $r = 0.27$; group X, $y = 0.84x - 7.1$, $r = 0.97$; group XII, $y = 0.635x - 0.68$, $r = 0.88$; group XIII, $y = 0.83x - 19.55$, $r = 0.93$.

Fig. 3.

Relationship between size of region at risk and size of infarction in TAN-670-, CCPA-treated WT, and 1400W-treated mice. In all groups, infarct size was positively and linearly related to risk region size. *A*: TAN-670-treated WT (*group IX*) and 1400W-treated mice (*group XI*). *B*: CCPA-treated WT (*group XII*) and 1400W-treated mice (*group XIV*). Linear regression equations were as follows: *group IX*, $y = 0.07x + 5.32$, $r = 0.27$; *group XI*, $y = 0.75x - 6.31$, $r = 0.88$; *group XII*, $y = 0.64x + 0.68$, $r = 0.88$; *group XIV*, $y = 0.60x + 0.17$, $r = 0.79$.

Table 1

Reasons for excluding mice from study

	Groups															Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	
Bleeding	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	2
Death	5	1	8	6	3	1	1	4	1	0	1	0	1	0	7	39
Technical problems	0	3	1	2	1	0	1	1	2	1	1	2	1	3	0	19
Poor postmortem staining	1	0	1	1	0	1	0	0	2	1	1	0	1	1	0	10
Mice instrumented	21	14	28	21	14	12	12	15	15	13	13	12	13	14	17	234
Mice excluded	6	4	10	10	4	2	2	5	5	2	3	2	3	5	7	70
Mice included in study	15	10	18	11	10	10	10	10	10	11	10	10	10	9	10	162
Mice included in study, %	71	71	64	52	71	83	83	67	67	85	77	83	77	64	59	69

Table 2

Blood pressure and heart rate before, during, and after drug administration

	n	Baseline	During Infusion, min			After Administration, min				
			10	30	60	10	30	40	50	60
Mean arterial pressure, mmHg										
DETA/NO	3	95 ± 6	92 ± 8	90 ± 6	86 ± 5	88 ± 6	85 ± 8	82 ± 8		
NTG	2	97	97	98	99	102	94			
SNAP	3	87 ± 5	82 ± 6	82 ± 5	85 ± 6	83 ± 2	86 ± 6			
TAN-670	3	89 ± 2				86 ± 5	92 ± 3	88 ± 2	90 ± 2	
CCPA	4	92 ± 2	84 ± 2			84 ± 2	85 ± 5	87 ± 2	88 ± 2	92 ± 2
1400W	3	88 ± 6				82 ± 5	83 ± 5	80 ± 6		
Heart rate, beats/min										
DETA/NO	3	520 ± 18	522 ± 15	505 ± 26	532 ± 12	510 ± 9	508 ± 9	485 ± 12		
NTG	2	504	510	500	510	490	488			
SNAP	3	504 ± 8	508 ± 9	500 ± 5	530 ± 8	556 ± 8	504 ± 15			
TAN-670	3	510 ± 6				456 ± 9	440 ± 12	480 ± 14	485 ± 10	
CCPA	4	552 ± 15	470 ± 15			500 ± 16	515 ± 15	540 ± 15	550 ± 8	580 ± 8
1400W	3	512 ± 17				515 ± 10	480 ± 16	484 ± 16		

Values are means ± SE. Measurements of blood pressure and heart rate were taken before administration of drugs and for up to 70 min after administration. Doses were diethylenetriamine (DETA/NO, 0.5 mg/kg iv every 20 min), nitroglycerine (NTG, 24 µg·kg⁻¹·min⁻¹ iv for 60 min), S-nitroso-N-acetyl-penicillamine (SNAP, 2.5 µg·kg⁻¹·min⁻¹ iv for 60 min), TAN-670, (60 mg/kg ip), 2-chloro-N⁶-cyclopentyladenosine (CCPA, 10 µg·kg⁻¹·min⁻¹ iv for 10 min), and 1400W (10 mg/kg ip).

Table 3

Rectal temperature and heart rate on the day of the 30-min coronary occlusion

Group	Preocclusion	Occlusion, min			Reperfusion, min		
		5	15	30	1	10	15
Temperature, °C							
<i>I</i> (B6129, AMI)	36.8 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	37.2 ± 0.1	37.0 ± 0.1	36.8 ± 0.1	37.0 ± 0.1
<i>II</i> (<i>iNOS</i> ^{-/-} , AMI)	36.9 ± 0.1	37.2 ± 0.1	37.2 ± 0.1	37.0 ± 0.1	37.0 ± 0.1	37.0 ± 0.1	37.0 ± 0.1
<i>III</i> (B6129, DETA/NO)	37.1 ± 0.1	37.1 ± 0.0	37.2 ± 0.0	37.1 ± 0.0	37.1 ± 0.0	37.1 ± 0.1	37.1 ± 0.1
<i>IV</i> (<i>iNOS</i> ^{-/-} , DETA/NO)	37.0 ± 0.1	37.1 ± 0.1	37.2 ± 0.1	37.2 ± 0.1	37.2 ± 0.1	37.2 ± 0.1	37.1 ± 0.1
<i>V</i> (B6129, NTG)	37.0 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	37.0 ± 0.0	37.0 ± 0.1	37.1 ± 0.1	37.0 ± 0.0
<i>VI</i> (<i>iNOS</i> ^{-/-} , NTG)	37.0 ± 0.1	37.2 ± 0.1	37.2 ± 0.0	37.2 ± 0.1	37.1 ± 0.1	37.0 ± 0.1	37.1 ± 0.1
<i>VII</i> (B6129, SNAP)	37.1 ± 0.1	37.2 ± 0.1	37.2 ± 0.1	37.0 ± 0.1	37.1 ± 0.1	37.0 ± 0.0	37.1 ± 0.0
<i>VIII</i> (<i>iNOS</i> ^{-/-} , SNAP)	36.9 ± 0.1	37.2 ± 0.1	37.1 ± 0.1	37.2 ± 0.1	37.1 ± 0.1	36.9 ± 0.1	37.0 ± 0.1
<i>IX</i> (B6129, TAN-670)	36.9 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	37.1 ± 0.0	37.0 ± 0.1	36.9 ± 0.1
<i>X</i> (<i>iNOS</i> ^{-/-} , TAN-670)	37.0 ± 0.1	37.0 ± 0.1	37.2 ± 0.0	37.2 ± 0.1	37.1 ± 0.0	37.0 ± 0.1	37.1 ± 0.0
<i>XI</i> (1400W, TAN-670)	37.1 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	37.0 ± 0.0	37.0 ± 0.0	36.9 ± 0.1	37.0 ± 0.1
<i>XII</i> (B6129, CCPA)	37.0 ± 0.1	37.1 ± 0.1	37.2 ± 0.1	37.1 ± 0.0	37.0 ± 0.1	37.1 ± 0.0	37.1 ± 0.1
<i>XIII</i> (<i>iNOS</i> ^{-/-} , CCPA)	37.0 ± 0.1	37.1 ± 0.0	37.0 ± 0.1	37.1 ± 0.0	37.0 ± 0.0	37.0 ± 0.0	37.1 ± 0.1
<i>XIV</i> (1400W, CCPA)	36.8 ± 0.1	37.2 ± 0.1	37.0 ± 0.1	37.1 ± 0.0	36.8 ± 0.1	37.0 ± 0.1	37.0 ± 0.1
<i>XV</i> (B6129, LPC)	36.9 ± 0.1	37.3 ± 0.1	37.1 ± 0.1	37.0 ± 0.1	37.3 ± 0.1	36.8 ± 0.1	37.0 ± 0.1
Heart rate, beats/min							
<i>I</i> (B6129, AMI)	544 ± 12	570 ± 14	585 ± 22	588 ± 22	584 ± 23	585 ± 20	600 ± 24
<i>II</i> (<i>iNOS</i> ^{-/-} , AMI)	523 ± 13	522 ± 14	515 ± 18	517 ± 15	502 ± 19	548 ± 21	567 ± 15
<i>III</i> (B6129, DETA/NO)	556 ± 12	573 ± 10	563 ± 10	569 ± 10	578 ± 9	597 ± 10	614 ± 13
<i>IV</i> (<i>iNOS</i> ^{-/-} , DETA/NO)	548 ± 17	567 ± 17	556 ± 16	547 ± 15	552 ± 12	570 ± 9	571 ± 13
<i>V</i> (B6129, NTG)	576 ± 17	592 ± 14	615 ± 16	612 ± 11	610 ± 14	628 ± 18	631 ± 18
<i>VI</i> (<i>iNOS</i> ^{-/-} , NTG)	540 ± 11	563 ± 18	575 ± 21	596 ± 20	593 ± 22	603 ± 20	603 ± 23
<i>VII</i> (B6129, SNAP)	556 ± 16	566 ± 19	570 ± 19	566 ± 14	562 ± 16	573 ± 17	576 ± 19
<i>VIII</i> (<i>iNOS</i> ^{-/-} , SNAP)	547 ± 8	588 ± 8	574 ± 17	584 ± 15	577 ± 15	563 ± 16	571 ± 14
<i>IX</i> (B6129, TAN-670)	485 ± 10	538 ± 12	547 ± 7	556 ± 11	543 ± 11	527 ± 13	527 ± 13
<i>X</i> (<i>iNOS</i> ^{-/-} , TAN-670)	517 ± 9	579 ± 13	594 ± 18	602 ± 21	601 ± 21	598 ± 19	597 ± 21

Group	Preocclusion	Occlusion, min			Reperfusion, min		
		5	15	30	1	10	15
<i>XI</i> (1400W, TAIN-670)	511 ± 15	546 ± 15	562 ± 11	573 ± 11	559 ± 15	556 ± 15	547 ± 17
<i>XII</i> (B6129, CCPA)	547 ± 14	560 ± 13	571 ± 20	580 ± 17	574 ± 20	573 ± 14	565 ± 18
<i>XIII</i> (<i>iNOS</i> ^{-/-} , CCPA)	513 ± 21	579 ± 13	596 ± 14	606 ± 15	599 ± 16	593 ± 15	578 ± 17
<i>XIV</i> (1400W, CCPA)	532 ± 17	536 ± 17	587 ± 23	606 ± 28	592 ± 29	595 ± 29	592 ± 28
<i>XV</i> (B6129, LPC)	551 ± 14	566 ± 16	565 ± 17	572 ± 16	572 ± 17	581 ± 21	576 ± 18

Values are means ± SE. Measurements of rectal temperature and heart rate were taken before 30-min coronary occlusion (preocclusion), at 5, 15 and 30 min into 30-min occlusion and at 1, 10, and 15 min after reperfusion. Rectal temperature was continuously monitored and carefully controlled throughout experiment. AML, acute myocardial infarction; *iNOS*^{-/-}, inducible nitric oxide synthase knockout mice; LPC, late preconditioning.

Table 4

Size of left ventricle, risk region, and infarct

Group	Body Weight, g	Heart Weight, mg	LV Weight, mg	Heart Weight/Body Weight	Risk Region, mg	Infarct Weight, mg	Risk Region, % of LV	Infarct, % of Risk Region	Infarct, % of LV
<i>I</i>	29.2 ± 1.0	161.1 ± 7.8	118.6 ± 6.3	0.55 ± 0.01	43.2 ± 3.0	25.6 ± 2.3	36.8 ± 2.1	58.6 ± 1.8	21.7 ± 1.5
<i>II</i>	30.8 ± 2.4	166.6 ± 14.6	120.7 ± 9.4	0.54 ± 0.02	44.3 ± 2.4	23.5 ± 1.9	38.0 ± 2.8	53.4 ± 3.5	20.4 ± 2.1
<i>III</i>	32.3 ± 0.8	166.8 ± 6.5	124.1 ± 5.4	0.52 ± 0.02	42.0 ± 2.8	13.3 ± 2.0 ^{ab}	33.9 ± 1.7	31.0 ± 3.4 ^{ab}	10.4 ± 1.3 ^{ab}
<i>IV</i>	31.6 ± 1.7	161.4 ± 12.3	121.8 ± 9.8	0.51 ± 0.03	40.1 ± 3.5	21.7 ± 2.5	33.1 ± 1.8	53.3 ± 3.3	17.6 ± 1.4
<i>V</i>	33.7 ± 1.1	195.1 ± 8.1	150.7 ± 7.5	0.58 ± 0.02	48.6 ± 3.9	14.1 ± 2.4 ^{ac}	33.0 ± 3.4	27.9 ± 4.1 ^{ac}	9.5 ± 1.8 ^{ac}
<i>VI</i>	27.5 ± 0.8	150.8 ± 6.6	110.9 ± 6.1	0.55 ± 0.02	40.6 ± 2.7	22.7 ± 2.2	36.9 ± 2.0	55.1 ± 2.5	20.5 ± 1.9
<i>VII</i>	30.1 ± 0.7	160.2 ± 7.7	117.1 ± 5.1	0.53 ± 0.02	35.5 ± 3.7	7.92 ± 1.4 ^{ad}	30.1 ± 2.5	22.6 ± 3.5 ^{ad}	6.8 ± 1.1 ^{ad}
<i>VIII</i>	28.4 ± 1.2	167.4 ± 10.7	127.1 ± 7.3	0.59 ± 0.03	42.0 ± 4.1	28.6 ± 3.1	33.0 ± 2.6	67.5 ± 2.5	22.4 ± 2.1
<i>IX</i>	30.1 ± 0.8	160.1 ± 6.0	119.3 ± 4.5	0.53 ± 0.01	36.1 ± 3.1	8.0 ± 0.83 ^{ae}	30.1 ± 1.9	23.1 ± 2.7 ^{ae}	6.8 ± 0.8 ^{ae}
<i>X</i>	31.5 ± 0.9	177.2 ± 5.6	130.7 ± 3.7	0.57 ± 0.02	38.1 ± 3.9	24.9 ± 3.3	29.2 ± 2.9	63.2 ± 2.8	19.1 ± 2.5
<i>XI</i>	31.8 ± 1.0	174.4 ± 5.5	129.3 ± 4.1	0.55 ± 0.01	40.7 ± 3.1	24.3 ± 2.6	31.4 ± 2.1	58.6 ± 3.2	18.8 ± 2.0
<i>XII</i>	31.0 ± 1.0	158.1 ± 5.4	115.6 ± 4.4	0.51 ± 0.01	40.7 ± 2.1	14.1 ± 2.87 ^{af}	35.15 ± 1.2	33.3 ± 3.5 ^{af}	12.0 ± 1.4 ^{af}
<i>XIII</i>	31.8 ± 0.9	186.1 ± 7.9	143.7 ± 6.4	0.59 ± 0.02	47.1 ± 3.9	30.6 ± 2.8	32.59 ± 1.9	65.0 ± 3.3	21.2 ± 1.7
<i>XIV</i>	28.7 ± 0.72	162.2 ± 7.3	121.3 ± 5.4	0.57 ± 0.03	40.8 ± 2.5	23.2 ± 1.7	34.0 ± 2.0	57.6 ± 4.1	19.5 ± 1.8
<i>XV</i>	31.7 ± 1.5	145.2 ± 8.6	109.4 ± 6.1	0.46 ± 0.02	37.7 ± 4.4	6.32 ± 0.8 ^a	34.2 ± 2.76	19.3 ± 3.4 ^a	6.2 ± 1.0 ^a

Values are means ± SE. Heart weight, total heart weight (ventricles and atria); LV, left ventricle

^a $P < 0.05$ vs. groups *I* and *II*^b $P < 0.05$ vs. group *IV*^c $P < 0.05$ vs. group *VI*^d $P < 0.05$ vs. group *VIII*^e $P < 0.05$ vs. groups *X* and *XI*^f $P < 0.05$ vs. group *XIII* and group *XIV*.