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Administration of a CO-releasing molecule induces late preconditioning against myocardial infarction

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Abstract

Mounting evidence suggests that carbon monoxide (CO) exerts powerful cytoprotective actions. CO-releasing molecules (CORMs) offer an effective means of delivering CO to tissues in vivo. The goal of the present study was to determine whether a water-soluble CORM, tricarbonylchloro(glycinato)ruthenium(II) (CORM-3), induces delayed protection against myocardial infarction 24 h later and to explore the duration of this protection. Mice received a 60-min i.v. infusion of CORM-3 or inactive CORM-3 (which does not release CO) and then, 24, 72, or 120 h later, underwent a 30-min coronary occlusion followed by 24 h of reperfusion. Pretreatment with CORM-3 24 h prior to coronary occlusion markedly reduced infarct size (24.8% ± 2.9% of the risk region vs. 43.8% ± 4.4% with inactive CORM-3). The infarct-sparing effect of CORM-3 was still evident 72 h after administration of the CO donor (20.4% ± 3.7% of the risk region vs. 41.9% ± 2.5% with inactive CORM-3) but was no longer apparent at 120 h. Both at 24 and 72 h, the protective effects of CORM-3 were equivalent to those afforded by the late phase of ischemic preconditioning (PC; 27.0% ± 2.9% and 30.3% ± 3.9% of the risk region, respectively). We conclude that the novel CO-releasing compound, CORM-3, induces delayed protection against myocardial infarction which is similar to that afforded by the late phase of ischemic PC, and that this salubrious effect is sustained for 72 h. To our knowledge, this is the first report that exposure to CO causes the heart to shift to a preconditioned phenotype. In addition, this study provides the first evidence that the cardioprotective actions of ischemic PC persist for 72 h in the mouse.

Keywords

Carbon monoxide-releasing molecules; Preconditioning; Myocardial infarction

1. Introduction

Although CO has historically been viewed as toxic to biological systems [1], recent studies suggest that this byproduct of heme oxygenase-1 (HO-1) exerts an important regulatory role in many cellular and biological processes. Specifically, CO has been shown to promote vasorelaxation [2,3] and to inhibit proliferation of smooth muscle cells [4], transplant

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rejection [5], inflammation [6,7], platelet aggregation and microvascular thrombosis [8], cytokine production [9,10], and oxidative stress [11]. CO has also been shown to alleviate hypoxia/reoxygenation injury in isolated cells and ischemia/reperfusion injury in liver [12], in isolated hearts [5], and in an in vivo murine model of myocardial infarction [13]. In addition, numerous studies suggest that CO plays an important role in suppressing apoptosis [11,14–16].

Recent advances have enhanced the clinical feasibility of delivering CO in vivo. Thus far, most in vivo studies have utilized inhalation of CO to deliver this gas to tissues. However, administering CO gas is a very nonspecific approach, as most of the free CO reaching the bloodstream reacts rapidly with hemoglobin and other heme proteins prior to reaching the target tissue. This can potentially result in toxic effects. Carriers of CO that transport and deliver this gas to a target tissue would clearly increase both the clinical feasibility and the specificity of CO therapy. Motterlini et al. [5,17] have shown that transition metal carbonyls can deliver CO in biological systems and have recently developed a new molecule, namely, tricarbonyldichloro(glycinato)ruthenium(II), referred to as CO-releasing molecule-3 (CORM-3), which is water-soluble [5]. Clark et al. [5] has demonstrated that CORM-3 effectively delivers CO to tissues under physiological conditions and limits anoxia/reoxygenation or ischemia/reperfusion injury in isolated rat hearts and cardiomyocytes. We [13] have reported that administration of CORM-3 5 min before reperfusion in an in vivo murine model of myocardial ischemia/reperfusion results in a decrease in infarct size without increased CO levels in the blood. These results suggest that CORM-3 provides a clinically relevant tool for delivering therapeutic amounts of CO in an in vivo system; however, the long-term effects of CO donor administration on myocardial ischemia/reperfusion injury are unknown.

The powerful anti-apoptotic actions of CO suggest that, in addition to its immediate beneficial effects on myocardial ischemia/reperfusion injury [5,13], this gas may also produce sustained cytoprotection similar to that associated with the late phase of ischemic preconditioning (PC). Indeed, in non-cardiac cells CO has been reported to upregulate iNOS and HO-1 [18], two enzymes that are known to mediate the infarct-sparing actions of late PC [19,20]. In the heart, CO has been shown to participate in cardioprotective signaling during ischemia [21,22]. The potential clinical significance of late PC stems from the fact that it affords long-lasting (up to 72 h) protection against both myocardial stunning and myocardial infarction [23]. Previous studies have demonstrated that a delayed cardioprotective effect similar to the late phase of ischemic PC can be elicited by a variety of pharmacologic agents including nitric oxide donors [24–28], adenosine receptor agonists [29,30], opioid receptor agonists [31,32], reactive oxygen species [33], endotoxin derivatives [34,35], the K_{ATP} channel opener diazoxide [36,37], and nicorandil [38]. Unfortunately, most of these interventions are not clinically applicable or have significant side effects. Although CO donors are an effective means of delivering CO in vivo, the ability of these drugs to induce a delayed and sustained cardioprotective effect similar to that seen with the late phase of ischemic PC has not been tested.

Accordingly, the goal of the present study was to determine whether pretreatment with CORM-3, in the absence of ischemia, can reproduce the protective effect of the late phase of ischemic PC against myocardial infarction. All experiments were performed in a well-established murine model of ischemia/reperfusion injury [39].

2. Materials and methods

This study was performed in accordance with the guidelines of the Animal Care and Use Committee of the University of Louisville School of Medicine (Louisville, KY) and with the

Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services, National Institutes of Health, Publication No. 86-23).

2.1. Animals

Male ICR mice (body wt. 35.5 ± 1.3 g, age 10.2 ± 0.3 weeks) were used for this study. All mice were purchased from Jackson Laboratory (Bar Harbor, ME) and maintained in microisolator cages under specific pathogen-free conditions in a room with a temperature of 24 °C, 55–65% relative humidity, and a 12-h light–dark cycle.

2.2. Experimental protocol

The overall experimental design is summarized in Fig. 1. Mice were assigned to nine groups. On day 1, mice in group I received sham PC (1 h of open-chest state with no coronary occlusion), whereas mice in groups II and III underwent six cycles of 4-min coronary occlusion/4-min reperfusion according to the methods described below. On day 1, mice in groups V, VII, and IX received a 60-min i.v. infusion of CORM-3 (total dose, 3.54 mg/kg); to preserve the CO-releasing activity, CORM-3 was dissolved in distilled water (pH 7.0). This dose of CORM-3 was selected because it affords robust cardioprotection in this murine model [13]. Mice in groups IV, VI, and VIII received inactive CORM-3 at the same dose used in groups V, VII, and IX. CORM-3 was inactivated by dissolving it in PBS (0.35 mg/ml) and leaving it at room temperature for 24 h; under these conditions, 1 mol of CO per mole of compound is released in the solution and, as a result, no additional CO is liberated upon administration of the drug [5]. On day 2 (24 h after the six occlusion/reperfusion cycles, after sham PC, or after CORM-3 or inactive CORM-3 infusion), mice in groups I, II, IV, and V underwent a 30-min coronary occlusion followed by 24 h of reperfusion [39]. Mice in groups III, VI and VII underwent the 30-min occlusion/24-h reperfusion sequence 72 h after the six occlusion/reperfusion cycles, after infusion of inactive CORM-3, or after infusion of CORM-3. Mice in groups VIII and IX underwent the 30-min occlusion/24-h reperfusion sequence 120 h after infusion of inactive CORM-3 or CORM-3.

2.3. Studies of myocardial infarction

The experimental preparation has been described in detail previously [20,39]. Briefly, mice were anesthetized with sodium pentobarbital (50 mg/kg i.p.), intubated, and ventilated with room air supplemented with oxygen at a rate of 105 strokes per min and with a tidal volume of 0.6 ml using a small rodent ventilator. These respiratory settings result in physiological values of arterial pH (7.39 ± 0.01) and adequate oxygenation [20,39]. After administration of antibiotics, the chest was opened through a midline sternotomy, and a non-traumatic balloon occluder was implanted around the mid-left anterior descending coronary artery by using an 8-0 nylon suture [39]. To prevent hypotension, blood from donor mice was given during surgery. Physiologic variables, including heart rate, core body temperature, and arterial pH were monitored and carefully maintained within the normal range throughout the experiments [20,39]. In all groups, myocardial infarction was produced by a 30-min coronary occlusion followed by 24 h of reperfusion [20,39]. After the coronary occlusion/reperfusion protocol, the chest was closed in layers and the mice were allowed to recover.

2.4. 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 from viable myocardium, the heart was then perfused with 1% 2,3,5-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 [20,39]. As a result of this procedure, the region at risk was identified by the absence of blue dye, whereas the rest

of the LV 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. Infarct size was calculated by computerized planimetry of the infarcted, ischemic/reperfused and nonischemic regions in serial heart slices [20,39]. From these measurements, infarct size was calculated as a percentage of the region at risk [20,39].

2.5. Statistical analysis

Data are reported as mean ± S.E.M. Heart rate, risk region, and infarct size were analyzed with a one-way or a two-way repeated-measures (time and group) ANOVA, as appropriate, followed by Student's *t*-tests for unpaired data with the Bonferroni correction [40]. The relationship between infarct size and risk region size was compared between groups with ANCOVA, with the size of the risk region as the covariate. The correlation between infarct size and risk region size was assessed by linear regression with the least-squares method. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS (version 8.0) statistical software (SPSS Inc., Chicago, IL).

3. Results

A total of 94 mice were used. Twelve mice were excluded for the reasons specified in Table 1.

3.1. Body temperature and heart rate

By experimental design [20,39], rectal temperature remained within a narrow, physiologic range (36.7–37.3 °C) in all groups. Five minutes before the 30-min coronary occlusion, the average heart rate in groups I–IX ranged from 490 to 636 beats/min (Table 2). In group II, the average heart rate 5 min before occlusion was higher (636 ± 33 beats/min, $P < 0.05$ vs. other groups), likely due to the trauma from the surgery that had been performed 24 h previously [39]. During the 30-min occlusion and ensuing reperfusion, heart rate did not differ significantly among groups I and III–IX but remained elevated in group II compared with the other groups (Table 2).

3.2. Infarct size

There were no significant differences among the nine groups with respect to heart weight or weight of the region at risk (Table 3). In the sham PC mice (group I), infarct size averaged 49.9% ± 4.0% of the region at risk (Fig. 2). As expected [39], at 24 h after ischemic PC (group II) infarct size was smaller than in the sham PC group (27.0% ± 2.9%, $P < 0.05$). A similar reduction in infarct size was noted 72 h after ischemic PC (group III) (30.3% ± 3.9%, $P < 0.05$).

In mice pretreated with inactive CORM-3 (groups IV and VI), infarct size was similar to the sham PC group (group I) (43.8% ± 4.4%, 41.9% ± 2.5%, and 49.9% ± 4.0% of the region at risk, respectively), indicating that administration of CORM-3 alone does not affect the extent of cell death. However, in mice pretreated with CORM-3 and subjected to coronary occlusion 24 h later (group V), infarct size (24.8% ± 2.9% of the region at risk) was significantly ($P < 0.05$) smaller than that observed in group IV (Fig. 2). This infarct-sparing effect persisted at 72 h after CORM-3 (20.4% ± 3.7% of the region at risk vs. 41.9% ± 2.5% in mice pretreated with inactive CORM-3), but was no longer observed at 120 h (45.5% ± 3.4% of the region at risk vs. 44.6% ± 3.0% in mice pretreated with inactive CORM-3). The infarct size measured in groups V and VII was similar to that measured in groups II and III, indicating that administration of CORM-3 24 or 72 h before ischemia/reperfusion resulted in a protective effect that was comparable to that induced by the late phase of ischemic PC at corresponding time-points (24 and 72 h).

In groups IV–IX, the size of the infarction was positively and linearly related to the size of the region at risk (r = 0.75, 0.41, 0.83, 0.26, 0.49, and 0.77, respectively). The regression line, however, was shifted down and to the right in group V compared with group IV ($P < 0.05$) and in group VII compared with group VI ($P < 0.05$; Fig. 3), indicating that for any given size of the region at risk, the resulting infarction was smaller in CORM-3-treated mice than in inactive CORM-3-treated mice at both 24 and 72 h. The relationship between infarct size and region of risk did not differ between groups VIII and IX (data not shown).

4. Discussion

In recent years there has been a remarkable paradigm shift with respect to our understanding of the role of CO in biological systems. Mounting evidence indicates that this gas, which has been traditionally regarded as a toxic byproduct of HO-1 activity, exerts an important homeostatic function and plays a cytoprotective role in many pathophysiological conditions [16]. In the heart, the effects of CO have been examined only in the context of acute CO treatment [5,13]. The present study is the first to explore the long-term effects of CO exposure on myocardial ischemia/reperfusion injury.

Our results demonstrate that administration of the water-soluble CO donor CORM-3 24 or 72 h prior to ischemia/reperfusion reduces infarct size in vivo. The magnitude of the delayed cardioprotective effects of CORM-3 was similar to that observed during the late phase of ischemic PC, both at 24 and 72 h. We have previously shown that this dose of CORM-3 has no effect on arterial pressure [13], and in the present study heart rate during coronary occlusion was similar in CORM-3-treated and inactive CORM-3-treated mice (Table 2). Consequently, the late PC-mimetic actions of CORM-3 cannot be attributed to alterations in arterial pressure or heart rate, nor can they be ascribed to nonspecific actions of the drug, since administration of the same moiety lacking the ability to release CO (inactive CORM-3) had no effect on infarct size. Previous investigations have documented the ability of CO to elicit immediate cardioprotective effects [5,13]. To our knowledge, this is the first study to demonstrate that a relatively brief exposure to CO can produce a sustained, long-term cardioprotective effect that mimics the late phase of ischemic PC. These findings advance our understanding of the role of CO in cardiovascular homeostasis by shifting the focus from its immediate, short-lived actions to its long-term effects.

To better assess the cardioprotective actions of CORM-3, we compared them with those of ischemic PC, both at 24 and 72 h. Since all previous studies of ischemic PC in murine models have been performed at 24 h after the stimulus [20,39,41–49], the duration of ischemia-induced late PC in this species is unknown. To our knowledge, the data obtained in group III provide the first evidence that the cardioprotective actions of ischemic PC persist for 72 h in the mouse. Fig. 2 clearly demonstrates that the infarct-sparing actions of CORM-3 are at least as robust as those of ischemic PC at 24 and 72 h, further supporting the potential utility of CO donors for cardioprotection.

The implicit goal of studying PC is to exploit this phenomenon for the protection of ischemic myocardium in patients with coronary artery disease. Although various pharmacological agents have been shown to induce a late PC phenotype in a variety of animal models, many of these agents are not clinically applicable or have significant side effects [24–38]. The recent development of CORMs has enabled administration of CO in biological systems in a predictable, effective, and safe manner. We have previously shown that CORM-3, administered in vivo in this same murine model, releases CO at concentrations sufficient to elicit protection without increasing blood carboxyhemoglobin levels [13]. The present investigation suggests that, in addition to their immediate infarct-sparing actions [13], CO donors may also be useful as pharmacologic tools to promote the

shift of the heart from a naïve to a defensive (preconditioned) phenotype. Unlike other late PC-mimetic agents, CORM-3 reduces infarct size even when given at the time of reperfusion [13]. These facts provide a rationale for testing the potential utility of CO donors as late PC mimetics in patients at risk for myocardial infarction.

The mechanism whereby brief exposure to CO induces sustained cardioprotection remains to be elucidated. CO modulates many potentially protective signaling pathways. For example, CO may alleviate ischemia/reperfusion injury by activating mitochondrial ATP-sensitive (mitoK_{ATP}) potassium channels. This mechanism has been suggested by Clark et al. [5], who demonstrated that the cardioprotection induced by CORM-3 in cardiac cells and isolated hearts was abrogated by 5-hydroxydecanoic acid. Whether this immediate effect of CO can account for its delayed cytoprotective actions, however, is unknown. Because CO exerts powerful anti-apoptotic effects in many cell types [11,14–16], it is also plausible that CO-induced cardioprotection may be associated with upregulation of anti-apoptotic proteins and/or downregulation of pro-apoptotic proteins. Another pathway that may potentially mediate CO donor-induced cardioprotection is the p38 MAPK signaling pathway, which has been previously implicated in the protective effects of ischemic PC [50]. Activation of p38 MAPK has been shown to underlie CO-dependent alleviation of hepatic ischemia/reperfusion injury [12] and CO-dependent inhibition of apoptosis during lung ischemia/reperfusion injury [51]. Considerable work will be necessary to unravel the molecular basis of CO-induced delayed cardioprotection.

In conclusion, our understanding of the cardiovascular functions of CO continues to evolve rapidly. The results of this study indicate, for the first time, that CO induces a sustained cardioprotective phenotype that confers resistance to myocardial ischemia/reperfusion injury. We propose the novel idea that CO plays a critical role in the adaptation of the heart to stress by virtue of its ability to reprogram the heart in a manner that promotes cell survival. This concept implies that, far from being a toxic waste product, CO exerts a fundamental (and heretofore unrecognized) function in protecting the heart against ischemia. Besides these conceptual implications, the present findings have potential practical reverberations, since CO donors may become a new class of antiischemic drugs [5]. The fact that CORM-3 has now been shown to induce cardioprotection not only when administered at the time of reperfusion [13] but also when given 24 or 72 h prior to coronary occlusion suggests that CO confers a broad range of salubrious actions in myocardial ischemia/reperfusion. The observations reported herein, coupled with prior reports [13], suggest that CO-releasing agents may be potentially useful for inducing a sustained cardioprotective phenotype in patients at risk for myocardial infarction.

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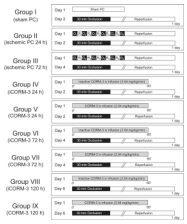

Fig. 1.

Experimental protocol. Nine groups of mice were used. On day 1, mice in group I (n = 6, sham PC) received sham PC, while mice in group II (n = 10, late PC 24 h) and group III (n = 10, late PC 72 h) underwent six cycles of 4-min coronary occlusion (O)/4-min reperfusion (R). Mice in groups IV (n = 9),VI (n = 9), and VIII (n = 7) received 3.54 mg/kg of inactive CORM-3 (iCORM-3) as an i.v. infusion for 60 min. Mice in groups V (n = 10), VII (n = 10), and IX (n = 11) received 3.54 mg/kg of CORM-3 as an i.v. infusion for 60 min. In groups I, II, IV, and V, mice underwent a 30-min coronary occlusion followed by 24 h of reperfusion on day 2 (24 h after sham PC, ischemic PC, inactive CORM-3, or CORM-3). In groups III, VI, and VII, mice underwent a 30-min coronary occlusion followed by 24 h of reperfusion on day 4 (72 h after ischemic PC, inactive CORM-3 or CORM-3). In groups VIII and IX, mice underwent a 30-min coronary occlusion followed by 24 h of reperfusion on day 6 (120 h after inactive CORM-3 or CORM-3).

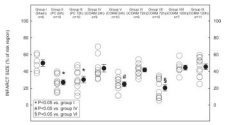

Fig. 2.

Myocardial infarct size in groups I–IX. Infarct is expressed as a percentage of the region at risk of infarction, ○, Individual mice; ●, mean ± S.E.M. for respective groups; CORM, CORM-3; iCORM, inactive CORM-3.

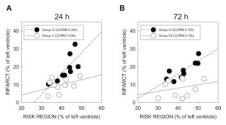

Fig. 3.

Relationship between size of the region at risk and size of the infarction. ***Panel A.*** Individual values and regression lines obtained by linear regression analysis in groups IV and V. In both groups, infarct size was positively and linearly related to risk region size. The linear regression equations were as follows: group IV, $y = -31 + 1.2x$, $r = 0.75$; group V, $y = -1.4 + 0.28x$, $r = 0.41$. Analysis of covariance demonstrated that the regression line for group IV was significantly different from group V ($P < 0.05$), indicating that for any given risk region size, infarct was smaller in CORM-3-treated mice than in inactive CORM-3-treated mice at 24 h after treatment. ***Panel B.*** Individual values and regression lines obtained by linear regression analysis in groups VI and VII. In both groups, infarct size was positively and linearly related to risk region size. The linear regression equations were as follows: group VI, $y = -11.5 + 0.69x$, $r = 0.83$; group VII, $y = 0.72 + 0.72x$, $r = 0.26$. Analysis of covariance demonstrated that the regression line for group VI was significantly different from group VII ($P < 0.05$), indicating that for any given risk region size, infarct was smaller in CORM-3-treated mice than in inactive CORM-3-treated mice at 72 h after treatment; CORM, CORM-3; iCORM, inactive CORM-3.

Table 1

Reasons for excluding mice

	Group I	Group II	Group III	Group IV	GroupV	Group VI	Group VII	Group VIII	Group IX	Total
Death	0	2	1	1	1	1	0	0	0	6
Technical problems	0	1	0	1	2	0	0	0	0	4
Poor postmortem staining	0	1	0	1	0	0	0	0	0	2
Mice excluded	0	4	1	3	3	1	0	0	0	12
Mice operated upon	6	14	11	12	13	10	10	7	11	94
Mice included in study	6	10	10	9	10	9	10	7	11	82
Mice included in study (%)	100	71	91	75	77	90	100	100	100	87

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Table 2

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

Group	Preocclusion	Occlusion		Reperfusion	
		5 min	30 min	5 min	15 min
Temperature (°C)					
I	37.2 ± 0.1	37.2 ± 0.2	37.0 ± 0.2	36.9 ± 0.1	36.9 ± 0.1
II	36.8 ± 0.0	37.0 ± 0.0	37.1 ± 0.1	37.0 ± 0.1	36.9 ± 0.1
III	37.0 ± 0.1	37.2 ± 0.0	37.0 ± 0.1	37.0 ± 0.1	37.1 ± 0.1
IV	37.0 ± 0.1	37.2 ± 0.0	37.0 ± 0.1	37.0 ± 0.1	37.1 ± 0.1
V	36.9 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	36.9 ± 0.1	37.0 ± 0.1
VI	36.8 ± 0.1	37.0 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	37.0 ± 0.1
VII	36.9 ± 0.1	37.0 ± 0.1	37.0 ± 0.1	37.2 ± 0.1	36.9 ± 0.0
VIII	37.0 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	37.0 ± 0.1	36.9 ± 0.1
IX	37.0 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	36.9 ± 0.0
Heart rate (bpm)					
I	584 ± 16	635 ± 17	639 ± 23	641 ± 30	626 ± 32
II	636 ± 33*	675 ± 25*	689 ± 17*	679 ± 11*	662 ± 13*
III	550 ± 25	620 ± 20	635 ± 25	634 ± 20	603 ± 19
IV	580 ± 20	600 ± 16	579 ± 20	560 ± 16	566 ± 23
V	600 ± 22	628 ± 22	608 ± 28	600 ± 31	612 ± 31
VI	503 ± 14	583 ± 22	552 ± 25	557 ± 21	534 ± 17
VII	526 ± 27	561 ± 28	544 ± 23	579 ± 24	558 ± 26
VIII	490 ± 20	569 ± 15	541 ± 23	552 ± 25	537 ± 34
IX	538 ± 18	549 ± 22	531 ± 22	542 ± 22	534 ± 25

Mice underwent a 30-min coronary occlusion followed by 24 h of reperfusion. Heart rate was measured 5 min before coronary occlusion (preocclusion), at 5 and 30 min into the 30-min coronary occlusion, and at 5 and 15 min after reperfusion. The experimental protocols are specified in the legend to Fig. 1. Data are mean ± S.E.M. Note that the heart rate in group II was higher than that in groups I and III–IX, possibly reflecting the effect of surgical trauma 24 h earlier.

*$P < 0.05$ vs. groups I, III–IX).

Table 3

Size of left ventricle, risk region, and infarct

Group	Body Wt (g)	Heart Wt (mg)	LVWt (mg)	Heart Wt/body Wt	Risk region Wt (mg)	Infarct Wt (mg)	Risk region (% of LV)	Infarct (% of risk region)	Infarct (% of LV)
I	30.7 ± 1.3	141.5 ± 7.2	101.4 ± 5.9	0.47 ± 0.03	40.0 ± 5.7	20.2 ± 3.3	39.3 ± 4.7	49.9 ± 4.0	19.9 ± 3.1
II	34.3 ± 0.8	164.1 ± 5.8	122.3 ± 4.1	0.48 ± 0.01	53.8 ± 4.9	14.1 ± 1.7*	44.0 ± 3.9	27.0 ± 2.9*	11.4 ± 1.1*
III	35.1 ± 1.9	172.8 ± 6.4	122.0 ± 4.2	0.50 ± 0.02	46.0 ± 2.6	14.0 ± 1.9*	37.5 ± 1.3	30.3 ± 3.9*	11.5 ± 1.6*
IV	40.0 ± 0.7	171.9 ± 6.1	125.7 ± 3.5	0.43 ± 0.02	53.2 ± 2.4	23.9 ± 3.5	42.3 ± 1.5	43.8 ± 4.4	18.9 ± 2.4
V	37.8 ± 1.3	162.2 ± 4.3	114.2 ± 4.5	0.43 ± 0.01	45.2 ± 2.9	11.4 ± 1.6#	39.5 ± 1.7	24.8 ± 2.9#	9.8 ± 1.2#
VI	37.2 ± 0.9	161.9 ± 5.8	113.8 ± 4.1	0.44 ± 0.01	48.8 ± 3.3	20.6 ± 2.1	42.6 ± 2.0	41.9 ± 2.5	18.0 ± 1.7
VII	35.1 ± 0.5	148.4 ± 6.3	105.8 ± 3.9	0.42 ± 0.02	43.5 ± 3.0	9.11 ± 1.8^	41.0 ± 2.2	20.4 ± 3.7^	8.4 ± 1.5^
VIII	35.4 ± 1.0	169.4 ± 7.1	118.7 ± 6.2	0.48 ± 0.02	41.2 ± 1.9	18.4 ± 1.6	34.9 ± 1.4	44.6 ± 3.0	15.6 ± 1.1
IX	36.6 ± 0.9	165.1 ± 6.4	118.0 ± 5.4	0.45 ± 0.02	49.2 ± 1.7	22.3 ± 1.7	42.3 ± 1.8	45.5 ± 3.4	19.2 ± 1.7

Data are mean ± S.E. Heart Wt, total heart weight (ventricles and atria); LV, left ventricle.

*$P < 0.05$ vs. group I.

#$P < 0.05$ vs. group IV.

^$P < 0.05$ vs. group VI.