Noradrenaline Protects *In Vivo* Rat Heart Against Infarction and Ventricular Arrhythmias *Via* Nitric Oxide and Reactive Oxygen Species

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**Background.** Our previous study showed that pretreatment with noradrenaline *via* opening of the mitochondrial ATP-sensitive potassium channel protects myocardium against ischemia/reperfusion injuries. We have hypothesized that production of nitric oxide (NO) and generation of reactive oxygen species (ROS) are involved in noradrenaline-induced cardioprotection in rat heart.

**Methods.** All anesthetized rats underwent 25 min of regional ischemia followed by 120 min of reperfusion. Animals were randomized to receive one of the following treatments: saline, noradrenaline (2 μg/kg, i.v.), noradrenaline plus prazosin (an α1-adrenoceptor blocker, 0.5 mg/kg, i.v.), noradrenaline plus L-NAME (a nonspecific NOS inhibitor, 10 mg/kg, i.v.), noradrenaline plus tempol (a membrane-permeable radical scavenger, 30 mg/kg, i.v.), Prazosin alone, only L-NAME and tempol alone.

**Results.** Infarct size (% of risk area) was reduced from 49.6 ± 2.4 in saline-control group to 18.2 ± 1.5 in noradrenaline preconditioned group. Administration of prazosin, L-NAME, or tempol prior to noradrenaline injection abolished the observed cardioprotection of noradrenaline (45.5 ± 3, 41.7 ± 4.5 and 38.7 ± 5.4, respectively) and restored infarct size to saline-control rats’ level. Incidences and severity of ventricular arrhythmias during ischemia and early reperfusion significantly decreased in noradrenaline preconditioned group compared with saline-control group. This cardioprotective effect of noradrenaline against ventricular arrhythmia was abrogated by administration of prazosin, L-NAME, or tempol alone.

**Conclusion.** Cardioprotection effect of the α1-adrenoceptor stimulation by noradrenaline was inhibited by L-NAME or tempol in anesthetized rat heart.

**Key Words:** α1-adrenoceptor; ischemia/reperfusion; nitric oxide; oxygen radicals; infarction; ventricular arrhythmias, preconditioning.

**INTRODUCTION**

Preconditioning the myocardium with nonlethal short episodes of ischemia/reperfusion exerts potent endogenous cardioprotection and protects the heart against damage resulting from subsequent prolonged period of ischemia-reperfusion by reducing infarct size [1] and by attenuating ventricular arrhythmias [2]. This phenomenon displays two distinctive phases: an early (classic) phase, which is protective for 1–3 h after the initial insult; and a late phase, which is efficient 12–72 h following the initial ischemic insult [3].

In recent years, much research has been designed to explain the mechanisms that are responsible for the preconditioning-induced protection to ischemia/reperfusion injury. In this way, although endogenous nitric oxide (NO) is important to the triggering of the late preconditioning, its involvement in the signaling of early preconditioning until recently is less clear [4]. In the isolated hearts treated with a NO synthase (NOS) inhibitor, no effect against early cardioprotection was seen [5]. In contrast, in the *in vivo* hearts, it has been indicated that NO is involved in early phase of ischemic preconditioning [4].

Oxidative damage mediated by endogenous production of reactive oxygen species (ROS) is an important contributing factor to ischemia/reperfusion-induced injuries in myocardium [6]. On the other hand, it has been...
shown that the generation of endogenous reactive oxygen species contributes to the signaling pathways in early preconditioning and administration of radical scavenger blocks the cardioprotection afforded by some pharmacological agents [7].

There are many pharmacologic agents that have been reported to mimic ischemic preconditioning and protect the heart by modulating various signaling pathways. An effective pharmacologic approach to achieve cardioprotection against infarction [8] and arrhythmias [9, 10] is the α1-adrenoceptor stimulation with administration of exogenous noradrenaline prior to long-lasting ischemia.

In order to explore the mechanisms involved in the noradrenaline-induced early preconditioning against infarction and ventricular arrhythmias in the rat heart in vivo, we investigated the potential role of NO, using the nonspecific NOS inhibitor N-nitro-L-arginine methyl ester (L-NAME). We also determined the role of ROS, using a membrane-permeable radical scavenger 4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl (tempol).

**MATERIALS AND METHODS**

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication no.85-23, revised 1996).

**Surgical Procedure**

Male Wistar rats (250–350 g) were anesthetized with pentobarbital sodium (50 mg/kg i.p.). The rats were tracheotomized in middle of the neck, intubated, and ventilated with room air by a Parvalux (London, England) rodent ventilator (15 mL/kg stroke volume and 60–70 breaths/min). Respiratory rate was adjusted to maintain arterial oxygen saturation in the normal level.

Body temperature was measured with a rectal thermometer and maintained at 37 ± 1 °C by the use of a lamp. The right carotid artery was cannulated and connected to a pressure transducer to record mean arterial blood pressure (MBP). The tail vein was cannulated to allow administration of saline or drugs. A standard limb lead-II electrocardiogram (ECG) was monitored with subcutaneous stainless steel electrodes. Power Lab monitoring system (ML750 PowerLab/4 sp, Castle Hill, Australia) was used for recording of MBP, heart rate (HR) and ECG. After injection of heparin (200 IU/kg, i.v.), a left lateral thoracotomy was performed in the fifth intercostal space to expose the heart. The pericardium was incised and a 6–0 silk suture placed around the left anterior descending coronary artery (LAD), close to its origin. The ends of the silk thread were passed through a plastic tube. Applying tension to the suture caused regional ischemia following coronary artery occlusion, and reperfusion was achieved by releasing the tension on the ligature. After completion of the surgical procedure, the animals were allowed to stabilize for 30 min before starting the experimental protocol. Ischemia was confirmed by ST elevation and increase in R-wave amplitude in ECG and decrease in arterial blood pressure. All animals were subjected to a 25 min of coronary artery occlusion followed by 120 min of reperfusion. At the end of surgical procedure, any rat with a constant fall in MBP to less than 80 mmHg or ventricular fibrillation lasting for more than 5 min was discarded from the study.

**Assessment of Infarct and Risk Regions**

At the end of reperfusion period, the LAD was reoccluded and Evans Blue dye (3 mL of 2% solution) was injected into the tail vein to stain the normally perfused region of the heart, whereas the area at risk (AAR) remained unstained. The rats were then killed and their hearts were excised and frozen overnight. The atria and right ventricle were removed, and the left ventricle (LV) was cut into transverse slices of 2 mm thickness from apex to base. The anatomic uncolored AAR (pink) was separated from the colored nonischemic area (blue), and then incubated with a 1% solution of 2,3,5 triphenyltetrazolium chloride (TTC, in 0/1 M phosphate buffer, pH 7.4) for 20 min at 37 °C to identify the unstained infarcted region (as pale yellow color) from stained ischemic myocardium (as red color). The slices were then immersed in 10% phosphate-buffered formalin to enhance the contrast of the stain. In each slice, the infarct size (IS) and AAR were determined by using an image processing software program (Photoshop, ver. 7.0, Adobe system, San Jose, CA, USA). IS was expressed as a percentage of the AAR (%IS/AAR).

**Evaluation of Ventricular Arrhythmias**

Ventricular arrhythmias during ischemia and reperfusion periods were measured in accordance with the Lambeth Conventions [11]. Ventricular ectopic beat (VEB) was diagnosed as a distinctive and identifiable premature QRS complex. Ventricular tachycardia (VT) was defined as a run of four or more consecutive VEBs. Ventricular fibrillation (VF) was defined as unidentifiable and low voltage QRS complexes. Other multipart forms of VEBs, such as bigeminy and salvos (two or three consecutive VEBs), were not determined separately and they were included as single VEB. VF lasting for more than 5 min was measured as irreversible.

A scoring system was used to calculate the severity of arrhythmias [12]: where hearts with 0–50 VEBs were given score 0; 50–500 VEBs a score of 1; more than 500 VEBs, or one episode of spontaneously reversible VT and/or VF a score of 2; 2–30 episodes of spontaneously reversible VT and/or VF a score of 3; more than 30 episodes of spontaneously reversible VT and/or VF a score of 4, and irreversible VF was given a score of 5.

**Experimental Protocols**

Drugs and saline were injected as an intravenous bolus. After stabilization period, the rats were randomly assigned into one of the following groups:

- Control group (n = 9): rats received 0.9% saline 10 min before the coronary artery ligation. Noradrenaline preconditioned group (n = 6): noradrenaline (2 μg/kg) was given 10 min before coronary artery occlusion. Prazosin plus noradrenaline group (n = 6): rats received prazosin (0.5 mg/kg) 5 min prior to noradrenaline injection. L-NAME plus noradrenaline group (n = 6): L-NAME (10 mg/kg) was injected 15 min before noradrenaline administration. Tempol plus noradrenaline group (n = 6): tempol (30 mg/kg) was administered 10 min prior to noradrenaline injection. Prazosin alone group (n = 6): rats received prazosin (0.5 mg/kg) 15 min prior to ischemia/reperfusion. L-NAME alone group (n = 6): rats received L-NAME (10 mg/kg) 25 min prior to ischemia/reperfusion. Tempol alone group (n = 6): rats received tempol (30 mg/kg) 20 min prior to ischemia/reperfusion.

**Measurement of Plasma Creatine Kinase-MB (CK-MB)**

Blood samples were collected in heparinized tube at the end of reperfusion period (prior to Evans Blue staining). The samples were centrifuged and the plasma was removed and frozen until assayed. Plasma CK-MB activity was measured with a specific CK-MB Kit (Pars Azmoon), using an autoanalyzer (Roche Hitachi Modular DP Systems, Mannheim, Germany).
Materials

Pentobarbital sodium, noradrenaline, prazosin hydrochloride (a specific α1-adrenoceptor blocker), L-NAME (a nonspecific NOS inhibitor, N-nitro-L-arginine methyl ester), and tempol (a membrane-permeable radical scavenger, 4-hydroxy-2,2,6,6-tetramethyl piperidinoxy) were obtained from Sigma Chemical Co (St. Louis, MO, USA). Prazosin was dissolved in distilled water and the rest were dissolved in saline immediately before use.

Statistical Analysis of Data

Data are expressed as means ± SEM or the percentage of incidence. Statistical comparison of means between groups was made by one-way ANOVA and a subsequent tukey test. Within each group, differences between means in hemodynamic parameters were compared by one-way repeated measures ANOVA. The arrhythmia scores were analyzed with Kruskal-Wallis test and the incidences of VT or VF were compared using the Fisher exact test. Significant differences were determined as P < 0.05.

RESULTS

Hemodynamic Parameters

Table 1 summarizes hemodynamic data obtained during the experiments. Basal MBP and HR values were similar in all groups of animals. Injection of noradrenaline was led to increase of MBP and HR, but these parameters returned to the baseline values prior to the 25-min index ischemia. Although, administration of tempol or prazosin caused a rapidly transient fall in MBP, there were no statistically considerable differences in hemodynamic parameters between preoclusion and baseline periods. A bolus injection of L-NAME significantly increased MBP in preocclusion period compared with baseline. Coronary artery ligation led to significant decrease in MBP during 25-min ischemia in all groups compared with baseline. During reperfusion period, MBP was significantly reduced in all groups compared with baseline, but HR did not change.

Infarct Size and Area at Risk

Figure 1 shows the myocardial area at risk (as red color) and infarction area (as pale yellow color) in the experimental groups. Figure 2 illustrates the ratio of area at risk to total left ventricular areas (AAR/LV) and ratio of infarct size to area at risk (IS/AAR). There were no significant differences in AAR/LV between the rats in all experimental groups. IS/AAR decreased considerably from 49.6 ± 2.4 in control group to 18.2 ± 1.5 in noradrenaline preconditioned group. Noradrenaline-induced infarct limitation was inhibited by administration of each prazosin, L-NAME, or tempol, where IS/AAR was increased to 45.5 ± 3, 41.7 ± 4.5 and 38.7 ± 5.4, respectively. Treatment with prazosin alone, L-NAME alone, or tempol alone had no effect on IS/AAR compared with control group.

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<thead>
<tr>
<th>Table 1</th>
<th>Hemodynamic Data</th>
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<td>Baseline</td>
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<tr>
<td>CON MBP</td>
<td>93 ± 3</td>
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<td>HR</td>
<td>424 ± 21</td>
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<td>NA MBP</td>
<td>98 ± 4</td>
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<tr>
<td>HR</td>
<td>413 ± 34</td>
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<tr>
<td>PRAZ-NA MBP</td>
<td>98 ± 6</td>
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<tr>
<td>HR</td>
<td>372 ± 46</td>
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<tr>
<td>TEMP-NA MBP</td>
<td>105 ± 10</td>
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<tr>
<td>HR</td>
<td>322 ± 20</td>
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<tr>
<td>L-NAME-NA MBP</td>
<td>116 ± 9</td>
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<tr>
<td>HR</td>
<td>363 ± 13</td>
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<tr>
<td>PRAZ MBP</td>
<td>108 ± 4</td>
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<td>TEMP MBP</td>
<td>101 ± 5</td>
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<tr>
<td>HR</td>
<td>310 ± 18</td>
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<tr>
<td>L-NAME MBP</td>
<td>97 ± 5</td>
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<td>HR</td>
<td>322 ± 24</td>
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HR = heart rate (beats/min); MBP = mean arterial blood pressure (mmHg); CON = control; NA = noradrenaline; PRAZ = prazosin; TEMP = tempol.

Data are presented as mean ± SEM.

*P < 0.05.

**P < 0.01.

***P < 0.001 compared with baseline within group.
Ventricular Arrhythmias

Arrhythmias During the 25-Min Index Ischemia

In this study, regional ischemia with occlusion of LAD coronary artery produced rigorous ventricular arrhythmia. Compared with control group, addition of noradrenaline 10 min prior to the 25 min index ischemia reduced significantly incidence of VT (44.5% versus 100%), incidence of VF (0% versus 55.5%), duration of VF + VT (4.7 ± 2.1 s versus 52.9 ± 6 s), severity of arrhythmias (1.7 ± 0.5 versus 3.9 ± 0.3), and number of episodes of VEB/min (2.5 ± 0.5 versus 9.5 ± 1.5).

This protection of noradrenaline against ventricular arrhythmias was abrogated by administration of each prazosin, L-NAME, or tempol, where incidence of VT (100%, 100%, and 100%, respectively), incidence of VF (67% (4/6), 50% (3/6), and 67% (4/6)), duration of VT + VF (70 ± 10.5 s, 80 ± 19.6 s, and 52.5 ± 16.7 s), severity of arrhythmias (3.8 ± 0.3, 3.4 ± 0.2 and 3.7 ± 0.3), and number episodes of VEB/min (7.5 ± 1.5, 9 ± 1, and 12.5 ± 1.5) virtually returned to control group levels. Administration of prazosin alone, L-NAME alone, or tempol alone had no effect on arrhythmias during ischemia compared with control group. These results are illustrated in Fig. 3.

Arrhythmias During Early Coronary Artery Reperfusion

Within the first 5 min of reperfusion, 77.8% (7/9) of the hearts in control group exhibited VT, and pretreatment with noradrenaline markedly decreased occurrence of VT (33% or 3/9). Application of each prazosin, L-NAME or tempol abolished cardioprotective effect of noradrenaline and restored incidence of VT [83% (5/6), 67% (4/6), and 67% (4/6), respectively] to level as seen in control group (Fig. 4A).

Severity of arrhythmias during the first 5 min of coronary artery reperfusion was significantly improved by noradrenaline-induced preconditioning compared with control group (1 ± 0.5 versus 2.7 ± 0.2), and this protective effect of noradrenaline was attenuated with pretreatment by each prazosin, L-NAME, or tempol (2.7 ± 0.5, 2.6 ± 0.3, and 3 ± 0.1, respectively). Administration of prazosin alone, L-NAME alone, or tempol alone had no effect on arrhythmias during early reperfusion compared with control group (Fig. 4B).

Plasma CK-MB Activity

Plasma level of CK-MB was markedly declined by noradrenaline-induced preconditioning compared
with control group (1 ± 0.5 versus 2.7 ± 0.2), and this was returned to level as observed in control group by injection of each prazosin, L-NAME, or tempol prior to noradrenaline (2.7 ± 0.5, 2.6 ± 0.3, and 3 ± 0.1, respectively). Treatment with prazosin alone, L-NAME alone, or tempol alone did not change plasma level of CK-MB compared with control group (Fig. 5).

**DISCUSSION**

The present study indicates that the intravenous administration of noradrenaline in anesthetized rat reduced myocardial ischemia-reperfusion injuries by reduction in infarct size and ventricular arrhythmias. These cardioprotective effects of noradrenaline were inhibited when each of prazosin (an \( \alpha_1 \)-adrenoceptor blocker), L-NAME (as a nonspecific NOS inhibitor), or tempol (a membrane-permeable radical scavenger) was administrated prior to noradrenaline injection. Although it has been confirmed that endogenous NO was critical to the trigger of the late preconditioning, few studies are available about the involvement of endogenous NO in the area of pharmacologically-induced early preconditioning. Although it has been shown that treatment with NOS inhibitor blocked compared with ischemia-induced early preconditioning.
preconditioning effect of bradykinin and acetylcholine in the open-chest anesthetized dog and in the isolated rat heart, respectively [13, 14], other studies reported that cardioprotection effect of bradykinin [15] or acetylcholine [16] was blocked by NOS inhibitors in the anesthetized rats. In our study, noradrenaline apparently did not exert its protective effects when administered in the presence of the NO-synthase inhibitor L-NAME and, therefore, this finding is undoubtedly consistent with the hypothesis that NO generation is crucial to the effects of noradrenaline. Previously, it has been found that α1-adrenoceptor stimulation produces late preconditioning through NOS in mouse heart [17], and activation of this receptor can release NO during ischemia in the non-preconditioned canine heart [18]. In the present study, treatment with L-NAME alone did not change infarct size or ventricular arrhythmias in the rat heart. Previously, it has also been indicated that treatment with L-NAME alone had no effect on ischemia/reperfusion-induced apoptotic death in the cultured rat myocytes [19] and infarct size in the rat heart in vivo [15, 20]. Therefore, it seems that L-NAME did not induce deleterious effects of its own that would cancel any benefits due to noradrenaline.

It is generally believed that the generation of ROS contributes to ischemia and reperfusion injuries in the myocardium, and attenuation of ROS generation has a beneficial effect on reduction of these injuries [21]. On the other hand, it is noted that ROS are essential in the eliciting of preconditioning [22]. Tempol is an intracellular free radical scavenger with low molecular weight, which easily permeates biological membranes and accumulates in cytosol [23]. Although it has been reported that treatment with tempol before the onset of reperfusion and throughout the reperfusion period can attenuate infarct size in the rat heart [24], we showed that bolus injection of tempol alone prior to the ischemia period had no effect on ischemia/reperfusion-induced injuries. It has been also shown that pharmacologic preconditioning by angiotensin II was eliminated by tempol [25]. In this study, treatment of the hearts with tempol prior to noradrenaline injection significantly abolished cardioprotective effect of noradrenaline and restored myocardial injuries (infarct size and ischemia/reperfusion-induced ventricular arrhythmias) to level as seen in control rats. Thus, this result indicates that generation of ROS is involved in the noradrenaline-induced early preconditioning in the rat heart in vivo. This is in agreement with the study in which protective effect of phenylephrine (as an α1-adrenoceptor agonist) was blocked by a free radical scavenger in the isolated rabbit heart [7].

The electrophysiologic changes due to ischemia or reperfusion in the myocardium were not explored in this study. Since triggered activity and reentry are thought to be involved in arrhythmogenesis [26], it seems that stimulation of α1-adrenoceptor leads to inhibition of reentry or triggered activity throughout ischemia or reperfusion periods, and administration of L-NAME or tempol eliminates these antiarrhythmic properties of noradrenaline.

The extent of the ischemic zone is an important factor in the arrhythmogenesis [27]. However, this factor was excluded in our study, and the extent of the ischemic zone (AAR) was not different among the experimental groups.

In an early study, we concluded the contribution of mitochondrial K+ATP channels to the protection of noradrenaline-induced preconditioning [28]. On the other hand, it has been proposed that NO can open the mitochondrial K+ATP channel [29], and opening of this channel triggers the preconditioning by generating ROS [4]. Therefore, stimulation of α1-adrenoceptor by noradrenaline could possibly activate mitochondrial K+ATP channel via endogenous release of NO and then generate ROS from mitochondria.

In summary, we show that stimulation of α1-adrenoceptor by administration of noradrenaline via endogenous release of NO and generation of ROS reduces ischemia/reperfusion injuries in the rat heart in vivo.

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