Ischemic Preconditioning in the Intact Rat Heart Is Mediated by $\delta_1$- But Not $\mu$- or $\kappa$-Opioid Receptors

Jo El J. Schultz, PhD; Anna K. Hsu, BS; Garrett J. Gross, PhD

**Background**—Our laboratory has previously shown that $\delta$-opioid receptors are involved in the cardioprotective effect of ischemic preconditioning in the rat heart. However, this class of receptors consists of two subtypes, $\delta_1$ and $\delta_2$, and $\mu$- or $\kappa$-opioid receptors may also exist in the heart. Therefore, the purpose of the present study was to test the hypothesis that ischemic preconditioning is mediated through stimulation of one or both $\delta$-opioid receptor subtypes.

**Methods and Results**—Anesthetized, open chest, male Wistar rats were assigned to 1 of 14 groups. All animals were subjected to 30 minutes of occlusion and 2 hours of reperfusion. Ischemic preconditioning was elicited by three 5-minute occlusion periods interspersed with 5 minutes of reperfusion. Two doses of 7-benzylidenenaltrexone (BNTX; 1 and 3 mg/kg IV), a selective $\delta_1$-opioid receptor antagonist, or naltriben (NTB; 1 and 3 mg/kg IV), a selective $\delta_2$-opioid receptor antagonist, were given before ischemic preconditioning. To test for a role of $\mu$-opioid receptors, rats were pretreated with $\beta$-funtaltrixamine ($\beta$-FNA; 15 mg/kg SC), an irreversible $\mu$-opioid receptor antagonist, 24 hours before ischemic preconditioning or given the $\mu$-opioid receptor agonist D-Ala$^2$N-Me-Phe$^4$glycerol$^5$-enkephalin (DAMGO) as three 5-minute infusions (1, 10, and 100 $\mu$g/kg per infusion IV, respectively) interspersed with 5-minute drug-free periods before the prolonged ischemic and reperfusion periods (lowDAMGO, medDAMGO, and hiDAMGO, respectively). The involvement of $\kappa$-opioid receptors was tested by administering one of two doses of nor-binaltorphimine (nor-BNI; 1 and 5 mg/kg IV) before ischemic preconditioning. Infarct size (IS) as a percent of the area at risk (AAR) was measured by triphenyltetrazolium stain. Ischemic preconditioning markedly reduced IS/AAR (14% ± 6%; $P<.05$) compared with control (55% ± 4%). NTB, $\beta$-FNA, and nor-BNI were unable to block the cardioprotective effect of ischemic preconditioning. In addition, DAMGO had no effect on IS/AAR. However, the high dose of BNTX (3 mg/kg IV) significantly attenuated the cardioprotective effect of ischemic preconditioning (39% ± 5%; $P<.05$ versus control and ischemic preconditioning).

**Conclusions**—These results indicate that $\delta_1$-opioid receptors play an important role in the cardioprotective effect of ischemic preconditioning in the rat heart. (Circulation. 1998;97:1282-1289.)

**Key Words:** receptors ■ ischemia ■ myocardial infarction ■ signal transduction ■ heart diseases

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The idea of multiple opioid receptors is an accepted concept, and a number of subtypes for each class of opioid receptors has been identified.1–4 Through biochemical and pharmacological methods, the $\mu$-, $\delta$-, and $\kappa$-opioid receptors have been characterized.5,3 Pharmacologically, it is well known that $\delta$-opioid receptors consist of two subtypes, $\delta_1$ and $\delta_2$.6–8

Opioid receptor activation has been implicated to elicit a protective effect during situations of stress produced by hypoxia, ischemia, cold, or acidic environments.5–9,20 and the $\delta$-opioid receptor has been demonstrated to play a major role in this protection.5,15–18 Mayfield and D’Aleyc4,15 showed, using DPDPE (selective $\delta_1$-opioid receptor agonist) and BNTX (selective $\delta_2$-opioid receptor antagonist), that the $\delta_1$-opioid receptor mediated the adaption or increased survival time of mice to hypoxic environments. Furthermore, Chien et al13 demonstrated that the time before organ transplantation was increased significantly from a 6-hour window to a 48-hour window after the administration of a synthetic $\delta$-opioid receptor agonist, DADLE. Recently, our laboratory showed that $\delta$-opioid receptors were involved in the cardioprotective effect of ischemic preconditioning (PC) in the intact rat heart.18 We demonstrated that administration of naltrindole, a nonselective $\delta$-opioid receptor antagonist, attenuated the cardioprotective effect of ischemic PC and morphine.18 However, the role of the specific $\delta$-opioid receptor subtype ($\delta_1$ and $\delta_2$) as well as a role for $\mu$- and $\kappa$-opioid receptors in the cardioprotective effect of ischemic PC remains unknown. Therefore, the focus of the present study was to determine the role of these four opioid receptor subtypes in mediating the cardioprotective effect of ischemic PC in the intact rat heart.

**Methods**

This study was performed in accordance with the guidelines of the Animal Care Committee of the Medical College of Wisconsin, which
is accredited by the American Association of Laboratory Animal Care.

General Surgical Preparation
Male Wistar rats weighing 350 to 450 g were used. The rats were anesthetized by intraperitoneal administration with the long-acting thiobutabarbital inactin (100 mg/kg IV). A tracheotomy was performed and the rat was intubated with a cannula connected to a rodent ventilator (model 683, Harvard Apparatus) and ventilated with room air at 65 to 70 breaths/min. Atelectasis was prevented by maintaining a positive end-expiratory pressure of 5 to 10 mm of H2O. Body temperature was monitored (Yellow Springs Instruments, Tele-Thermometer) and maintained at 37 ± 1°C (mean ± SEM) by use of a heating pad.

The right carotid artery was cannulated to measure blood pressure and heart rate with a Gould PE50 or Gould PE23 pressure transducer, which was connected to a Grass (model 7) polygraph. The right jugular vein was connected to infuse saline or drugs. A left thoracotomy was maintained within a normal physiological range (pH 7.35 to 7.45; pCO2 35 to 40 mm Hg; pO2 80 to 110 mm Hg) by adjusting the respiratory rate and/or tidal volume (2 to 4 mL/100 g). Body temperature was monitored (Yellow Springs Instruments, Tele-Thermometer) and maintained at 37 ± 1°C (mean ± SEM) by use of a heating pad.

Drugs
Inactin, (-)-trans-(1S,2S)-U-50488H, nor-binaltorphimine (nor-BNI) and [D-Pen2, D-Pen5]-enkephalin (DPDPE) were purchased from Research Biochemicals International. 7-benzylidenenaltrexone (BNTX), naltriben (NTB), and β-funaltrexamine (β-FNA) were generously donated as gifts from Dr Hiroshi Nagase, Toray Industries, Inc, Kanagawa, Japan. D-Ala2,N-Me-Phe2,glycerol2- enkephalin (DAMGO) was purchased from Bachem Bioscience, Inc. 2,3,5-triphenyltetrazolium chloride (TTC) was purchased from Sigma Chemical Co. Inactin, NTB, and DAMGO were dissolved in 0.9% saline. BNTX and DPDPE were dissolved in distilled water and brought up to volume with saline. β-FNA, U-50488H, and nor-BNI were dissolved in distilled water. TTC was dissolved in a 100 mmol/L phosphate buffer.

Study Groups and Experimental Protocols
All protocols contained control (group I) and three 5-minute ischemic PC (group II) groups. The control group was subjected to 30 minutes of occlusion and 2 hours of reperfusion. Ischemic PC was elicited by three 5-minute occlusion periods interspersed with 5 minutes of reperfusion after the prolonged occlusion and reperfusion periods. Fig 1 represents the experimental protocol designed to demonstrate the specific δ1 (δ1) opioid receptor involved in the cardioprotective effect of ischemic PC. In group II, NTB (1 mg/kg IV) was given 10 minutes before the long occlusion period in nonpreconditioned animals. Groups IV and V showed a dose-response effect of BNTX to antagonize ischemic PC (1 and 3 mg/kg IV; lowBNTX + PC, BNTX (1 mg/kg IV) given 10 minutes before ischemic PC; VIII, hNTB + PC, BNTX (3 mg/kg IV) given 10 minutes before ischemic PC; VI, NTB, naltriben (1 mg/kg IV), a δ2-opioid receptor antagonist, given 10 minutes before the 30 minutes of occlusion; VII, lowNTB + PC, NTB (1 mg/kg IV) given 10 minutes before ischemic PC; and VIII, hNTB + PC, naltriben (3 mg/kg IV) infused for 50 minutes before ischemic PC.

Because μ- and κ-opioid receptors have been implicated in many cardiovascular physiological and pathophysiological responses, several pharmacological antagonists and one agonist were used to determine if these two opioid receptors were involved in ischemic PC in the intact rat heart (Fig 2). In group IX, animals were pretreated 24 hours before ischemic PC with β-funaltrexamine (β-FNA; 15 mg/kg SC), an irreversible μ-opioid receptor antagonist (β-FNA + PC). To test if the μ-opioid receptor mimicked ischemic PC, groups X through XII consisted of three 5-minute infusions (1, 10, and 100 μg/kg per infusion IV, respectively) of DAMGO, a selective μ-opioid receptor agonist, interspersed with 5-minute drug-free periods before the prolonged ischemic and reperfusion periods (lowDAMGO, medDAMGO, hiDAMGO, respectively). Last, to test if κ-opioid receptors mediated the cardioprotective effect of ischemic PC, a dose-response effect of nor-binaltorphimine (nor-BNI), a κ-opioid receptor antagonist, was studied. In groups XIII (low nor-BNI + PC) and XIV (hi nor-BNI + PC), nor-BNI (1 and 5 mg/kg IV, respectively) was given 15 minutes before ischemic PC. In previous studies, we have shown that saline or distilled water had no effect on infarct size in rat hearts.

Specificity of the Opioid Receptor Agonist and Antagonists
To demonstrate that the effect of the antagonists and agonists occurred at a specific opioid receptor, BNTX, and NTB, the δ1- and δ2-opioid receptor antagonists, respectively, β-FNA, the irreversible μ-opioid receptor antagonist, nor-BNI, the κ-opioid receptor antagonist,
DAMGO, the μ-opioid receptor agonist, DPDPE, the δ-opioid receptor agonist, and U-50488H, the κ-opioid receptor agonist, were used. DAMGO produced a transient hypotensive effect during infusion of the three doses (1, 10, and 100 μg/kg IV) studied. Therefore, animals that were pretreated with β-FNA (group 9 from Fig 2) were subjected to a dose response of DAMGO (3, 30, and 300 μg/kg IV) before ischemic PC to test the specificity of β-FNA for the μ-opioid receptor. Similarly, a dose response of DAMGO (3, 30, and 300 μg/kg IV) was studied with BNTX or NTB (groups III and VI from Fig 1) in which either δ-opioid receptor antagonist was given and then a DAMGO dose response was performed before the long occlusion period to demonstrate the lack of effect of BNTX and NTB for the μ-receptor and their specificity for the δ1-(BNTX) and δ2-(NTB) opioid receptor.

In addition, specificity of the δ1-, δ2-, and κ-antagonists to their respective opioid receptors was further demonstrated with a δ1- and κ-opioid receptor agonist, A dose response of DPDPE (1, 3, and 10 mg/kg IV), the δ1-opioid receptor agonist, was performed and a transient decrease in blood pressure was observed. BNTX (3 mg/kg IV), the δ2-opioid receptor antagonist, was given 10 minutes before the next DPDPE dose response. Similarly, a dose response of U-50488H (1, 5, and 10 mg/kg IV), the κ-opioid receptor agonist, was performed followed by the administration of nor-BNI (5 mg/kg IV), the κ-opioid receptor antagonist, 15 minutes before the next U-50488H dose response.

Pretreatment for 24 hours with β-FNA resulted in a complete blockade of the vascular response to DAMGO, whereas neither BNTX nor NTB had any effect on the response to DAMGO (data not shown). The decrease in blood pressure caused by DPDPE was antagonized by BNTX but not by NTB (data not shown). In addition, hypotensive responses to U-50488H were blocked by nor-BNI (data not shown). These data demonstrate that the doses of opioid antagonists used are specific for their respective receptors.

**Determination of Infarct Size**

After each experiment, the left coronary artery was reoccluded and Patent blue dye was injected into the venous catheter to stain the normally perfused region of the heart. The rat was euthanized with 15% KCl through the arterial catheter. The heart was excised and the left ventricle removed and sliced into five cross-sectional pieces. The heart was excised and the left ventricle removed and sliced into five cross-sectional pieces. TTC was used as an indicator to separate out viable and nonviable tissue. The tissue was stored overnight in a 10% formaldehyde solution. The following day, the infarcted tissue was separated from the AAR by use of the dissecting scope. The different regions (nonischemic, AAR, and infarct) were determined by gravimetry, and infarct size (IS) was calculated as a % of the AAR (IS/AAR).

**Exclusion Criteria**

A total of 107 animals were assigned to the present study. Animals were excluded from the study because of unacceptable blood gases, intractable ventricular fibrillation (VF), or hypotension (mean arterial blood pressure <30 mm Hg). Three animals in the control group, two in the ischemic PC group, two animals in the hDAMGO+PC group, one in the hiDAMGO group, and four animals in the β-FNA+PC group were excluded because of intractable ventricular fibrillation. In addition, three animals in the hiNTB+PC group and one animal in the low nor-BNI+PC group were excluded because of hypotension. A total of 91 animals completed the study.

**Statistical Analysis of the Data**

All values are expressed as mean±SEM. One-way ANOVA was used to determine differences among groups for IS and AAR. Differences between groups in hemodynamics at various time points were compared by use of two-way ANOVA for time and treatment with repeated measures and Fisher’s least significant difference test if significant F ratios were obtained. Statistical differences were considered significant if the probability value was <.05.

**Results**

**Hemodynamics**

Tables 1 and 2 summarize the mean±SEM values for the hemodynamic parameters of heart rate (HR), mean arterial blood pressure (MBP), and rate-pressure product (RPP) analyzed at baseline, 30 minutes of occlusion, and 2 hours of reperfusion. In Table 1, HR, MBP, and RPP at baseline were not significantly different among the groups. However, at 30 minutes of occlusion, the MBP in the lowBNTX+PC group was significantly higher compared with control, but by 2 hours of reperfusion, no differences in MBP were found between groups. In addition, RPP at 30 minutes of occlusion was significantly higher in the ischemic PC, BNTX, lowBNTX+PC, and hiBNTX+PC groups; however, the RPP at 2 hours of reperfusion was not significantly different in any...
TABLE 1. Hemodynamic Data Obtained in the Presence of Specific δ-Opioid Receptor Subtype Antagonists

<table>
<thead>
<tr>
<th>n</th>
<th>HR (beats/min)</th>
<th>MBP (mm Hg)</th>
<th>RPP (mm Hg/min per 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>375 ± 11</td>
<td>95 ± 14</td>
</tr>
<tr>
<td>Ischemic PC</td>
<td>6</td>
<td>375 ± 9</td>
<td>94 ± 10</td>
</tr>
<tr>
<td>BNTX</td>
<td>5</td>
<td>350 ± 3</td>
<td>81 ± 9</td>
</tr>
<tr>
<td>lowBNTX + Ischemic PC</td>
<td>6</td>
<td>360 ± 5</td>
<td>103 ± 9</td>
</tr>
<tr>
<td>hiBNTX + Ischemic PC</td>
<td>6</td>
<td>352 ± 12</td>
<td>85 ± 8</td>
</tr>
<tr>
<td>NTB</td>
<td>5</td>
<td>356 ± 16</td>
<td>92 ± 3</td>
</tr>
<tr>
<td>lowNTB + Ischemic PC</td>
<td>6</td>
<td>370 ± 8</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>hiNTB + Ischemic PC</td>
<td>6</td>
<td>350 ± 8</td>
<td>91 ± 4</td>
</tr>
<tr>
<td>Ischemic PC</td>
<td>6</td>
<td>375 ± 11</td>
<td>95 ± 14</td>
</tr>
<tr>
<td>30-Min Occlusion</td>
<td></td>
<td>368 ± 17</td>
<td>69 ± 6</td>
</tr>
<tr>
<td>2-Hour Reperfusion</td>
<td></td>
<td>458 ± 18</td>
<td>84 ± 11</td>
</tr>
</tbody>
</table>

HR indicates heart rate (beats/min); MBP, mean arterial blood pressure (mm Hg); RPP, rate-pressure product (mm Hg/min per 1000); Ischemic PC, three 5-minute ischemic periods; BNTX (3 mg/kg IV), a selective δ₁-opioid receptor antagonist, given 10 minutes before the 30-minute occlusion; lowBNTX + Ischemic PC, BNTX (1 mg/kg IV) given 10 minutes before ischemic PC; hiBNTX + Ischemic PC, BNTX (3 mg/kg IV) given 10 minutes before ischemic PC; NTB, naltriben (1 mg/kg IV), a selective δ₁-opioid receptor antagonist, given 10 minutes before the 30-minute occlusion; lowNTB + Ischemic PC, naltriben (1 mg/kg IV) given 10 minutes before ischemic PC; and hiNTB + Ischemic PC, naltriben (3 mg/kg IV) infused for 60 minutes before ischemic PC.

Values given as mean ± SEM; *P < .05 vs control.

of these groups. The HR in the NTB group was significantly lower at 2 hours of reperfusion, but there was no significant difference in MBP or RPP.

Table 2 shows that the baseline HR in the ischemic PC group was significantly lower than control; however, HR in this group was not significantly different from control at 30 minutes of occlusion or 2 hours of reperfusion. The hi-DAMGO group had a significantly lower HR compared with control at 2 hours of reperfusion. The medDAMGO and β-FNA + PC groups had a significantly lower MBP at 30 minutes of occlusion and 2 hours of reperfusion. In addition, MBP at 2 hours of reperfusion was significantly lower in the hiDAMGO and low nor-BNI + PC groups. RPP at 30 minutes of occlusion was significantly lower in the medDAMGO and β-FNA + PC groups. However, there were no significant differences in RPP among the groups at 2 hours of reperfusion.

TABLE 2. Hemodynamic Data Obtained in the Presence of μ- and κ-Opioid Receptor Agonists or Antagonists

<table>
<thead>
<tr>
<th>n</th>
<th>HR (beats/min)</th>
<th>MBP (mm Hg)</th>
<th>RPP (mm Hg/min per 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>378 ± 13</td>
<td>90 ± 7</td>
</tr>
<tr>
<td>Ischemic PC</td>
<td>6</td>
<td>343 ± 11</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>30-Min Occlusion</td>
<td></td>
<td>378 ± 15</td>
<td>80 ± 7</td>
</tr>
<tr>
<td>2-Hour Reperfusion</td>
<td></td>
<td>453 ± 21</td>
<td>85 ± 7</td>
</tr>
<tr>
<td>β-FNA + PC</td>
<td>6</td>
<td>364 ± 17</td>
<td>75 ± 8</td>
</tr>
<tr>
<td>lowDAMGO</td>
<td>5</td>
<td>366 ± 13</td>
<td>81 ± 7</td>
</tr>
<tr>
<td>medDAMGO</td>
<td>6</td>
<td>392 ± 14</td>
<td>89 ± 5</td>
</tr>
<tr>
<td>hiDAMGO</td>
<td>7</td>
<td>350 ± 8</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>lownorBNI + PC</td>
<td>6</td>
<td>372 ± 6</td>
<td>78 ± 3</td>
</tr>
<tr>
<td>hinarBNI + PC</td>
<td>6</td>
<td>378 ± 10</td>
<td>80 ± 5</td>
</tr>
</tbody>
</table>

HR indicates heart rate (beats/min); MBP, mean arterial blood pressure (mm Hg); RPP, rate-pressure product (mm Hg/min per 1000); Ischemic PC, three 5-minute ischemic periods; β-FNA, β-funaltrexamine (15 mg/kg SC, 24-hour pretreatment), irreversible μ-opioid receptor antagonist given before ischemic preconditioning (PC); lowDAMGO, three 5-minute DAMGO infusions (1 μg/kg per infusion), μ-opioid receptor antagonist; medDAMGO, three 5-minute DAMGO infusions (10 μg/kg per infusion), μ-opioid receptor antagonist; hiDAMGO, three 5-minute DAMGO infusions (100 μg/kg per infusion), μ-opioid receptor antagonist; lownorBNI, nor-binaltorphine (1 mg/kg IV) given 15 minutes before ischemic PC; and hinarBNI, nor-binaltorphine (5 mg/kg IV) given 15 minutes before ischemic PC (3 × 100 μg/kg per 5-minute infusion).

Values given as mean ± SEM; *P < .05 vs control.

Infarct Size and Area at Risk

δ₂- and δ₁-Opioid Receptors

Left ventricular (LV) weight, AAR, and IS weights are shown in Table 3. The LV and AAR weights were not significantly different between groups. Infarct size was significantly smaller in the ischemic PC, lowBNTX + PC, and both NTB + PC groups. The IS/AAR data for the individual rat hearts are depicted in Fig 3 as well as the mean ± SEM for each group. The control group had an average IS/AAR of 53.2 ± 2.9%. Ischemic PC markedly reduced infarct size to 14.1 ± 5.1% (P < .05 versus control). The low dose of the selective δ₁-receptor antagonist BNTX (1 mg/kg IV) did not attenuate the effect of ischemic PC (19.0 ± 3.4%; P < .05), whereas the high dose of BNTX (3 mg/kg IV) significantly attenuated the cardioprotective effect of ischemic PC (38.7 ± 5.4%; P < .05 versus control and ischemic PC). Similarly, a dose response to NTB, a δ₁-opioid receptor antagonist, was performed. Neither dose (1 and 3 mg/kg IV) of NTB inhibited the response to ischemic PC.
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The results shown in Table 4 indicate that there were no significant differences in LV and AAR weights between the groups. Also, the results in Table 4 demonstrate that the IS weights in ischemic PC, β-FNA+PC–treated animals, and low and hi nor-BNI+PC groups were significantly lower compared with control. In addition, the results in Fig 4 show the infarct sizes of the individual rat hearts and the mean±SEM for each group. The average IS/AAR in the control group was 54.7±3.7%. Ischemic PC significantly reduced IS/AAR (12.0±3.2%; P<.05 versus control). Twenty-four–hour pretreatment (15 mg/kg SC) with β-FNA, an irreversible μ-opioid receptor antagonist, did not abolish the cardioprotective effect of ischemic PC (8.0±1.7%; P<.05 versus control). Furthermore, three doses (1, 10, and 100 μg/kg per 5-minute infusion IV) of DAMGO, a selective μ-opioid receptor agonist, did not mimic the cardioprotective effect of PC (53.9±4.3%, 52.9±4.7%, and 52.0±8.1%, respectively). Finally, two doses (1 and 5 mg/kg IV) of nor-BNI, a κ-opioid receptor antagonist, given 15 minutes before ischemic PC did not block its protective effect (20.2±5.1% and 20.2±2.5%, respectively).

Discussion

The class of δ-opioid receptors consists of two subtypes, δ1 and δ2.26–28 There are a number of pharmacological agents available to distinguish these two subtypes of δ-opioid receptor.2 BNTX, a nonpeptidic δ1-opioid receptor antagonist, and NTB, a nonpeptidic δ2-opioid receptor antagonist, were used in the present study to clarify the role of these two subtypes to mediate the cardioprotective effect of ischemic PC. A number of studies have demonstrated the selectivity and specificity of BNTX and NTB toward its respective opioid receptor.26–28 Dose responses of both BNTX and NTB were performed in the present study, and the results demonstrate that the high dose (3 mg/kg IV) of BNTX but not the low dose (1 mg/kg IV) partially abolished the protective effect of ischemic PC. Conversely, neither dose of NTB (1 or 3 mg/kg IV) blocked ischemic PC. Our results demonstrating that BNTX at the low dose (1 mg/kg) did not block the cardioprotective effect of ischemic PC confirm the results obtained by Mayfield and D’Alecy.15 This group observed that 1 mg/kg of NTB did not decrease the hypoxic survival time of mice; however, at a higher dose of BNTX (10 mg/kg), the hypoxic survival time was significantly decreased.15 Similarly, we observed that the cardioprotective effect of ischemic PC in the rat was blocked at a higher dose of BNTX (3 mg/kg). BNTX (3 mg/kg IV) and NTB (1 mg/kg IV) in combination when given 10 minutes before ischemic PC did not have an additive effect to block the cardioprotective effect (data not shown). In fact, the IS/AAR observed when the combination of BNTX and NTB was given was no larger than the IS/AAR observed when BNTX alone was given before ischemic PC. This observation further suggests that ischemic PC in the rat heart is predominantly mediated by the δ1-opioid

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**Table 3. Infarct Size Data in the Presence of BNTX and NTB, Specific δ-Opioid Receptor Subtype Antagonists**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>LV</th>
<th>AAR</th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>0.847±0.073</td>
<td>0.408±0.064</td>
<td>0.221±0.039</td>
</tr>
<tr>
<td>Ischemic PC</td>
<td>6</td>
<td>0.777±0.053</td>
<td>0.409±0.035</td>
<td>0.056±0.019*</td>
</tr>
<tr>
<td>BNTX</td>
<td>5</td>
<td>0.791±0.030</td>
<td>0.396±0.041</td>
<td>0.261±0.048</td>
</tr>
<tr>
<td>lowBNTX+Ischemic PC</td>
<td>6</td>
<td>0.726±0.052</td>
<td>0.324±0.061</td>
<td>0.063±0.016*</td>
</tr>
<tr>
<td>hiBNTX+Ischemic PC</td>
<td>6</td>
<td>0.832±0.058</td>
<td>0.433±0.069</td>
<td>0.167±0.037</td>
</tr>
<tr>
<td>NTB</td>
<td>5</td>
<td>0.776±0.031</td>
<td>0.427±0.028</td>
<td>0.248±0.022</td>
</tr>
<tr>
<td>lowNTB+Ischemic PC</td>
<td>6</td>
<td>0.777±0.042</td>
<td>0.411±0.045</td>
<td>0.091±0.027*</td>
</tr>
<tr>
<td>hiNTB+Ischemic PC</td>
<td>6</td>
<td>0.710±0.058</td>
<td>0.315±0.022</td>
<td>0.057±0.009*</td>
</tr>
</tbody>
</table>

n indicates number of animals; LV, left ventricle in grams; AAR, area at risk in grams; and IS, infarct size in grams. Protocols are described in "Methods" and in footnote to Table 1.

Values given as mean±SEM. There were no significant differences among the groups for the LV and AAR sizes. IS in PC, lowBNTX+PC, low- and hiBNTX+PC hearts showed a significant difference compared with control (%P<.05 vs control).

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**Figure 3.** Infarct sizes in rat hearts subjected to control (CON); ischemic preconditioning (PC) elicited by three 5-minute occlusion periods interspersed with 5 minutes of reperfusion; BNTX (3 mg/kg IV), a selective δ1-opioid receptor antagonist, given 10 minutes before the 30 minutes of occlusion; lowBNTX+PC, BNTX (1 mg/kg IV) given 10 minutes before ischemic PC; hiBNTX+PC, BNTX (3 mg/kg IV) given 10 minutes before ischemic PC; NTB, naltriben (1 mg/kg IV), a δ2-opioid receptor antagonist, given 10 minutes before the 30 minutes of occlusion; lowNTB+PC, NTB (1 mg/kg IV) given 10 minutes before ischemic PC; and hiNTB+PC, naltriben (3 mg/kg IV) infused for 50 minutes before ischemic PC. ○, Infarct sizes from individual hearts; •, group mean infarct size; mean±SEM, with *%P<.05 vs control and #%P<.05 vs control and ischemic PC.
The overall data demonstrate that the δ-opioid receptor is the important δ-opioid receptor subtype involved in the cardioprotective effect of ischemic PC in the rat. Further studies with selective δ-opioid receptor agonists are necessary to demonstrate that stimulating this δ-opioid receptor produces a cardioprotective effect to reduce infarct size in the rat heart.

Involvement of μ- and κ-opioid receptors were studied with the use of the μ-receptor agonist DAMGO, the irreversible μ-receptor antagonist β-FNA, and the κ-receptor antagonist nor-BNI. Many studies have provided evidence that DAMGO and β-FNA are selective for the μ-opioid receptor, and nor-BNI is selective for κ-opioid receptors. We demonstrated that β-FNA (15 mg/kg SC) when administered 24 hours preceding ischemic PC did not block its cardioprotective effect. Similarly, DAMGO at any of the doses studied did not mimic the cardioprotection induced by brief periods of ischemia. Previously, we showed that morphine reduced infarct size and elicited a cardioprotective effect in the rat heart, indicating that this cardioprotection may involve μ-opioid receptors. However, our laboratory recently showed that naltrindole, a nonselective δ-opioid receptor antagonist, abolished the cardioprotective effect of morphine, demonstrating that its protection is mediated by a δ-opioid receptor. It is known that morphine has high affinity for the μ-opioid receptor; however, morphine has also been shown to interact with the δ- and κ-opioid receptors. In addition, a number of investigators have demonstrated that cross-talk can occur between μ- and δ-opioid receptors. Therefore, we used a more selective μ-opioid receptor agonist, DAMGO, to further demonstrate and clarify the role, if any, of μ-opioid receptors in the cardioprotective effect of ischemic PC in the rat. The present results clearly suggest that the μ-opioid receptor does not mediate ischemic PC. The lack of μ-opioid receptor activity in the cardioprotective effect of ischemic PC has also been supported by a number of functional and receptor binding studies in ventricular myocytes, indicating an absence of this particular opioid receptor in this tissue. In addition, the hypoxic conditioning study by Mayfield and D’Alecy showed that β-FNA (48-hour pretreatment with 1 to 20 mg/kg SC) did not decrease hypoxic survival time in mice, indicating that the μ-opioid receptor was not involved.

The role of κ-opioid receptors in the protective effect of ischemic PC was tested by the use of nor-BNI, a selective κ-opioid receptor antagonist. Two doses of nor-BNI (1 and 5 mg/kg IV) were tested. Neither dose of nor-BNI abolished ischemic PC, suggesting that κ-opioid receptors are not involved in cardioprotection in the rat. As with the BNTX and NTB combination, there was no additive effect to block ischemic PC in the rat when BNTX (3 mg/kg IV) and nor-BNI (1 mg/kg IV) were given together (data not shown). In support of the lack of involvement of the κ-opioid receptor in ischemic PC, Xia et al demonstrated that antiarrhythmic effect of ischemic PC in the isolated rat heart may be due to a decreased affinity of κ-opioid

### Table 4. Infarct Sizes in Rat Hearts Treated With μ- or κ-Opioid Receptor Agonists and Antagonists

<table>
<thead>
<tr>
<th>Group</th>
<th>LV (g) ± SEM</th>
<th>AAR (g) ± SEM</th>
<th>IS (g) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.842±0.056</td>
<td>0.377±0.048</td>
<td>0.208±0.034</td>
</tr>
<tr>
<td>Ischemic PC</td>
<td>0.755±0.043</td>
<td>0.380±0.035</td>
<td>0.204±0.017*</td>
</tr>
<tr>
<td>β-FNA + PC</td>
<td>0.785±0.045</td>
<td>0.462±0.045</td>
<td>0.040±0.11*</td>
</tr>
<tr>
<td>lowDAMGO</td>
<td>0.827±0.051</td>
<td>0.476±0.051</td>
<td>0.265±0.046</td>
</tr>
<tr>
<td>medDAMGO</td>
<td>0.831±0.057</td>
<td>0.456±0.034</td>
<td>0.245±0.034</td>
</tr>
<tr>
<td>hiDAMGO</td>
<td>0.807±0.046</td>
<td>0.409±0.036</td>
<td>0.220±0.047</td>
</tr>
<tr>
<td>lownorBNI + PC</td>
<td>0.819±0.040</td>
<td>0.430±0.023</td>
<td>0.086±0.21*</td>
</tr>
<tr>
<td>hinorBNI + PC</td>
<td>0.782±0.042</td>
<td>0.380±0.040</td>
<td>0.076±0.011*</td>
</tr>
</tbody>
</table>

n indicates number of animals; LV, left ventricle in grams; AAR, area at risk in grams; and IS, infarct size in grams. Protocols are described in "Methods" and in footnote to Table 2.

Values given as mean±SEM. There were no significant differences among the groups for the LV and AAR sizes. IS in PC, β-FNA, low- and hinorBNI hearts showed a significant difference (*P<.05 vs control).
receptor binding by U69593, a highly selective κ-agonist, during reperfusion. Also, Mayfield and D’Alecy were unable to decrease hypoxic survival time of mice with nor-BNI (1 to 20 mg/kg SC) when animals were subjected to hypoxic preconditioning. Furthermore, Niroomand et al indicated that κ-opioid receptors may not be present on canine cardiac sarcolemma because the κ-agonist U50488H did not inhibit adenylate cyclase activity.

In summary, our results indicate that the beneficial effect of brief periods of ischemia are partially mediated by the δ-opioid receptor. BNTX, the δ-opioid receptor antagonist, attenuated the cardioprotection, whereas NTB, the δ-opioid receptor antagonist, did not inhibit ischemic PC. Neither μ- nor κ-opioid receptors seem to be involved in eliciting cardioprotection in the rat heart because antagonists to these two receptors did not prevent PC and DAMGO, a selective μ-opioid receptor agonist, did not mimic ischemic PC. Also, combinations of the δ2- or κ-antagonist with BNTX did not produce an additive inhibition of ischemic PC in comparison to the results with BNTX alone, suggesting that ischemic PC in the rat occurs primarily through activation of δ-opioid receptors.

The results of this study suggest that there may be significant clinical potential for stimulating δ-opioid receptors with regard to treating cardiac ischemia in patients with coronary artery disease; however, more studies need to be performed to demonstrate a universal role for δ-opioid receptors in ischemic PC in all species. Opioids have been used clinically to manage pain after surgery. The demonstration that opioid receptors, most notably δ1, which not only have analgesic properties but may have the potential to protect the myocardium during cardiac surgical interventions, suggests a possible new pharmacological approach for the treatment of patients with acute myocardial infarction.

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