Effects of Naloxone on Myocardial Ischemic Preconditioning in Humans

Fabrizio Tomai, MD, FACC,* Filippo Crea, MD, FACC,† Achille Gaspardone, MD, FACC,* Francesco Versaci, MD, FACC,* Anna S. Ghini, MD,* Claudio Ferri, MD,‡ Giovambattista Desideri, MD,‡ Luigi Chiarriello, MD, FACC,* Pier A. Gioffré, MD*

Rome, Italy

OBJECTIVES

We attempted to establish whether naloxone, an opioid receptor antagonist, abolishes the adaptation to ischemia observed in humans during coronary angioplasty after repeated balloon inflations.

BACKGROUND

Experimental studies indicate that myocardial opioid receptors are involved in ischemic preconditioning.

METHODS

Twenty patients undergoing angioplasty for an isolated stenosis of a major epicardial coronary artery were randomized to receive intravenous infusion of naloxone or placebo during the procedure. Intracoronary electrocardiogram and cardiac pain (using a 100-mm visual analog scale) were determined at the end of the first two balloon inflations. Average peak velocity in the contralateral coronary artery during balloon occlusion, an index of collateral recruitment, was also assessed by using a Doppler guide wire in the six patients of each group with a stenosis on the left anterior descending coronary artery.

RESULTS

In naloxone-treated patients, ST-segment changes and cardiac pain severity during the second inflation were similar to those observed during the first inflation (12 ± 6 vs. 11 ± 7 mm, p = 0.3, and 58 ± 13 vs. 56 ± 12 mm, p = 0.3, respectively), whereas in placebo-treated patients, they were significantly less (6 ± 3 vs. 13 ± 6 mm, p = 0.002 and 31 ± 21 vs. 55 ± 22 mm, p = 0.008, respectively). In both naloxone- and placebo-treated patients, average peak velocity significantly increased from baseline to the end of the first inflation (p = 0.04 and p = 0.02, respectively), but it did not show any further increase during the second inflation.

CONCLUSIONS

The adaptation to ischemia observed in humans after two sequential coronary balloon inflations is abolished by naloxone and is independent of collateral recruitment. Thus, it is due to ischemic preconditioning and is, at least partially, mediated by opioid receptors, suggesting their presence in the human heart. (J Am Coll Cardiol 1999;33:1863–9) © 1999 by the American College of Cardiology

Ischemic preconditioning, a powerful form of protection against myocardial infarction (1), has been shown in several animal species (1–4) and, more recently, in humans (5–10). Several experimental studies have shown that preconditioning results from a complex series of events, involving various G protein-coupled receptors (11,12). The stimulation of these receptors would result in the activation of protein kinase C (PKC). This, in turn, leads to the translocation of PKC from the cytoplasm to the sarcolemma, where it phosphorylates a substrate protein (possibly the adenosine triphosphate–sensitive K+ [K_ATP] channel), which confers resistance to ischemia (11,12). Recent studies have confirmed that A1-adenosine receptors (13,14), alpha-adrenergic receptors (15) and K_ATP channels (16) play an important role in mediating preconditioning also in humans.

The activation of opioid receptors has been shown to trigger ischemic preconditioning in experimental models (17–22). Indeed, their stimulation mimics and their blockade abolishes ischemic preconditioning in rat (17,18,20–22) and rabbit (19) hearts. Of note, the mu- and delta-opioid receptors are coupled to G proteins (23–25) and can activate PKC in chicken neuron cultures (26) and K_ATP channels in pig cerebral arteries (27) and in the rat heart (18). To establish the role played by opioid receptors in preconditioning in humans, we assessed the effects of naloxone (a nonselective opioid receptor antagonist) (28) in patients undergoing repeated coronary occlusions in the setting of elective angioplasty of an isolated stenosis of a major
Abbreviations and Acronyms

<table>
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<th>Abbreviation</th>
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<tr>
<td>ECG</td>
<td>electrocardiogram</td>
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<tr>
<td>$K_{ATP}$</td>
<td>adenosine triphosphate–sensitive K⁺ channel</td>
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<td>PKC</td>
<td>protein kinase C</td>
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epicardial coronary artery. Because collateral recruitment can occur during coronary angioplasty (29,30), changes in blood flow velocity in the contralateral coronary artery during balloon occlusion (an accepted index of collateral recruitment) (31–33), were also measured by using an intracoronary Doppler guide wire.

METHODS

Patients. We studied 20 consecutive patients (17 men and 3 women; age range, 48 to 70 years; mean age, 60 years) who underwent successful uncomplicated elective coronary angioplasty for an isolated obstructive lesion (internal diameter reduction of 50% to 90% on the basis of the use of the quantitative cardiovascular software program ACA, Philips, DCI, Best, The Netherlands.) (34) in the proximal two thirds of a major epicardial coronary artery. Patients with stenoses >90% were not included in the study, to avoid “preinflation ischemia” due to obstruction from the guide wire across the lesion, which would prolong the ischemic time of the first inflation compared with the second (35). All patients fulfilled the entry criteria of: 1) history of chronic stable angina pectoris lasting ≥3 months; 2) no history of previous myocardial infarction nor pathologic Q waves on the electrocardiogram (ECG); 3) no angiographic evidence of coronary collateral vessels (grade 0, according to Rentrop’s classification) (29), and 4) right dominant coronary circulation. No patient had evidence of left ventricular hypertrophy or of wall motion abnormalities on the echocardiogram or conduction defects on the ECG that could have interfered with the interpretation of ST-segment changes. All patients had normal hepatic and renal function and fasting blood glucose levels. All patients gave written informed consent for participation in the study, which was approved by the Institutional Ethics Committee.

Study protocol. In this single-blind study, which was performed within five days of the diagnostic coronary angiography, patients were randomly allocated to two groups. One group consisted of 10 patients (nine men and one woman; age range, 48 to 68 years; mean age, 62 years) who received an intravenous infusion of naloxone (naloxone hydrochloride 0.4 mg/ml; DuPont Pharmaceuticals, Wilmington, Delaware). Naloxone was administered as a loading dose (6-mg bolus over 5 min), followed by an intravenous infusion at a rate of 0.1 mg/min. The infusion was started 15 min before coronary angioplasty and was stopped at the end of the second inflation. Naloxone was adminis-tered at a dose previously shown to increase beta-endorphin plasma levels, which indicates effective opiate receptor blockade (36–38). The other group consisted of 10 patients (8 men and 2 women; age range, 53 to 70 years; mean age, 59 years) who received an intravenous infusion of placebo (6 ml of 0.9% NaCl as bolus over 5 min, followed by an intravenous infusion at a rate of 1 ml/min), started 15 min before coronary angioplasty and stopped at the end of the second inflation. Beta-adrenergic blocking agents were withdrawn five days before the study. All patients were on oral aspirin (100 mg o.d.), diltiazem (60 mg t.i.d.) and isosorbide dinitrate (40 mg b.i.d.) for ≥48 h before coronary angioplasty. All patients received the morning dose of treatment before coronary angioplasty, which was performed within the next 4 h. No patient had angina in the last 24 h before the study. No patient received sublingual or intravenous nitrates in the last 24 h before the study or throughout the study. Patients were not premedicated with diazepam or other sedatives.

Coronary angioplasty of the stenosed artery was performed by a standard technique using the right femoral approach, as previously described (15,16). Briefly, after placement of the guiding catheter through a 8-F femoral sheath in the right femoral artery and performance of baseline angiography, the guide wire was placed across the lesion in the distal segment of the stenosed artery. The balloon catheter was then placed within the stenosis, and the balloon was inflated for 2 min. After balloon deflation and withdrawal proximal to the lesion, with the guide wire still across the lesion, a recovery period of ≥5 min was allowed to reestablish baseline hemodynamic and ECG conditions. A second balloon inflation for 2 min was then performed. In each individual patient balloon pressure during the first and second inflation was identical. After the first two inflations, coronary angioplasty was completed on the basis of the specific needs of individual patients.

Assessment of myocardial ischemia. Standard surface 12-lead and intracoronary ECGs derived from the angioplasty guide wire were continuously monitored and simultaneously recorded (Mingograph 7, Siemens, Solna, Sweden) at a paper speed of 25 mm/s throughout the study. The ECGs were analyzed by a cardiologist who had no knowledge of the study protocol. At baseline (with just the guide wire across the lesion) and at the end of the first two inflations, ST-segment shift was measured 80 ms after the J point. The severity of myocardial ischemia was expressed as: 1) the summation of the absolute values of the ST-segment elevation or ST-segment depression from baseline, on surface ECG, from all 12 leads; and 2) the absolute values of the ST-segment elevation or ST-segment depression from baseline on intracoronary ECG. ST-segment shifts were expressed in millimeters (1 mm = 0.1 mV).

Assessment of cardiac pain. At the beginning of each coronary angioplasty procedure, patients were informed that they might develop chest pain. At the end of the first two
balloon inflations, the intensity of cardiac pain was assessed by using a visual analog scale (39). Patients were asked to put a mark on a 100-mm scale marked from no symptoms (0) to severe symptoms (100). Time to pain onset (in seconds) was also assessed.

Assessment of coronary blood flow velocity. In the six patients of each group with a stenosis on the left anterior descending coronary artery, a 5-F femoral sheath was also inserted in the left femoral artery. A 5-F right Judkins femoral catheter was advanced through the left femoral sheath into the ostium of the right coronary artery for guidance of a 0.014-in. (0.036 cm) Doppler-tipped guide wire (FloWire, Cardiometrics, Mountain View, California). After heparinization (10,000 U IV) and placement of the angioplasty guiding catheter into the ostium of the left main coronary artery and before administration of naloxone or placebo infusion, a 0.014-in. Doppler-tipped intracoronary guide wire (FloWire and FloMap, Cardiometrics) was advanced through the 5-F right Judkins catheter into the medium tract of the right coronary artery and positioned until an optimal and stable Doppler signal, not in the proximity of a side branch, was obtained. Blood flow velocity was calculated as previously described (40,41). Average peak velocity in the contralateral artery was measured at baseline, before the first (15 min after naloxone or placebo infusion) and the second balloon inflations and at the end of the first two inflations. Collateral recruitment was expressed as the changes in average peak velocity in the contralateral coronary artery during the first and second balloon inflations.

Statistical analysis. Two-factor repeated measures analysis of variance with repeated measures on one factor was used to compare ischemic ECG and average peak velocity changes during balloon inflations in the two groups of patients. When significant differences were detected, pairwise comparisons were made using the Scheffé F test. Comparisons of the remaining continuous or discrete variables between the two groups were performed using an unpaired Student t or a chi-square test, respectively. Visual analog scales were analyzed using the Wilcoxon signed rank test or the Mann-Whitney U test as appropriate. Correlations between changes in average peak velocity from the first to the second inflation and changes in ST-segment shift were assessed by univariate linear regression analysis. Data are expressed as mean ± 1 SD; values of p < 0.05 were considered significant.

RESULTS

There was no significant difference in clinical, anatomic or hemodynamic features between the two groups (Table 1). In both naloxone- and placebo-treated patients, the values of systolic arterial pressure and heart rate were similar at baseline, 15 min after naloxone or placebo infusion (immediately before the first balloon inflation), before the second balloon inflation and at the end of the first two inflations (Table 1). During intravenous administration of naloxone as a bolus, two patients complained of transient nausea not associated with hemodynamic changes. No patient reported any symptom during placebo infusion.

Coronary angioplasty was successfully performed in all 20 patients (residual stenosis <30%) (Table 1). The mean balloon pressure and the recovery period between the two balloon inflations were similar in naloxone- and placebo-treated patients (5.6 ± 1.6 vs. 4.7 ± 1.8 atm, p = 0.3, and 361 ± 69 vs. 392 ± 61 s, p = 0.3, respectively).

Myocardial ischemia. The values of ST-segment shift from baseline at the end of the first two inflations are expressed as mean ± 1 SD; values of p < 0.05 were considered significant.

### Table 1. Clinical, Anatomic and Hemodynamic Features in the Two Groups of Patients

<table>
<thead>
<tr>
<th></th>
<th>Naloxone (n = 10)</th>
<th>Placebo (n = 10)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>62 ± 7</td>
<td>59 ± 6</td>
<td>0.3</td>
</tr>
<tr>
<td>Male/female (n)</td>
<td>9/1</td>
<td>8/2</td>
<td>1.0</td>
</tr>
<tr>
<td>Vessel disease (%)</td>
<td></td>
<td></td>
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<tr>
<td>LAD</td>
<td>14 ± 4</td>
<td>14 ± 4</td>
<td>1.0</td>
</tr>
<tr>
<td>LCx</td>
<td>11 ± 4</td>
<td>11 ± 4</td>
<td>1.0</td>
</tr>
<tr>
<td>RCA</td>
<td>11 ± 4</td>
<td>11 ± 4</td>
<td>1.0</td>
</tr>
<tr>
<td>Degree of stenosis (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before PTCA</td>
<td>86 ± 10</td>
<td>84 ± 11</td>
<td>0.8</td>
</tr>
<tr>
<td>After PTCA</td>
<td>18 ± 5*</td>
<td>16 ± 6*</td>
<td>0.4</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before infusion</td>
<td>69 ± 14</td>
<td>73 ± 12</td>
<td>0.5</td>
</tr>
<tr>
<td>15 min after inflation (baseline 1)</td>
<td>69 ± 10</td>
<td>74 ± 13</td>
<td>0.4</td>
</tr>
<tr>
<td>Inflation 1</td>
<td>69 ± 13</td>
<td>74 ± 10</td>
<td>0.3</td>
</tr>
<tr>
<td>Baseline values (baseline 2)</td>
<td>67 ± 12</td>
<td>71 ± 11</td>
<td>0.4</td>
</tr>
<tr>
<td>Inflation 2</td>
<td>68 ± 13</td>
<td>73 ± 9</td>
<td>0.3</td>
</tr>
<tr>
<td>Systolic arterial pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before infusion</td>
<td>136 ± 25</td>
<td>139 ± 32</td>
<td>0.8</td>
</tr>
<tr>
<td>15 min after inflation (baseline 1)</td>
<td>129 ± 26</td>
<td>136 ± 28</td>
<td>0.6</td>
</tr>
<tr>
<td>Inflation 1</td>
<td>128 ± 20</td>
<td>128 ± 20</td>
<td>1.0</td>
</tr>
<tr>
<td>Baseline values (baseline 2)</td>
<td>136 ± 22</td>
<td>135 ± 23</td>
<td>1.0</td>
</tr>
<tr>
<td>Inflation 2</td>
<td>125 ± 19</td>
<td>130 ± 21</td>
<td>0.6</td>
</tr>
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*p < 0.001 vs. stenosis before PTCA.
LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; PTCA = percutaneous transluminal coronary angioplasty; RCA = right coronary artery.
both the surface ECG (6 ± 3 vs. 11 ± 6 mm, p = 0.002) and the intracoronary ECG (6 ± 3 vs. 13 ± 6 mm, p = 0.002) (Fig. 1). The drug–inflation interaction for ST-segment changes on the surface and the intracoronary ECGs was highly significant (p = 0.004 and p < 0.001, respectively). There was no significant difference between the two groups of patients in the degree of ST-segment shift at the end of the first inflation on either surface (p = 0.5) or intracoronary ECG (p = 0.6) (Table 2). Finally, in both naloxone- and placebo-treated patients, changes in average peak velocity from the first to the second inflation did not correlate with those in ST-segment shift on surface (r = 0.12, p = 0.7, and r = 0.22, p = 0.6, respectively) or intracoronary ECG (r = 0.15, p = 0.7, and r = 0.11, p = 0.8, respectively).

Cardiac pain. In naloxone-treated patients, the severity of cardiac pain and time to pain onset at the end of the second inflation were similar to those at the end of the first inflation (58 ± 13 vs. 56 ± 12 mm, p = 0.3, and 48 ± 20 vs. 54 ± 20 s, p = 0.1, respectively). Conversely, in placebo-treated patients, the severity of cardiac pain and time to pain onset at the end of the second inflation were less and, respectively, longer than those at the end of the first inflation (31 ± 21 vs. 55 ± 22 mm, p = 0.008, and 65 ± 22 vs. 56 ± 31 s, p = 0.02, respectively) (Fig. 1). There was no significant difference between the two groups of patients in cardiac pain severity (p = 0.9) or time to pain onset (p = 0.9) at the end of the first inflation (Table 2).

Coronary blood flow velocity. The values of average peak velocity in the contralateral artery in the six patients of each group with a stenosis on the left anterior descending coronary artery are reported in Table 2.

In both naloxone- and placebo-treated patients, average peak velocity in the right coronary artery significantly increased from baseline to the end of the first inflation (from 22 ± 7 to 26 ± 6 cm/s, p = 0.04, and from 21 ± 6 to 25 ± 6 cm/s, p = 0.02, respectively), but it did not show a further increase during the second inflation (27 ± 6 to 26 ± 7 cm/s, respectively; p = 0.2 and p = 0.1, vs. the first inflation). There was no significant difference between the two groups of patients in average peak velocity at the end of the first (p = 0.7) and the second inflation (p = 0.7) (Table 2).

DISCUSSION

Several previous studies carried out in vitro on human myocardial tissue (5,6) and in patients undergoing coronary artery bypass surgery (9) have consistently demonstrated that ischemic preconditioning does occur in man. This concept is further strengthened by the observation that preinfarction angina is a powerful and independent predictor of better short- and medium-term prognosis after acute myocardial infarction (10). Our results, in agreement with those of previous studies based on the same model (7,8,13–16), confirm that ischemic preconditioning also occurs in humans during repeated balloon inflations in the setting of coronary angioplasty. More important, our study indicates
that opioid receptors are present in the human heart and that they play an important role in ischemic preconditioning. Indeed, the latter was prevented by pretreatment with naloxone, a powerful nonselective antagonist of opioid receptors (28).

The statistical power of the study in the assessment of ST-segment changes in naloxone-treated patients was sufficiently high (\(>90\%)\) for pairwise comparisons). Thus, although the number of patients was small, it is unlikely that significant changes in ST-segment shift during the first and second inflation were not detected.

Role of opioid receptors in ischemic preconditioning. Schultz et al. (17) were the first to demonstrate that opioid receptors are involved in the signaling pathway of ischemic preconditioning, using naloxone, the nonselective opioid receptor antagonist, in the intact rat heart. Subsequently, Chien and Van Winkle (19) have demonstrated that opioid receptors are also involved in ischemic preconditioning in the rabbit heart and that naloxone blockade of ischemic preconditioning-induced infarct size limitation is stereospecific and, therefore, is opioid receptor-mediated. More recently, Gross and his coworkers have also shown that ischemic preconditioning in the intact rat heart is mediated by delta\(_{1}\)- but not mu- or kappa-opioid receptors (22). In agreement with experimental studies (17–22), our results indicate that pretreatment with naloxone abolishes ischemic preconditioning in patients undergoing coronary angioplasty, a finding consistent with the concept that the endogenous opioid system mediates ischemic preconditioning also in humans. Our findings are supported by the early results by Bolli’s group (42), who found that pretreatment with morphine sulphate mimics ischemic preconditioning during coronary angioplasty.

As naloxone has been shown to cross the blood–brain barrier thus having the capability of antagonizing both central and peripheral opioid receptors (28,43), our study does not provide any direct evidence whether the prevention of preconditioning during coronary angioplasty was of a central or peripheral origin. However, it seems unlikely that our results are due to a central opioid receptor blockade by naloxone, because the infusion of naloxone did not change heart rate, blood pressure or coronary blood flow. Thus, it is likely that, in our study, naloxone prevented preconditioning during repeated coronary occlusions through the blockade of a peripheral opioid receptor mechanism. This is in agreement with a recent study by Gross and his coworkers (20), in which the involvement of central or peripheral opioid receptor pathways in ischemic preconditioning in the intact rat heart has been addressed. To this end, they compared the effect of naloxone to that of its quaternary derivative with a positively charged nitrogen, naloxone methiodide, which has been shown not to cross the blood–brain barrier (44,45). They found that naloxone methiodide like naloxone, abolished the infarct limitation of ischemic preconditioning, thus suggesting a peripheral involvement of opioid receptors in myocardial protection (20).

At the present time, the mechanism by which opioid receptors produce their cardioprotective actions is not clear and cannot be deduced by the results of the present study. It is known that mu- and delta-opioid receptors are coupled to G proteins (23–25) and can activate \(K_{\text{ATP}}\) channels in regulating antinociception (46–49) and cerebral vascular control (27). It is worth noting that glibenclamide has been recently shown to abolish morphine-induced cardioprotection in the rat heart, thus suggesting an involvement of the myocardial \(K_{\text{ATP}}\) channel as an important component of this cardioprotective effect (18). As \(K_{\text{ATP}}\) channels have been previously shown to be involved in ischemic preconditioning during coronary angioplasty (16), opioid receptor-induced cardioprotection is probably mediated via \(K_{\text{ATP}}\) channel also in the human heart.

In the present study, naloxone infusion did not affect cardiac pain during the first coronary balloon occlusion. These findings are in agreement with the results of several previous studies (36,50,51) and, in particular, with those of Marchant et al. (36), who failed to modify the time to onset of angina during exercise test at a dose of naloxone identical...
to that used in our study. The inability of naloxone to affect the severity of angina might suggest that endogenous opiates do not play a major role in the modulation of cardiac ischemic pain. Alternatively, it is possible that in the central nervous system naloxone did not reach adequate concentrations at the site of opioid receptors potentially able to affect angina severity.

Limitation of the angioplasty model of preconditioning. There are three major concerns with the angioplasty model of preconditioning: 1) the adaptation to ischemia observed after repeated coronary balloon occlusions might be due to progressive collateral recruitment, rather than to a metabolic myocardial adaptation, that is, ischemic preconditioning; 2) the electrocardiographic changes might not reflect a preconditioning state, and 3) patients might have experienced silent ischemia in the 24 h before the study. With regard to the first point, we assessed changes in blood flow velocity in the contralateral coronary artery during balloon occlusion by using a Doppler guide wire. In the absence of significant changes in arterial pressure or heart rate, as was the case in our study at the end of both inflations, blood flow velocity changes in the contralateral coronary artery have been shown to be a reliable index of collateral perfusion and function during coronary angioplasty, more accurate than thermodilution, measurement of coronary occlusion pressure through the balloon catheter or angiographic visualization of collateral vessels (31–33). We found that coronary blood flow velocity significantly increased at the end of the first inflation in both groups of patients, whereas it did not exhibit any further increase during the second inflation. These findings confirm our previous results (15) and are in agreement with those of Kyriakidis et al. (33) and Cribier et al. (52). We measured right-to-left collateral recruitment only, thus probably underestimating total collateral flow. However, intracoronary collateral recruitment is unlikely to explain the adaptation to ischemia observed in our study for two reasons. First, patients were randomized to placebo and naloxone and, therefore, it is unlikely that contribution of intracoronary collateral recruitment was different in placebo- and naloxone-treated patients. Second, to the best of our knowledge, naloxone does not influence collateral flow.

With regard to the second point, Shattock et al. (53), who measured ST-segment changes in open-chest pigs, subjected to two cycles of 8-min ischemia and 8-min reperfusion followed by 60-min ischemia and 2-h reperfusion, found that ST-segment changes provide a reliable index of preconditioning during the first few minutes of coronary occlusion. More recently, Cohen et al. (54) have shown that pharmacologic induction or blockade of protection altered the electrocardiographic response accordingly, suggesting that the attenuation of ST-segment changes during repetitive coronary occlusions truly reflects the protection of ischemic preconditioning and is not merely an epiphenomenon.

With regard to the third point, an episode of silent ischemia in the last 24 h before the study cannot be ruled out; however, as patients were randomized to placebo and naloxone, possible episode of silent ischemia should, in theory, be equally distributed between groups.

Clinical implications. The experimental and clinical findings showing that opioid receptors are involved in ischemic preconditioning suggest that opioid agonists may possess, aside from their specific analgesic and anesthetic effects, a previously unrecognized beneficial cardioprotective effect in the clinical setting. A better knowledge of the specific opioid receptor subtypes involved in ischemic preconditioning might allow the development of new opioid agonists with specific cardioprotective properties and without any potential adverse effects on central nervous system or cardiac contractility.

Reprint requests and correspondence: Dr. Fabrizio Tomai, Divisone di Cardiochirurgia, Universita di Roma Tor Vergata, European Hospital, via Portuense 700, 00149 Rome, Italy.

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