Ferrous Ion Diminished the Antiarrhythmic Effect of Naloxone in Myocardial Ischemia of Isolated Rat Hearts

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This investigation was to examine the effect of ferrous ion (a prooxidant) on the antiarrhythmic effect of naloxone (an endogenous opioid receptor antagonist) in isolated rat hearts. Isolated Sprague-Dawley rat hearts were perfused in the Langendorff mode and myocardial ischemia was performed by ligating the left descending coronary artery. Cardiac rhythm was recorded. Heart α-tocopherol concentrations were analyzed. Naloxone (1.2 μmol/heart) was effective in reducing the severity of arrhythmia (arrhythmia score; mean ± S.E.M: 2.82±0.69 for naloxone vs. 5.18±0.38 for control, p<0.01). Fe²⁺ (100 nmol/heart) alone did not significantly affect the arrhythmia score (5.63±0.32) when compared with the control, however, Fe²⁺ administration did cause significant early onset of ventricular premature contraction and ventricular tachycardia. Additionally, Fe²⁺ administration diminished the naloxone's antiarrhythmic effect (arrhythmia score 4.12±0.40). α-Tocopherol, a major free radical scavenger that exerts protective functions on heart tissues during myocardial ischemia/reperfusion, was significantly higher in the naloxone-treated group (59.05±3.30 nmol/g wet wt) than in the control group (43.84±4.17 nmol/g wet wt, p<0.05). These results suggest that endogenous opioid peptides and reactive oxygen species might be related to ischemia-induced arrhythmia.

Key words: endogenous opioid peptide; oxygen-derived free radical; myocardial ischemia; arrhythmia; α-tocopherol; naloxone

Myocardial ischemia and reperfusion can generate free radicals (or reactive oxygen species, ROS) that are responsible for either primary or secondary damage to the ischemic tissues.²⁻³ ROS-induced damage can be widespread and devastating.³ Following myocardial ischemia alone or myocardial ischemia and reperfusion, the size of infarction and incidence of ventricular fibrillation can be improved or exacerbated by reducing or increasing the amount of ROS, respectively.⁴⁻⁵ Although it is generally believed that the reperfusion following myocardial ischemia generates ROS, there is also evidence indicating that myocardial ischemia alone can also produce ROS.⁶⁻⁷ The possible source being residual oxygen molecules left in the ischemic tissue.⁸

Endogenous opioid peptides (EOP) are believed to be released at the onset of myocardial ischemia.⁹ Interactions of EOPs with their receptors are possible causes of arrhythmia during both myocardial ischemia and reperfusion.¹⁰ α-Tocopherol antagonists such as naloxone are capable of significantly reducing, and agonists such as US0488H are capable of significantly increasing, the severity of arrhythmia in both myocardial ischemia¹¹⁻¹³ and reperfusion.¹⁴ Furthermore, plasma levels of β-endorphin are correlated with the ischemia-induced arrhythmia in patients with acute myocardial infarction.¹⁵ The arrhythmogenic mechanisms of EOPs are currently under investigation.

The formation of ROS and the release of EOP might be related to the damage often seen following myocardial ischemia. Allopurinol, a superoxide formation inhibitor, was found in isolated working rat hearts to partially relieve dynorphin-induced exacerbation of ischemic arrhythmia.¹⁶ Additionally, naloxone, an EOP receptor antagonist, was found to reduce the myocardial ischemia-induced production of hydroxyl radicals in the left ventricular myocardium of anesthetized cats.¹⁷ These results implicated the interrelations of ROS and EOP in myocardial ischemia-induced arrhythmias. Therefore, we administered ferrous ion, a prooxidant, to isolated rat hearts to investigate its influence on naloxone's antiarrhythmic effect. Additionally, heart tissue α-tocopherol levels were measured, since α-tocopherol exerts a protective function during myocardial ischemia and reperfusion.

MATERIALS AND METHODS

Female Sprague-Dawley rats (210—270 g) were used. The heart was perfused in the Langendorff mode. Animal handling and heart preparation methods have been described elsewhere.¹⁸ Naloxone (1.2 μmol/heart) and Fe²⁺ solutions (500 μM) were administered in a 200 μl volume over a one minute period via a separate cannula leading directly into the aorta. The drugs were infused 6 minutes prior to myocardial ischemia. Myocardial ischemia was induced by placing a ligature (6—0 silk suture) around the left coronary artery. All drugs were administered through a sidearm attached to the Langendorff apparatus.

To enable a quantitative comparison, an arrhythmia scoring system modified from that of Curtis and Walker was used.¹⁷ Each heart was given one score representing the most severe type of arrhythmia observed anytime during the entire ischemia period (30 min). The arrhythmia was scored as follows: 0, for no arrhythmia; 1, occasional ventricular premature contraction (VPC); 2, frequent VPC when there were three or more VPC occurring within one minute; 3, ventricular tachycardia (VT; one or two episodes); 4, VT (three to five episodes); 5, VT (more than five episodes); 6, ventricular fibrillation (VF, one to two episodes); 7, VF (three to five episodes); 8, VF (more than five episodes).

At the end of the 30-min ischemia, the hearts were removed from the Langendorff apparatus and homogenized with a Teflon-pestle homogenizer in 50 mM phosphate buffer solution containing 2 mM EDTA (0.3 g/5 ml). The homogenates were added to an equal volume of ascorbic acid (0.01 g in 10 ml absolute ethanol). α-Tocopherol was dissolved in the mixtures to serve as the internal standard. The mixture was extracted 2 or 3 times with an equal volume of

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Table 1. Effect of Naloxone (Nal) and/or Fe²⁺ on Myocardial Ischemia-Induced Arrhythmias

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Arrhythmia scores</th>
<th>VPC onset (min)</th>
<th>VT onset (min)</th>
<th>VT incidence</th>
<th>VF incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11</td>
<td>5.18±0.33³</td>
<td>16.54±1.39³</td>
<td>17.54±1.65³</td>
<td>11/11</td>
<td>4/11</td>
</tr>
<tr>
<td>Naloxone</td>
<td>11</td>
<td>2.82±0.59ᵃᵃ</td>
<td>16.49±1.83</td>
<td>17.33±2.74</td>
<td>6/11ᵇᵇ</td>
<td>1/11ᵇᵇ</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>8</td>
<td>5.63±0.32ᵃᵃ</td>
<td>5.94±2.24ᵇᵇ</td>
<td>12.28±1.97ᵇᵇ</td>
<td>8/8</td>
<td>3/8</td>
</tr>
<tr>
<td>Nal+Fe²⁺</td>
<td>8</td>
<td>4.12±0.46ᵃᵃ</td>
<td>17.93±1.97ᵇᵇ</td>
<td>17.95±1.48</td>
<td>7/8</td>
<td>1/8</td>
</tr>
</tbody>
</table>

Naloxone and ferrous ion dosages were 1.2 μmol/heart and 100 nmol/heart respectively. a,b,c,d,e,f,g p<0.05 by ANOVA (Fisher PLSD) + p<0.05 vs. control * p<0.01 vs. control by chi-square test.

RESULTS

Naloxone, an EOP receptor antagonist, was effective in reducing the severity of ischemia-induced arrhythmia (Table 1), observed by lowered arrhythmia scores (2.82±0.59 for naloxone vs. 5.18±0.33 for controls) and lowered VT and VF incidences. (VT 6/11 for naloxone vs. 11/11 for controls; VF 1/11 for naloxone vs. 4/11 for controls). Administration of ferrous ion, a strong prooxidant, significantly shortened the VPC and VT onsets when compared with the controls (5.94±2.24 vs. 16.54±1.39 min for controls) (12.28±1.97 vs. 17.54±1.65 min for controls, p<0.05). However, ferrous ion had no significant effect on the arrhythmia score. Interestingly, when ferrous ion was co-administered with naloxone, it partially attenuated the antiarrhythmic effect of naloxone, (arrhythmia score 4.12±0.40 for ferrous ion with naloxone and 2.82±0.59 for naloxone alone).

Heart α-tocopherol concentrations are summarized in Fig. 1. Myocardial ischemia significantly decreased the heart α-tocopherol concentrations (43.84±4.17 nmol/g wet wt) when compared with the sham-operated hearts (55.02±3.00 nmol/g wet wt). Interestingly, the α-tocopherol concentrations were significantly higher in hearts that were administered naloxone (59.05±3.00 nmol/g wet wt) then in control hearts. The α-tocopherol concentrations in hearts, that were administered either ferrous ion alone or ferrous ion with naloxone, showed no significant differences when compared with controls (Fig 1).

DISCUSSION

Following myocardial ischemia, the release of endogenous opioid peptides and their interaction with receptors, and the increased formation of oxygen-derived free radicals have been reported as two major arrhythmogenic mechanisms. The relation between EOP and free radicals in myocardial ischemia has seldom been studied. Our earlier results provided preliminary evidence for the possible interrelations of EOP and ROS in ischemia-induced arrhythmia. In that study, we used superoxide formation inhibitor (allopurinol) and EOP agonist (dynorphin A-13). We observed that allopurinol reduced the severity of myocardial ischemia-induced arrhythmia in isolated rat hearts, and dynorphin diminished allopurinol's antiarrhythmic effect. In our present study, instead of using EOP receptor agonist and superoxide formation inhibitor, we investigated the effect of an EOP receptor antagonist (naloxone) and a prooxidant (Fe²⁺) on ischemia-induced arrhythmia. We observed that naloxone was...
indeed antiarrhythmic during myocardial ischemia, which was in accordance with published results. On the other hand, ferrous ion, a proxoidant that significantly shortened the onset of VPC and VF, diminished the arrhythmia-preventive effect of nalozone.

EOP may be released following myocardial ischemia. The interaction between EOP and their receptors may be responsible for the myocardial ischemia-induced arrhythmia since EOP receptor antagonists can effectively block the ischemia-induced arrhythmia. Inhibition of oxygen radical production also results in reduced arrhythmia and this protective effect can be diminished by the presence of EOP. On the contrary, the presence of proxoidant (ferrous ion) can also diminish the EOP receptor antagonist (nalozone) antiarrhythmic effect. These results strongly suggest a possible bilateral relation between EOP and oxygen radicals in the roles following myocardial ischemia. This relation can be further supported by the results that exogenous perfusion of EOP can result in hydroxyl radical production in anesthetized cats. However, the detailed linkage between EOP and oxygen radicals remains to be further investigated.

α-Tocopherol is an important free radical scavenger that has been reported to provide protective functions during myocardial ischemia/reperfusion. The results of decreased heart α-tocopherol levels following 30 min ischemia correspond with the results that oxygen radical may be produced during the ischemic period. Nalozone was found to increase heart α-tocopherol concentration following a 30-min ischemia. A possible mechanism for nalozone’s effect on increase the α-tocopherol levels following myocardial ischemia could be that nalozone attenuates the ROS production. Our earlier results demonstrated that nalozone was effective in reducing the hydroxyl radical production following myocardial ischemia. Thus, the level of α-tocopherol, which has been widely reported to be protective in myocardial ischemia by acting as an oxygen radical scavenger, could rise as a result of the reduced hydroxyl radical production. Therefore, the nalozone-induced increase in heart α-tocopherol levels following myocardial ischemia also implied the possible relation between EOP and ROS.

A possible linkage between EOP/EOP receptor interaction and ROS could be intracellular calcium concentration. Stimulation of EOP receptor has been shown to increase intracellular calcium levels, and increased intracellular calcium levels contribute to increased formation of ROS.

In conclusion, we found that ferrous ion can diminish the antiarrhythmic effect of nalozone, and that nalozone can increase heart α-tocopherol concentration following myocardial ischemia. These results imply that EOP/EOP receptor interaction and formation of ROS might be related in myocardial ischemia-induced damages. However, the detailed mechanism(s) and relations of these two factors require further examinations.

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REFERENCES