Biphasic protective effect of oxytocin on cardiac ischemia/reperfusion injury in anaesthetized rats

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ABSTRACT
Oxytocin (OT) is well known for its role in reproduction. However, evidence has emerged suggesting a role in cardiovascular system. The aim of this study was to investigate the cardioprotective effect of oxytocin on ischemia/reperfusion (I/R) injury in an in vivo rat. Myocardial ischemia, was surgically induced by means of left anterior descending coronary artery occlusion for 25 min followed by reperfusion for 120 min. Infarct size was evaluated using the staining agent 2,3,5-triphenyltetrazolium chloride. Creatine kinase-MB isoenzyme (CK-MB) and lactate dehydrogenase (LDH) levels in plasma were analyzed to assess the degree of cardiac injury. Intraperitoneal administration of OT 0.001, 0.01 and 0.1 μg significantly reduced infarct size, LDH and CK-MB levels as compared to control (I/R) group and it had a biphasic effect on the reduction of ischemia/reperfusion injury. This biphasic effect was revealed as a U-shaped curve in which efficacy was optimal between very low and very high doses. Furthermore there were no significant differences in mean arterial pressure or heart rate between the OT treatment groups and control group during I/R. Blockade of specific OT receptors by atosiban (10^-6 M) abolished or attenuated the effect of OT preconditioning. The result of this study shows that OT possess a dose-dependent cardioprotective effect against ischemia/reperfusion injury and so study of OT preconditioning may provide a new target site for therapeutic exploitation.

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1. Introduction

Intensive research is underway in effort to develop pharmacological means to control the morbidity and mortality arising from the ischemic heart disease. It has been suggested that the beneficial effects of reperfusion on the myocardium might be in part reversed by the occurrence of reperfusion injury and since the reperfusion is initiated by the treatment of myocardial infarction, it is important to limit the extent of this injury [36].

Ischemic preconditioning, in which brief episodes of ischemia are used to protect the heart, can limit the myocardial damage caused by subsequent sustained ischemic reperfusion. Evidences have indicated that the cardioprotection of ischemic preconditioning is mediated by endogenous active substances, including neurotransmitters and autacoids [1]. Few studies have considered the possible relation between hormones and preconditioning [13,31,39].

More recently, oxytocin (OT) has been considered to be a cardiovascular hormone [15,20].

Oxytocin, a nanopeptide, was the first hormone to have its biological activities established and chemical structure determined [12,35]. It was believed that OT is released from hypothalamic nerve terminals of the posterior pituitary into the circulation where it stimulates uterine contractions during parturition and milk ejection during lactation. However, equivalent concentrations of OT were found in the male pituitary, and stimuli for OT release was determined for both genders, suggesting other physiological functions for the hormone [12]. Indeed, recent studies indicate that OT is involved in cognition, tolerance, adaptation and complex sexual and maternal behavior as well as cardiovascular function [8,15].

Oxytocin is produced and released by the heart and acts on its cardiac receptors to decrease heart rate and force of contraction [15]. Systemic administration of OT has significant effects on blood pressure, vascular tone and cardiovascular regulation. Both increase and decrease in blood pressure in response to oxytocin have been reported [15,20,21,34]. Systemically administered oxytocin induces a short-lasting increase in blood pressure in rats [7,33] whereas the opposite is seen in humans [23,26,41]. Costa-e-Sousa et al. have reported that oxytocin is also able to modulate mechanical activity of atria and exerts negative inotropic and chronotropic effects both in unanesthetized rats and in
isolated rat heart [7]. Studies about the role of OT in cardiovascular regulation are limited and the mechanism by which OT induces its cardiovascular effects is not yet known [15].

A major goal of cardiovascular research is currently the identification of a reliable cardioprotective intervention that can salvage ischemic myocardium. Unfortunately, the clinical value of ischemic preconditioning itself is limited and none of several identified pharmacologic agents that appear to limit reperfusion injury is available for clinical use [49].

On the other hand, OT is produced and released by the heart and large vessels. It has been suggested that intrinsic OT system may play an important physiological role in regulation of vascular tone, as well as control of cardiac function [21]. OT is well known to exert potent physiological anti-stress effects [29] and stress increases the severity of cardiovascular disease [43]. Recently, increasing attention has been given to the potential role of oxytocin in cardiovascular functions [7,15,20,27]. Therefore, on the basis of this background the present study was designed: (1) to investigate the possible protective effect of OT against regional ischemic reperfusion (I/R) injury in heart, (2) to determine the dose–response relationship of OT on its cardioprotective effect and (3) to investigate the role of OT specific receptors in protection by three indices of injuries, infarct size, hemodynamic and biochemical factors in the same in vivo anesthetized rats.

2. Materials and methods

Male Sprague–Dawley rats (weighing 270–360 g) were maintained in the animal quarters under standardized conditions (12-h light/day cycle, 20–22°C room-temperature and 40–50% humidity) and received standard laboratory rat chow and water ad libitum.

2.1. Animals and surgical preparation

The animals were anesthetized with sodium pentobarbital (60 mg kg\(^{-1}\), i.p.) and anesthesia was maintained with supplementary injections (~30 mg kg\(^{-1}\), i.p.) as required. Body temperature was monitored by rectal thermometer and maintained at 37 ± 1°C.

Following tracheotomy, the rats were ventilated with air-and-oxygen mixture by a rodent ventilator (model 683, Harvard Apparatus, USA) with a stroke volume of approximately 1.2 ml 100 g\(^{-1}\) body weight at a rate of 60–70 stroke min\(^{-1}\). Atelectasis was prevented by the maintenance of positive end-expiratory pressure of 3–5 cm H\(_2\)O.

Catheter filled with heparinized saline (100 U/ml) was placed into the right carotid artery for blood sampling and blood pressure monitoring. A standard limb lead-II electrocardiogram (ECG) and arterial blood pressure were continuously monitored and recorded on a computer throughout the experiment, using a computerized data acquisition system (Power Lab data acquisition system, four channel, ADInstruments). For all groups, heart rate was extracted from the electrocardiogram recordings. The tail vein was cannulated for the administration of Evans blue to evaluate area at risk.

The left thoracotomy was performed to expose the heart by sectioning of the fourth rib approximately 3 mm from the sternum. The pericardium was incised and a sling (6-0 silk Ethicon) was placed around the left anterior descending coronary artery close to its origin immediately the left atrial appendage to the right part of the left ventricle (LV). Both ends of the ligature were passed through a small plastic tube and then chest was partially closed and the animal was allowed to recover for 30 min. Heart rate and blood pressure were allowed to stabilize for 30 min before protocols were initiated. Following stabilization, baseline and intervention periods, the coronary artery was occluded by applying tension to the plastic tube-silk string arrangement to induce transient regional myocardial ischemia for 25 min. Reperfusion was initiated by releasing the ligature and removing the tension. Coronary artery occlusion was verified by epicardial cyanosis and subsequent decrease in blood pressure, and reperfusion was confirmed by epicardial hyperemia.

2.2. Experimental protocol

The present study consisted of three protocols. Rats were randomly divided into eight groups and underwent LAD occlusion for 25 and 120 min of reperfusion, as shown in Fig. 1. On the day of the experiment, following stabilization period, basal hemodynamic parameters were measured for 15 min before drug administration. In protocol I (control group), saline was administered intraperitoneally 30 min before hearts were subjected to LAD occlusion. In protocol II, the second to sixth groups were given i.p. infusions of different doses of oxytocin (OT) 30 min before I/R. In protocol III, the selective antagonist atosiban was given i.p. 10 min before the infusions of 0.001, 0.01 and 0.1 μg oxytocin. TTC, triphenyltetrazolium chloride.

Fig. 1. Illustration of the experimental protocols. Animals in control group (protocol I) were subjected to 25 min of coronary artery occlusion followed by 120 min reperfusion (I/R). In protocol II, the second to sixth groups were given i.p. infusions of different doses of oxytocin (OT) 30 min before I/R. In protocol III, the selective antagonist atosiban was given i.p. 10 min before the infusions of 0.001, 0.01 and 0.1 μg oxytocin. TTC, triphenyltetrazolium chloride.
occlusion and reperfusion. In OT dose-response studies, in protocol II, oxytocin 0.0001, 0.001, 0.01,0.1 and 1 µg (Sigma Chemical Co. St. Louis, MO, USA) was given as an i.p. bolus 30 min before hearts were subjected to LAD occlusion and reperfusion. During protocol II it was found that OT had a biphasic (revealed as a U-shaped curve) shape: a low-phase, which was significantly effective in 0.001 µg and a high phase reappearing in 0.1 µg during the descending and ascending limbs of the dose–response curves respectively and optimal effects induced in 0.01 µg of OT. Therefore, in protocol III in order to explore the role of OT receptor-mediated effects, atosiban (Sigma Chemical Co. St. Louis, MO, USA), an OT-selective receptor antagonist, was used in 2 additional groups (OT 0.001 and 0.1) beside in OT 0.01 group. It was given as a bolus injection with a dose of 10−6 M intraperitoneally 10 min prior to the treatment of 0.001, 0.01 and 0.1 µg of OT.

2.3. Cardiac area at risk and infarct size determination

The preparation used in the present study was as described previously [17]. In brief, after the completion of the experimental protocols, blood samples were taken, then the coronary artery was reoxygenated and 2 ml of Evans blue dye in deionized water (2%) was injected intravenously to the tail vein for identification of area at risk (AAR). The Evans blue solution stains the perfused myocardium, while the occluded vascular bed remains unstained. Then the heart was excised, and both atria and the roots of the great vessels were removed. The heart was frozen for 24 h at −20 °C and then cut into slices of 2-mm-thick from the apex to the base. All slices were incubated with a 1% solution of 2,3,5-triphenyltetrazolium chloride (Sigma Chemical Co. St. Louis, MO, USA) (TTC, in 0.1 M phosphate buffer, pH 7.4) stain for 15 min at 37 °C, to visualize the infarct area and after that fixed for 2 days in 10% formalin to enhance the contrast of the Evans blue and TTC staining. Both surfaces of each transverse section were scanned and downloaded into Adobe PhotoShop (Adobe Systems Seattle, WA). The areas of the normal ventricle non-risk region (blue), area at risk (AAR, brick red) and area of necrosis (AN, pale) were determined by calculating the number of pixels occupying each area using the Adobe PhotoShop software. Total area at risk was expressed as a percentage of the left ventricles (AAR/LV). Infarct size was expressed as a percentage of the area at risk (AN/AAR).

All experimental procedures used in this study were approved by the guidelines of the animal and human ethical committee of Tehran Medical Sciences University.

2.4. Biochemical analysis

To measure the myocardial specific enzymes, including the activity of creatine kinase-MB isoenzyme (CK-MB) and lactate dehydrogenase (LDH), blood samples were collected at the end of each experiment. The heparinized samples were centrifuged at 5000 rpm, 4 °C, for 15 min, and the plasma was removed and stored at −70 °C until the time they were assayed. The activity of CK-MB and LDH were analyzed using commercial kits (Pars Azmoon, Iran) by employing an autoanalyzer (Roche Hitachi Modular DP Systems, Mannheim, Germany).

2.5. Hemodynamic functions

Hemodynamic data (arterial blood pressure and heart rate) were continuously monitored and recorded on a computer

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Baseline</th>
<th>Atosiban</th>
<th>Oxytocin</th>
<th>LAD occlusion 25 min</th>
<th>Reperfusion 120 min</th>
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<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>10</td>
<td>109 ± 4</td>
<td>106 ± 3</td>
<td>114 ± 10</td>
<td>114 ± 9</td>
<td>114 ± 9</td>
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<tr>
<td>OT 0.001</td>
<td>6</td>
<td>107 ± 5</td>
<td>99 ± 6</td>
<td>105 ± 9</td>
<td>105 ± 6</td>
<td>105 ± 6</td>
</tr>
<tr>
<td>OT 0.01</td>
<td>5</td>
<td>109 ± 6</td>
<td>120 ± 5</td>
<td>114 ± 9</td>
<td>112 ± 6</td>
<td>105 ± 7</td>
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<td>121 ± 5</td>
<td>121 ± 3</td>
<td>99 ± 10</td>
<td>99 ± 10</td>
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<tr>
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<td>9</td>
<td>115 ± 10</td>
<td>115 ± 5</td>
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<td>88 ± 10</td>
</tr>
<tr>
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<td>103 ± 4</td>
<td>107 ± 6</td>
<td>108 ± 6</td>
<td>108 ± 6</td>
</tr>
<tr>
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<td>7</td>
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<td>102 ± 5</td>
<td>112 ± 3</td>
<td>96 ± 8</td>
<td>73 ± 11</td>
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<td>365 ± 15</td>
<td>315 ± 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OT 0.001</td>
<td>6</td>
<td>375 ± 16</td>
<td>302 ± 13</td>
<td>276 ± 11**</td>
<td>291 ± 15**</td>
<td></td>
</tr>
<tr>
<td>OT 0.01</td>
<td>5</td>
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<td>339 ± 16</td>
<td>277 ± 15</td>
<td>277 ± 15</td>
</tr>
<tr>
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<td>369 ± 11</td>
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<td>359 ± 15</td>
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<td>362 ± 8</td>
<td>326 ± 17**</td>
<td>326 ± 17**</td>
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<td>380 ± 12</td>
<td>350 ± 10**</td>
<td>339 ± 10**</td>
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<tr>
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<td>314 ± 9</td>
<td>320 ± 6</td>
<td>320 ± 10**</td>
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<tr>
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<td>347 ± 9</td>
<td>353 ± 7</td>
<td>353 ± 6</td>
<td>323 ± 15**</td>
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<td>RPP (beat min−1 mmHg × 105)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td>43 ± 3</td>
<td>37 ± 4</td>
<td>27 ± 2***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OT 0.001</td>
<td>6</td>
<td>44 ± 2</td>
<td>33 ± 3</td>
<td>24 ± 2**</td>
<td>31 ± 1**</td>
<td></td>
</tr>
<tr>
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<td>5</td>
<td>39 ± 4</td>
<td>42 ± 4</td>
<td>35 ± 3</td>
<td>26 ± 2</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>OT 0.01</td>
<td>5</td>
<td>44 ± 2</td>
<td>49 ± 3</td>
<td>47 ± 4</td>
<td>41 ± 3</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>OT 0.01</td>
<td>8</td>
<td>46 ± 1</td>
<td>48 ± 2</td>
<td>40 ± 4</td>
<td>32 ± 4**</td>
<td>32 ± 4**</td>
</tr>
<tr>
<td>OT 0.01</td>
<td>6</td>
<td>44 ± 5</td>
<td>47 ± 2</td>
<td>36 ± 4</td>
<td>34 ± 2</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>OT 0.01 + atosiban</td>
<td>9</td>
<td>56 ± 8</td>
<td>47 ± 3</td>
<td>49 ± 2</td>
<td>35 ± 4</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>OT 0.01 + atosiban</td>
<td>6</td>
<td>50 ± 1</td>
<td>35 ± 2</td>
<td>40 ± 1</td>
<td>37 ± 3</td>
<td>37 ± 2**</td>
</tr>
<tr>
<td>OT 0.01 + atosiban</td>
<td>7</td>
<td>42 ± 1</td>
<td>37 ± 2</td>
<td>43 ± 9</td>
<td>38 ± 2</td>
<td>31 ± 3</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE. n: number of animals in each group; MAP: mean arterial pressure (mmHg); RPP: rate pressure product (beat min−1 mmHg × 105); control: prolonged equilibration time of 40 min prior to the 25 min period of regional ischemia followed by 120 reperfusion. Oxytocin (OT) at different doses (0.0001, 0.001, 0.01, 0.1 and 1 µg) was given as bolus intraperitoneally 30 min prior to the coronary occlusion in OT groups. Atosiban: 10−6 M a specific antagonist of OT receptor was given as bolus intraperitoneally 10 min prior to the OT treatment. *P < 0.05. **P < 0.01. ***P < 0.001 vs. baseline within the same group. *P < 0.05, vs. OT 0.01 group. **P < 0.05, ***P < 0.001 vs. control.
throughout the experiment: baseline, interaction period, 25 min LAD occlusion, and 120 min reperfusion. At the same time-points, the rate pressure product (RPP), defined as systolic blood pressure multiplied by the heart rate also were calculated by a computerized data acquisition system (Power Lab data acquisition system, four channels, ADInstruments).

2.6. Statistical analysis

Statistical analysis of hemodynamic data within and between groups was performed with analysis of variance for repeated measures followed by the Dunnett’s test. Differences in infarct size, plasma LDH and CK-MB levels were determined by one-way analysis of variance. Individual groups were compared using Dunnett’s test. All data were expressed as mean ± SE and statistical significance was defined as $P < 0.05$.

3. Results

3.1. Hemodynamic functions

Table 1 shows the time course of heart rate, mean arterial pressure and rate pressure product during the experiments. There were no significant differences among groups at baseline before treatment. At the end of reperfusion, heart function was significantly decreased in control group, as indicated by MAP, HR and RPP as compared with its baseline.

The results depicted in Table 1 show that at the end of the experiment, MAP decreased slightly in most OT groups but only in OT 0.1 group mean arterial pressure after 120 min reperfusion was significantly decreased compared to its baseline.

Although in some groups, administration of OT nonsignificantly increased HR, MAP and RPP at preocclusion period, only in OT 0.001 group the heart rate and RPP were significantly decreased compared to its baseline. On the whole, oxytocin itself had no significant effect on hemodynamic values at preocclusion.

No significant differences were observed in any of the hemodynamic parameters between control and OT treatment groups during ischemia and reperfusion with two exceptions: a significant drop in HR in OT 0.0001 group in LAD occlusion period, and a significant increase in RPP in OT 0.01 group at the end of 120 min.

In protocol III (Table 1) atosiban significantly decreased heart rate, mean arterial pressure and rate pressure product in OT 0.001 + atosiban and OT 0.1 + atosiban as compared to the baseline. Atosiban administration in OT 0.01 group did not cause any significant changes in hemodynamic values throughout the experimental protocol (Table 1). There were no significant differences in hemodynamic between oxytocin (0.001, 0.01 and 0.1), OT + atosiban and control groups. Only compared with OT
0.01 group, HR value was significantly reduced in OT 0.01 + atosiban group at LAD occlusion process. Also in OT 0.01 + atosiban group, atosiban caused significant changes in RPP after reperfusion for 120 min compared with the control group.

3.2. Area at risk and infarct size measurements

Fig. 2B presents risk zone size data. There were no significant differences in this parameter among the groups. Fig. 2A shows the infarct size for each group. Infarct size as a percentage of area at risk was 41.9 ± 1.8% in control group, whereas preconditioning with 0.001–0.1 µg oxytocin (OT 0.001, 0.01 and 0.1 groups) significantly and dose-dependently reduced infarct size to 25.3 ± 6.1% (P < 0.01), 16.2 ± 2.8% (P < 0.001), and 30.3 ± 2.2% (P < 0.05) respectively vs. control group (Fig. 2A).

In protocol III, the reduction in infarct size (AN/AAR) by OT 0.01 was completely abolished by pretreatment with atosiban (P < 0.05, Fig. 3A). Also atosiban abolished the anti-ischemic actions of oxytocin in OT 0.001 group (low-phase) and in OT 0.1 group (high phase) during descending and ascending limbs of the dose–response curves respectively (P < 0.05, Fig. 3A). Atosiban also increased infarct size when administered before 0.1 µg oxytocin (OT 0.1 group) as compared to the control group (55.7 ± 2.2%, and 41.9 ± 1.8%, respectively; P < 0.05, Fig. 3A).

3.3. Biochemical analysis

The activity of LDH and CK-MB in plasma was used to monitor the damage of myocardium. In protocol II, compared to the control group, LDH activity in plasma of treatment groups markedly decreased after the ischemia-reperfusion process. Treatment with oxytocin 0.001 (P < 0.05), 0.01 (P < 0.01) and 0.1 µg (P < 0.05) could prevent elevation of LDH activity in plasma after ischemia/reperfusion injury (Fig. 4A). Also OT 0.01 and 0.001 groups had significantly decreased CK-MB activity (P < 0.01, P < 0.05 respectively) as compared to the control group (Fig. 4B).

In protocol III, in addition the OT 0.01 + atosiban group, administration of the antagonist attenuated the cardioprotective effects of OT in both phases during descending and ascending limbs of the dose-response curve respectively (Fig. 5). The LDH activity in OT + atosiban groups significantly was increased compared to oxytocin 0.01 and 0.1 groups (P < 0.05, Fig. 5A). The LDH activity in OT 0.01 + atosiban and OT 0.1 + atosiban groups were even higher than control group (P < 0.05) after ischemia-reperfusion injury as shown in Fig. 5A.
There was a tendency towards a significant increase in the level of CK-MB in OT + atosiban groups as compared to oxytocin groups (P < 0.05) [Fig. 5B]. Administration of the antagonist before OT preconditioning also significantly (P < 0.05) altered the CK-MB levels as compared to the control (Fig. 5B).

3.4. Dose–response studies

Oxytocin decreased post-ischemic infarct size, LDH and CK-MB in a U-shaped dose-dependent manner. The minimum dose of OT which could reduce myocardial infarction and levels of the enzymes was 0.001 μg. The dose of OT that caused a maximum reduction in infarct size and enzymes concentrations was 0.01 μg (Figs. 2 and 4).

4. Discussion

The results of the present study show that pretreatment with OT can protect hearts from ischemic injury in anesthetized rats and to our knowledge, this is the first experimental evidence showing that OT can mimic preconditioning in a dose-dependent manner. OT with two exceptions caused no significant changes in the recorded hemodynamic profile, such as blood pressure, HR and RPP compared to the control group. This observation suggests that the effects of OT preconditioning on myocardial infarct are not related to alterations in hemodynamic parameters. So the in vivo effects of OT on the ischemic myocardium are likely dependent upon direct cytoprotection at the cellular level.

Furthermore, our study demonstrates that the treatment with oxytocin before regional myocardial ischemia reperfusion can provide myocardial protection against reperfusion injury by decreasing infarct size in a dose depending manner. The present in vivo observations are in agreement with and extend previous data obtained in isolated perfused hearts, which indicate that exogenous OT has cardioprotective effect against infarction [30]. Blockage of the cardioprotective effect of OT by atosiban, a selective OT receptor antagonist, suggests that this effect was mediated via activation of the OT specific receptors. In addition, administration of OT receptor antagonist (OT 0.1 + atosiban) increased infarct size compared to control group, indicating that endogenous OT contributes to protection against I/R injury.

In addition, OT reduced biochemical parameters in our experiments. Elevated levels of creatine kinase-MB (CK-MB) have been regarded as biochemical markers of myocyte necrosis [50] and OT when administrated before ischemia could induce a significant reduction in the level this enzyme compared to control group. Increase in plasma LDH level, which plays an important role in systemic tissue damage [9], was also attenuated by oxytocin treatment. In support of the current data, Tugtepe et al. showed that OT treatment attenuated the renal I/R-induced LDH response [42]. Yiylklı et al. have reported that elevated serum LDH levels in the pyelonephritic rats were reversed by OT treatment [3]. Similarly Iseri et al. showed that in the acetic acid-induced colitis, serum LDH level was elevated and this increase was significantly decreased by OT treatment [18].

In this study we clearly demonstrated that OT had a cardioprotective role against I/R injury in open chest anesthetized rats and induced its protective effect dose-dependently. Oxytocin treatment reveals a biphasic pattern, i.e., increasing doses correlate with the increasing efficacy of injury inhibition until an optimal dose is attained beyond which higher doses show less activity. Optimal effect in the reduction of the infarct size, biochemical changes and hemodynamic improvement was induced by i.p. bolus injection of 0.01 μg oxytocin. For all indices of protection, higher and lower doses of oxytocin showed a less protective effect. This pattern of the effect of OT has been shown in other reports, too. For example it was reported that oxytocin induced biphasic dose-dependent changes in mean arterial pressure [27], arteriolar dilation [2] and chronotropic/inotropic effects [7, 8, 27].

The effects of 0.01 μg oxytocin were abolished by atosiban, a selective OT-receptor antagonist. These observations provided evidence that OT receptor mediates the cardioprotective effects of OT. On the other hand, because in the present study, pretreatment with the OT antagonist, atosiban, abolished both the low-phase and high-phase beneficial effects of OT on ischemia/reperfusion injury, the mechanism of OT-cardioprotection in both phases after ischemia appears to be related to the OT specific-receptor activity.

In general, the receptor systems display such biphasic dose responses when a single agonist has differential affinities for two opposing receptor subtypes, a concept that was first described in detail by Szabadi [40]. By conducting more research about this concept, it is likely that even more mechanisms will be discovered that operate at the molecular, cellular and tissue levels, or total organism [6]. In spite of OT tendency for biphasic effects in different studies, it is not yet clarified whether different types of receptors are involved in the “low-” and “high-”dose effects of oxytocin [44] and also it remains to be determined whether receptor-dependent activity is the only cause related to the biphasic cardioprotection induced by OT against I/R injury.

In addition, atosiban, the OT antagonist, increased the severity of injury as compared to control group, suggesting a protective role for endogenous OT in the ischemia/reperfusion injury under experimental conditions. Therefore, the action of OT may be mediated, at least partly, via the cardiac OT receptors. Oxytocin receptors are cell surface receptors coupled with G-proteins and until now only one receptor is recognized [12, 22]. But Breton et al. and Petersson et al. have reported that the OT effect may be mediated by an unidentified OTR subtype that differs from the known OTR [4, 32].

Thus, the results of the present study add to the previous data [30] showing that OT under certain experimental conditions exerts a cardioprotective action.

There are only limited numbers of published studies regarding the anti-ischemic effects of OT [10, 42, 46], and these reported that OT exerts its beneficial effects in the different aspects of protection such as anti-inflammatory actions and prevention of free radical damaging cascades. Oxytocin protects sepsis-induced oxidative damage by acting as an antioxidant agent [19] and its protective effect in the colon, liver and kidney appears to be dependent on its inhibitory effect on neutrophil infiltration and associated production of reactive oxygen species [10, 42, 48]. In accordance with these, the anti-inflammatory and antioxidant effects of OT on cardiac tissue may be the cause of its protection.

Recent reports suggest that the protective effects of OT pretreatment on ischemia-reperfusion damage may be caused by the release of nitric oxide (NO) [10, 42]. The protective effects of NO on cardiac I/R have been repeatedly described [25, 28].

Another possibility to consider is that OT may release atrial natriuretic peptide (ANP) [14], which is a vasodilator and has antioxidative properties [14]. In previous studies, it was shown that ANP, released by heart in response to volume increase, protects kidney [10] and liver [11] from I/R injury. Since ANP acts as a mediator of ischemic preconditioning in rat [38], there is the possibility that the cardioprotective effects of OT preconditioning may be mediated through the release of ANP.

Oxytocin has also been suggested to be involved in the modulation of immune and inflammatory processes. Specifically, oxytocin decreases the release of interleukin-6 and releases prostacyclin, which inhibit platelet aggregation [18, 47]. On the other hand, it is generally accepted that OT activates two types of receptors, OT and V1 vasopressin receptors [7]. Both OT receptor [15] and vasopressin receptor [16], are present in the heart.
Activation of the V₁ receptor evokes severe coronary constriction whereas activation of the endothelial OT receptors leads to vasodilation [16,45]. However OT as a partial agonist of vasopressin may also evoke vasodilatation in different vascular beds. The action of high concentrations of OT may be caused by activation of V₂ receptors [21].

Petry et al. [34] reported that the intravenous administration of 10 µg OT produced an acute increase in mean arterial pressure of unanesthetized rats. Also, Costa-e-Sousa demonstrated that the intravenous administration of 1 µg OT (1 µg/ml to 10⁻⁶ M) produced a rise in mean arterial pressure of unanesthetized rats and that this effect resulted from the coupling of oxytocin to vasopressinergic V₁ receptors [7]. Supporting this, the in vitro vasconstrictor effect of OT can be completely blocked by a V₁a receptor antagonist [5,26].

In our study we showed that administration of a specific OT antagonist could not induce significant changes on hemodynamic parameters as compared to its absence. Our results are also in agreement with the results of Peterson and Uvnas-Moberg who found that the increase in blood pressure in response to peripherally administered oxytocin was only partially antagonized by an oxytocin antagonist. Thus it probably also involves vasopressin (V₁a) receptors, since oxytocin in high concentrations also binds to these receptors [33].

In addition, a possible mechanism of action may be that OT modulates the interaction between energy demand and the contractile state of the myocardium and thereby myocardial oxygen consumption [44]. Recently, it was demonstrated that OT exerted negative inotropic and chronotropic effects [7] and also exerted an energy-saving effect via its receptor, which might be of importance for the cardioprotective effect of OT under the present experimental conditions.

Another possibility is the involvement of central nervous system. Since 1–2% of peripherally given dose of OT passes the blood–brain barrier [32,44], the possible action of the hormone via central nervous system cannot be ruled out.

Moreover, on the basis of coronary effects of vasopressin during ischemia and reperfusion Martinez et al. suggested that vasopressin might be involved in the adverse effects of ischemia/reperfusion on coronary vasculature [24]. Therefore, it is possible that the interaction between exogenous OT in high doses and vasoconstrictor V₁ receptors during ischemia and reperfusion induces unfavorable effects in the ischemic/reperfused myocardium in OT 1 µg. Supporting this, previous studies have demonstrated that the vasoconstrictor effect of OT of more than 10⁻⁶ M could be completely blocked by V₁ receptor antagonists [7,26,34].

Thus, OT may induce PC-like cardioprotection via several different mechanisms. However, the exact mechanism(s) behind the cardioprotective effects of OT are beyond the aim of the present investigation and remain to be explored in future studies.

These findings illustrate the dose-dependent nature of OT protection against infarction and highlight the need to perform dose–response studies when determining the potential cardioprotective properties of novel agents.

5. Conclusion

In summary, the administration of OT before the induction of regional myocardial ischemia and reperfusion reduced the extent of irreversible myocardial injury by a mechanism involving activation of the OT specific receptors. The level of protection conferred by OT was manifested at a dose that did not result any changes in mean arterial blood pressure and heart rate values. Also in our study, reduction in infarct size was observed immediately after treatment with OT, indicating that induction of new genes is not necessary for its cardioprotective effect.

Our results suggest that OT protects the heart and may represent a novel approach for the protection of the heart during open surgeries.

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References


