Role of endogenous oxytocin in cardiac ischemic preconditioning

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A B S T R A C T

Background: The aim of the present study is to assess the role of endogenous oxytocin (OT) in cardioprotective effects of ischemic preconditioning (IPC) in anesthetized rat.

Materials and methods: Animals were divided into five groups: 1) ischemia-reperfusion (IR); (n = 6), hearts were subjected to 25 min regional ischemia and 60 min reperfusion. 2) OT; (n = 6), oxytocin was administered (0.03 μg/kg i.p) 10 min prior to ischemia. 3) IPC; (n = 7), IPC was induced via a 5 min regional ischemia followed by 5 min of reperfusion before IR. 4) IPC + ATO (Atosiban); (n = 6), atosiban (1.5 μg/kg i.p) was used as OT receptor selective antagonist in the beginning of IPC and 5) IR + ATO; (n = 6), atosiban was injected 10 min prior to ischemia-reperfusion.

Results: In our experiment, Infarct size was decreased significantly in OT and IPC groups compared to IR (23 ± 1.5% and 19 ± 0.6% vs. 45 ± 2.9% in IR group, P < 0.05). Administration of atosiban in IPC + ATO group increased infarct size to 39 ± 0.9% in comparison with OT and IPC groups (P < 0.05). The use of OT and IPC prior to ischemia significantly declined ventricular arrhythmias severity in compared to IR group (1.2 ± 0.4 and 1 ± 0.5 respectively, vs. 4 ± 0.4 in IR group, P < 0.05). Blockade of OT receptor by atosiban abolished the cardioprotecting effects of IPC.

Conclusion: This study shows that, in part, the cardioprotective effects of IPC can be induced by endogenous OT.

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1. Introduction

Ischemic preconditioning (IPC) of the heart, which was first described by Murry et al. [1], is an extremely complex physiological and pathophysiological phenomenon, resulting in a protection against ischemia–reperfusion (IR) injury [2]. Infarct size resulted from a subsequent sustained ischemia is reduced by brief episodes of ischemia–reperfusion. This has been confirmed in all species tested so far [3], and also in humans [4,5]. The signal transduction cascade of IPC involves mediators such as protein tyrosine kinases, mitogen-activated protein kinases, protein kinase C and endogenous triggers such as bradykinin, opioids, adenosine and free radicals [6,7]. Conceptually, triggers are important during the preconditioning phase, while mediators are important during the sustained ischemia [8,9]. The major effects of cardioprotection are reduction of infarct size (antinecrotic effect) [10], reduction of number and severity of cardiac arrhythmias (antiarrhythmic effect) [11,12] and improvement of contractile performance (protection against contractile dysfunction) [13].

More recently, oxytocin (OT) has been considered to be a cardiovascular hormone [14,15]. OT is produced and released by the heart and large vessels [16] which acts on its cardiac receptors to decrease cardiac rate and force of contraction [15]. The multiple established and proposed actions of OT are all mediated by one type of OT receptor. This receptor contains seven transmembrane domains and is a member of the class I family of G protein-coupled receptors [17,18]. Several studies showed that atosiban is a selective antagonist of OT receptor and blocks OT effects on tissues [19–21]. Systemic administration of OT has significant effects on blood pressure, vascular tone and cardiovascular regulation [16]. A major goal of cardiovascular research is the identification of a reliable cardioprotection intervention that can salvage ischemic myocardium [5]. It has been suggested that intrinsic OT system may play an important physiological role in regulating vascular tone, as well as control of cardiac function [16]. OT is well known to exert potent physiological anti-stress effects [22] and stress increases the severity of cardiovascular disease [23].

Recently, increasing attention has been given to the potential role of OT in cardiovascular functions [14,15,24]. Interestingly, it was shown that OT protects kidney and liver tissues against ischemia–reperfusion injuries [25,26]. Recently Ondrejčakova et al. reported its protective effects on ischemia–reperfusion-induced myocardial injury in the
isolated rat heart [10]. We have also depicted protective effects of OT on the ischemic reperfused heart injury [27] and involvement mechanism [28] in the anesthetized rat. The aim of the present study is to assess the role of endogenous OT in cardioprotective effects of IPC.

2. Methods and materials

2.1. Animals

Male Sprague–Dawley rats (300–350 g) were maintained in animal quarters under standardized conditions 12 h light/dark cycle, 20–22 °C ambient temperature and 40–50% humidity with free access to rat chow and water. All experimental procedures were done according to the guidelines of the animal and human ethical committee of Tehran University of medical sciences.

2.2. Surgical preparation

The preparation used in the present study was as described previously [28]. The animals were anesthetized with sodium Thiopental (60 mg kg$^{-1}$ i.p) and maintained with supplementary doses (-30 mg kg$^{-1}$ i.p) as required. Body temperature was measured by rectal thermometer and maintained at 37 ± 1 °C.

The rats were ventilated through a tracheotomy tub with air-and-oxygen mixture by a rodent ventilator (model 683, Harvard Apparatus) (stroke volume approximately 1.2 ml 100 g$^{-1}$, 60–70 stroke min$^{-1}$). A positive end-expiratory pressure was applied to prevent alveolar atelectasis (3–5 cm H$_2$O).

Heparinized catheter (100 U/ml) was fixed into the right carotid artery for blood sampling and pressure monitoring. The lateral tail vein was cannulated to inject Evans blue dye and other drugs. Electrocardiogram standard limb lead-II and arterial blood pressure were continuously monitored by using a computerized data acquisition system (Power Lab data acquisition system, four channels, AD Instruments).

The fourth rib was cut 3 mm below left lateral sternum border. The pericardium was incised and a sling (6–0 silk Ethicon) was placed around the left anterior descending (LAD) artery close to its origin right below the left atrial appendage. Both ends of the ligature were passed through a small plastic tube. Then the chest was partially closed and the animals were allowed to recover. Heart rate and blood pressure were allowed to stabilize for 20 min before the intervention protocols. To induce transient myocardial ischemia, LAD was occluded by applying tension to the plastic tube-silk string. Reperfusion was then initiated by releasing the ligature and removing the tension. Regional ischemia and reperfusion were confirmed by epicardial cyanosis and hyperemia, respectively.

2.3. Exclusion criteria

Data were excluded from the final analysis if arterial blood pressure was less than 60 mmHg before treatment or in case of inadequate blood gas.

2.4. Experimental protocols

The heart of all animals was subjected to 25 min regional ischemia and 60 min reperfusion (Fig. 1). Animals were divided into five groups: 1) IR; (n = 6), hearts were subjected to 25 min ischemia and 60 min reperfusion, 2) OT; (n = 6), Oxytocin was administered (0.03 μg/kg i.p) 10 min prior to ischemia [28], 3) IPC; (n = 7), IPC was induced via a 5 min regional ischemia followed by 5 min of reperfusion before IR, 4) IPC+ATO (Atosiban); (n = 6), atosiban (1.5 μg/kg i.p) was used as OT receptor selective antagonist in the presence of IPC [28] and 5) IR+ATO; (n = 6), atosiban was injected 10 min prior to ischemia–reperfusion.

Fig. 1. Illustration of the experimental protocols. Hearts in all groups were subjected to 25 min of ischemia followed by 60 min reperfusion. NS = normal saline; OT = oxytocin; IPC = ischemic preconditioning; IS = ischemia; Rep = reperfusion; IR = ischemia–reperfusion; and ATO = atosiban.

2.5. Hemodynamic functions

Hemodynamic parameters, arterial blood pressure and heart rate (HR) were continuously monitored and recorded. The rate pressure product (RPP); systolic blood pressure multiplied by the HR was also calculated by Power Lab data acquisition system.

2.6. Cardiac area at risk and infarct size determination

To identify area at risk (AAR) the coronary artery was reoccluded. Evans blue dye (2 ml, 2%) was then injected through lateral tail vein. The heart was excised; atria and the roots of the great vessels were removed. Remaining tissues were frozen for 24 h. Then they were cut into 2 mm slices. All pieces were incubated in 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC, 0.1 M phosphate buffer, pH 7.4, Sigma Chemical Co. St. Louis, MO, USA) at 37 °C for 15 min to visualize the infarct area. Then they were fixed for 2 days in 10% formalin to enhance the contrast. The non ischemic area, AAR and infarcted area were colored blue, brick red and pale respectively. Sections were scanned to determine normal area, AAR and infarct size (IS) by calculating pixels occupying each area using Adobe PhotoShop software (Adobe Systems Seattle, WA). Total AAR and IS are expressed as the percentage of total ventricle and AAR, respectively.

2.7. Assessment of ventricular arrhythmias

Ischemia-induced ventricular arrhythmias were counted during the occlusion period and determined in accordance with the Lambeth Conventions [29]. Ventricular ectopic beats (VEBs), ventricular tachycardia (VT), ventricular fibrillation (VF), multipart forms of VEBs such as bigeminy, couplet and salvos were counted at separate episodes [28]. The incidence, time of occurrence and duration of arrhythmias were used to identify arrhythmias severity according to the following scoring system [30]: 0: 0–49 VEBs, 1: 50–499 VEBs, 2: >499 VEBs and/or 1 episode of spontaneously reverting VT or VF, 3: >1 episode of VT or VF or both with a total duration <60 s, 4: VT or VF or both >60–120 s total duration, 5: VT or VF or both >120 s duration, 6: fatal VF starting at >15 min after occlusion, 7: fatal VF starting between 4 and 14 min 59 s, 8: fatal VF starting between 1 and 3 min 59 s, and 9: fatal VF starting <1 min after occlusion.

2.8. Biochemical analysis

Blood samples were collected at the end of each experiment. Plasma samples were extracted and stored at −70 °C until they were assayed. The creatine kinase-MB (CK-MB) isoenzyme and lactate dehydrogenase (LDH) levels were analyzed using ELECSYS System (ELECSYS 2010, Roche, Germany) and commercial kits (Pars Azmoon, Iran).
2.9. Materials

Oxytocin, atosiban, 2, 3, 5-triphenyltetrazolium chloride and Evans blue were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.10. Statistical analysis

Statistical analysis of hemodynamic data within and between groups was performed with two-way analysis of variance. Differences in infarct size, CK-MB and LDH plasma levels were determined by one-way analysis of variance. Groups were individually compared using Dunnet’s test. Arrhythmia scores were analyzed with Kruskal–Wallis test, and the incidences of VT or VF were compared by Fisher exact test. All data were expressed as mean±S.E.M. Statistical significance was defined as P<0.05.

3. Results

3.1. Hemodynamic data

The results depicted in Table 1 show that heart rate (HR), mean arterial pressure (MAP) and rate pressure product (RPP) slightly but not significantly decreased in all groups at the end of ischemia and reperfusion compared to their baseline. There were no significant differences of hemodynamic parameters in baseline, ischemia and reperfusion periods among groups (Table 1).

3.2. Area at risk and infarct size measurement

AAR was not different among groups. Infarct size was decreased significantly in OT and IPC groups compared to IR (P<0.05).

Administration of atosiban in IPC+ATO group increased infarct size to 39±0.9% in comparison with OT and IPC groups (P<0.05) (Fig. 2).

3.3. Ventricular arrhythmias during ischemia

3.3.1. Severity of arrhythmias

The use of OT and IPC prior to ischemia significantly declined ventricular arrhythmias severity in compared to IR group (P<0.05). Administration of atosiban in IPC+ATO group intensified severity of arrhythmias in compared with OT and IPC groups (P<0.05) (Fig. 3).

3.3.2. Incidences of VT and VF

In IR group, all animals were experienced VT, while VF occurred in 33% of hearts. The use of OT and IPC attenuated VT incidence to 50% and 43%, respectively (P<0.05) and abolished VF incidence completely. In comparison with OT and IPC groups, administration of atosiban restored the incidence of VT to 100% and VF to 50% in IPC+ATO group (P<0.05). Atosiban did not change significantly incidences of VT and VF in comparison with IR group (Fig. 4).

3.4. Biochemical analysis

The use of OT and IPC significantly decreased CK-MB and LDH plasma levels in comparison with IR group at the end of reperfusion period (P<0.05). Atosiban administration in IPC+ATO group significantly increased CK-MB and LDH plasma levels (P<0.05) (Fig. 5A and B).

4. Discussion

IPC has powerful cardioprotective effects against ischemia, which remains unmatched by other therapeutic agents. Our study shows

![Fig. 2. Myocardial area at risk (AAR/V%) and infarct size (IS/AAR%) in IR, OT, IPC, IPC+ATO and IR+ATO groups. Data are presented as mean±S.E.M. *P<0.05 vs. IR group. **P<0.05 vs. IPC group. ***P<0.05 vs. OT group. IR = ischemia–reperfusion; OT = oxytocin; IPC = ischemic preconditioning; and ATO = atosiban.]

![Fig. 3. Distribution of the arrhythmia score during 25 min ischemia in IR, OT, IPC, IPC+ATO and IR+ATO groups. Data are presented as mean±S.E.M. *P<0.05 vs. IR group. **P<0.05 vs. IPC group. ***P<0.05 vs. OT group. IR = ischemia–reperfusion; OT = oxytocin; IPC = ischemic preconditioning; and ATO = atosiban.]

Table 1

<table>
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<td>HR</td>
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<td>MBP</td>
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<tr>
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and ATO = atosiban.

Fig. 4. The incidence of ventricular fibrillation (VF) and ventricular tachycardia (VT) during 25 min ischemia in IR, OT, IPC, IPC + ATO and IR + ATO groups. Data are presented as mean ± S.E.M. *P < 0.05 vs. IR group. #P < 0.05 vs. IPC group. $P < 0.05 vs. OT group. IR = ischemia-reperfusion; OT = oxytocin; IPC = ischemic preconditioning; and ATO = atosiban.

Fig. 5. The effects of OT, IPC and ATO on plasma levels of LDH (A) and CK-MB (B) in IR, OT, IPC, IPC + ATO and IR + ATO groups. Data are presented as mean ± S.E.M. *P < 0.05 vs. IR group. #P < 0.05 vs. IPC group. $P < 0.05 vs. OT group. IR = ischemia-reperfusion; OT = oxytocin; IPC = ischemic preconditioning; and ATO = atosiban.

that cardioprotection induced by IPC can be abolished by an OT receptor selective antagonist. This suggests that endogenous OT may be involved in the protective effects of IPC in the intact rat heart.

More recently, OT has been considered to be a cardiovascular hormone [14,15]. It is produced and released by the heart and acts on its receptors to decrease cardiac rate and force of contraction [15]. Costa-E-Sousa et al. [24] have reported that endogenous OT may play an important role in regulation of vascular and cardiac function. The results of the present study show that pretreatment with OT receptor antagonist can prevent IPC-induced cardioprotection in the anesthetized rat. We also showed that atosiban administration cannot induce significant changes on hemodynamic parameters. This suggests that endogenous OT preconditioning effects on myocardial injury may not be related to hemodynamic parameters. One possible mechanism is that OT modulates energy demand and myocardial contractile state followed by a myocardial oxygen consumption reduction [31]. Recent study also showed exogenous OT has direct negative chronotropic effects [24]. Slow heart rate causes a decrease in myocardial consumption of oxygen, nutrients and an increase in subendocardial blood flow per beat that can improve regional contractile function [10]. It may be important for oxytocin cardioprotective effects in experimental condition.

In our study, changes in infarct size correlate well with the antiarrhythmic effects of IPC. Therefore, it appears that the extent of myocardial infarction may directly affect arrhythmia. Blockade of OT receptor rather offsets endogenous OT effects on ventricular arrhythmias and infarct size. On the other hand administration of atosiban significantly abolished the cardioprotective effects of IPC, but it did not completely increase infarct size to the level of IR group which means other mechanisms may be involved in IPC. However, the mechanism of antiarrhythmic effects may be different. The present investigation is in agreement with previous reports in which the cardioprotection induced by exogenous OT could be abolished when rats were treated with atosiban prior to ischemia [10, 27, 28].

Elevated level of CK-MB as a biochemical marker of myocyte necrosis [32] has also been increased by atosiban when administrated just before IPC. Increased serum LDH level, a systemic tissue damage marker [33], has been raised. OT treatment has also attenuated the renal IR-induced LDH response [26]. Similarly elevated serum LDH level in acetic acid-induced colitis was significantly decreased by OT treatment [34]. All of these findings suggest that endogenous OT exerts beneficial effects on the ischemic reperfused hearts. Since the effects of endogenous OT occur in the absence of detectable changes in systemic and coronary hemodynamics, it’s in vivo effects on ischemic myocardium are likely depends upon direct cytoprotection at the cellular level. There are only limited numbers of published studies showing the anti-ischemic effects of OT [25, 26, 35]. Based on anti-inflammatory actions and preventing free radical damaging cascades [34]. Its protective effect in colon, liver and kidney appears to be dependent on its inhibitory effect on neutrophil infiltration and associated production of reactive oxygen species [25, 26, 36]. Recently, a study also showed that OT may exert an alternate role by regulating and maintaining a balance of anti-inflammatory and pro-inflammatory cytokines within the injured heart in rat [37]. Accordingly, the anti-inflammation, antioxidant effect, and anti-inflammatory and pro-inflammatory cytokines balance of OT on cardiac tissue may be the cause of its protection.

The mechanism of protection of endogenous OT in cardiomyocytes requires further study. Nevertheless, the cytoprotective effect of endogenous OT on cardiomyocytes could have important therapeutic implications. Our study shows that endogenous OT can augment the effect of ischemic preconditioning. These results suggest that endogenous OT is not a major mediator of ischemic preconditioning, but the effect of ischemic preconditioning can be enhanced by endogenous OT. The major finding of the present study is that endogenous OT plays an important role in the cardioprotective effects of IPC.
5. Conclusion

In conclusion, this study shows that, in part, the cardioprotective effects of IPC can be induced by endogenous OT.

Acknowledgment

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References