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ORIGINAL ARTICLE

Role of Atrial Natriuretic Peptide in Oxytocin Induced Cardioprotection

Fariba Houshmand, PhD [a], Mahdieh Faghihi, PhD [b*], Saleh Zahediasl, PhD [c]

[a]Department of Physiology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Islamic Republic of Iran
[b]Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran
[c]Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran

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Background The purpose of this study was to determine whether endogenous atrial natriuretic peptide (ANP) contributes to the protective effect of neurohypophysial hormone oxytocin (OT) in heart preconditioning.

Methods Sprague–Dawley male rats were subjected to 25 min regional ischaemia and 120 min reperfusion and were divided into eight groups. Oxytocin or an equivalent volume of saline was administrated intraperitoneally, 30 min before ischaemia. The OT receptor antagonist (atosiban), ANP receptor antagonist (anantin) and nitric oxide synthase inhibitor (L-NAME) were injected 10 min before OT. In other groups, atosiban, anantin and L-NAME were only administered 40 min prior to ischaemia.

Results Compared with the ischaemia/reperfusion group (I/R), alterations in infarct size, biochemical parameters [LDH (lactate dehydrogenase), CK-MB (creatine kinase-MB), MDA (malondialdehyde) plasma levels] and severity of ventricular arrhythmia due to I/R injury were attenuated and VF was abolished by OT treatment. These OT effects were eliminated by OT and ANP receptor blockers and nitric oxide synthase inhibitor, but anantin did not reverse the effect of OT in lipid peroxidation.

Conclusions These findings demonstrate an important contributory role of ANP in the OT induced protection in myocardial ischaemia reperfusion. OT also reduced lipid peroxidation with a NO-dependent mechanism.

Keywords Oxytocin • Atrial natriuretic peptide • Ischaemia reperfusion • Oxidative stress • Preconditioning.

Introduction

Oxytocin, neurohypophyseal nonapeptide hormone, plays important roles in regulation of blood pressure, vascular tone and cardiovascular function [1]. Oxytocin is also synthesised in cardiac myocytes and exerts important cardiovascular effects directly via action on its receptors [2], which are coupled to G proteins [1].

Previous study has shown that hormone preconditioning with OT protects the heart against ischaemia and OT receptors contribute to the process of cardioprotection [3]. The cardioprotective effect of OT may include direct effects on the ischaemic myocardium. It may also indirectly impact the release of other mediators such as atrial natriuretic peptide (ANP). This was based on the observations that OT via neuroendocrine/endocrine/ paracrine pathways release ANP from the heart, which then exerts a negative chrono- and inotropic effect via activation of guanylyl cyclase and release of cGMP [4–6].

Natriuretic peptides are important factors involved in the regulation of hydroelectrolyte balance and vascular tone, inducing hypotension, natriuresis and diuresis as well as cardioprotection in myocardial ischaemia both as circulating hormones and as local autocrine and/or paracrine factors [7]. ANP exerts its biological actions through, and the interaction with, two specific membrane-bound guanylyl cyclase receptors: natriuretic peptide receptor A (NPR-A) and B (NPR-B) [8].

*Corresponding author at: Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.
Tel.: +98 21 66419484; facsimile: +98 21 66419484., Email: faghihim@tums.ac.ir

On the other hand, recent evidence demonstrated that the ANP exerts its vasodilatory action through nitric oxide (NO) generation [9].

As oxytocin and ANP act in concert to control vascular homeostasis and the body's internal environment, it is possible that the protective effect of OT is mediated through endogenous ANP; therefore, the purpose of this study was to determine whether endogenous ANP contributes to the protective effect of OT in the preconditioning of the heart. To test this hypothesis, we used the competitive NPR-A antagonist anantin to block any ANP-mediated effect. The reason for this was due to the fact that ANP exerts its cardioprotective effects by means of NPR-A/ cGMP pathway [7].

Haemodynamic parameters, infarct size, biochemical indexes as well as the ventricular arrhythmia assessments were used to evaluate the effects.

Materials and Methods

Animals

Male Sprague–Dawley rats weighing 280–310 g, were maintained under controlled conditions (temperature 20–22 °C, humidity 40–50% and 12 hrs light- dark cycle), fed on a standard diet with free access to water. All experimental procedures were carried in accordance with the institutional guidelines of Tehran University of Medical Sciences (Tehran, Iran).

Surgical Preparation

The animals were anaesthetised with sodium pentobarbital (60 mg/kg, i.p.). After tracheal intubation, artificial respiration with room air-and-oxygen mixture was instituted by use of a rodent ventilator (model 683, Harvard Apparatus) with a stroke volume of approximated 1.2 ml 100 g^{-1} body weight at a rate of 60–70 stroke min^{-1}. The right carotid artery was cannulated for blood sampling and cardiac monitoring throughout the experiment, using a computerised data acquisition system (ML750 PowerLab/4sp, ADInstruments). A left thoracotomy was performed at the fourth intercostal space followed by a pericardiotomy. A 6-0 silk suture was passed below the left anterior descending coronary artery (LAD) for later induction of coronary artery occlusion. After 25 min, the suture was removed, and animals were reperfused for 120 min. Heart rate and blood pressure were stabilised for 10 min before the following protocols were initiated. At the end of 120 min reperfusion, infarct size and myocardial enzyme leakage were measured.

Experimental Protocol

The animals were divided into eight groups. (i) The control group (cardiac I/R): following a 10 min stabilisation period, rats (n = 10) were submitted to 25 min of ischaemia, 120 min reperfusion. Saline was administered i.p. 30 min before hearts were subjected to LAD occlusion. (ii) Oxytocin treated group: six animals received oxytocin (Sigma Chemical Co. St. Louis, MO, USA) 0.01 µg/rat [3], 30 min before ischaemia. (iii) Atosiban treated group (ATO + OT, n = 6); this group was pretreated with OT-receptor antagonist, atosiban (0.5 µg/rat, i.p., Sigma Chemical Co. St. Louis, MO, USA) 10 min before OT. (iv) Anantine treated group (ANA + OT, n = 7): anantin (1.2 mg/kg, i.p., Sigma Chemical Co. St. Louis, MO, USA) was administrated 10 min before OT to explore the role of ANP receptor-mediated in OT-cardioprotection. (v) L-NAME treated group (L-NAME + OT, n = 7): this group received 0.2 mg/kg nitric oxide synthase inhibitor (Sigma Chemical Co. St. Louis, MO, USA), intraperitoneally 10 min prior to the OT treatment.

In (vi) ATO (n = 7), (vii) L-NAME (n = 8) and (viii) ANA (n = 8) groups alone, inhibitors were used 40 min prior to LAD occlusion.

Haemodynamic Variables

Arterial blood pressure and heart rate (HR) were continuously monitored and recorded throughout the experiment; 15 min baseline, preocclusion, 25 min LAD occlusion, and 120 min reperfusion. At the same time-points, the rate pressure product (RPP), systolic blood pressure multiplied by the heart rate, also were calculated by the data acquisition system.

Area at Risk and Myocardial Infarct Size Determination

The area at risk (ischaemic-reperfused region) and the infarct size were measured using Evans blue and 2,3,5- triphenyltetrazolium chloride double staining method (TTC). In brief, LAD was reoccluded and 2 ml of a 2% Evans blue solution was injected via the tail vein at the end of 120 min reperfusion. Then, the heart was removed, and immediately frozen at -20 °C. To define infarct size, hearts were sliced transversely then stained in triphenyltetrazolium chloride (TTC, in 0.1 M phosphate buffer, pH 7.4) at 37 °C for 15 min, followed by incubation in 10% formalin for 24 h to increase contrast. Total area at risk was expressed as a percentage of the left ventricles (AAR/LV). The infarct size was determined as the ratio between the pixel of necrotic area (NA) and ischaemic area at risk (AN/AAR), using Adobe Photoshop 7.0.

Biochemical Analysis

At the end of reperfusion period, blood samples were collected to measure the myocardial enzyme leakage, including lactate dehydrogenase (LDH), creatinine kinase (CK-MB) and malonyldialdehyde (MDA). The heparinised blood samples were centrifuged and obtained plasma was analysed by using an autoanalyser (Roche Hitachi Modular DP Systems, Mannheim, Germany) for determination of CK-MB and LDH levels, MDA was determined by spectrophotometrically.

Arrhythmia Analysis

Electrocardiograms (ECGs) were continuously monitored with standard limb lead-II throughout the experiment. Both ischaemia and reperfusion induced ventricular arrhythmias were determined in accordance with the Lambeth

Conventions [10]. Premature ventricular contractions (PVC) were defined as identifiable premature QRS complexes. Ventricular tachycardia (VT) was defined as four or more consecutive premature ventricular contractions (PVC). Ventricular fibrillation (VF) was defined as a signal that changed from beat to beat in rate and configuration or where individual QRS-deflections could not easily be distinguished from one another. The severity of arrhythmias was quantified by the following scoring system [11] **0**: <10 PVC, 1: ≥10 PVC, 2: VT (duration <30 s), 3: VT (duration ≥30 s), 4: VF starting 15 min after the onset of ischaemia, 5: VF starting 5–15 min after the onset of ischaemia, 6: VF starting within 5 min after the onset of ischaemia.

Statistical Analysis

All data were presented as mean ± SEM or the percentage of incidence. The repeated measures ANOVA were used to compare the haemodynamic parameters within groups. One-way ANOVA followed by the Duncan's test was used to compare differences in haemodynamic parameters, infarct size, CK-MB, LDH and MDA plasma content between groups. $P < 0.05$ was considered statistically significant.

Results

Haemodynamic Variables

There were no significant differences among groups at the baseline before treatment. In control animals (I/R group), coronary occlusion decreased RPP significantly ($P < 0.05$). During 120 min of reperfusion, systolic arterial pressure (SAP) and RPP reduced ($P < 0.05$) without a significant changes in the heart rate.

Before coronary artery occlusion, neither atosiban nor anantine nor L-NAME pretreatment influenced significantly the haemodynamic parameters in comparison with the baseline (data not shown). Anantin (ANA + OT group) induced a significant reduction in HR as compared to baseline when OT was administrated ($P < 0.05$, Table 1).

In the OT group, ischaemia had nearly no influence on haemodynamic variables except for SAP which was significantly increased compared to the control group, after a 120 min reperfusion period ($P < 0.05$). Pretreatment with anantin (ANA+OT group) significantly decreased HR and RPP during ischaemia/reperfusion compared to their baseline. It also had significantly reduced SAP during reperfusion. As compared to OT group, anantin induced significant reduction in HR during ischaemia. As shown in Table 1, L-NAME (L-NAME + OT) caused significant increase in RPP and SAP during reperfusion; anantin (ANA + OT) and atosiban (ATO + OT) also significantly enhanced SAP compared to control this time period. No significant differences of haemodynamic parameters were observed in other groups.

Area at Risk and Infarct Size Measurements

Fig. 1 represents the area at risk (AAR/LV) and the necrotic area (NA/AAR) as a percentage of area at risk. There were no significant differences in the area at risk among groups. Oxytocin significantly reduced myocardial infarction compared with control group ($P < 0.05$). This protective effect was totally abolished by atosiban, anantin and L-NAME. Treatment with atosiban, anantine and L-NAME alone had no significant effect on infarct size compared to I/R group.

Biochemical Analysis

Compared to the control group, administration of OT could prevent elevation of LDH and CK-MB activity in plasma after ischaemia reperfusion injury. This reduction was significantly abolished by atosiban, anantin and L-NAME

Table 1 **Haemodynamic values.**

Group	Basal			OT			Ischaemia			Reperfusion		
	SAP	HR	RPP	SAP	HR	RPP	SAP	HR	RPP	SAP	HR	RPP
Control	116 ± 7	366 ± 12	43 ± 3				104 ± 10	349 ± 16	37 ± 4†	89 ± 7†	324 ± 15	31 ± 3†
OT	126 ± 8	363 ± 8	46 ± 2	132 ± 5	360 ± 10	47 ± 3	128 ± 8	358 ± 13	43 ± 5	116 ± 6*	338 ± 16	38 ± 3
ATO + OT	115 ± 2	345 ± 11	40 ± 1	124 ± 4	320 ± 7	40 ± 1	117 ± 7	320 ± 10	37 ± 3	115 ± 5*	322 ± 8	37 ± 2
ANA + OT	124 ± 5	360 ± 10	45 ± 3	128 ± 7	340 ± 2†	44 ± 4	110 ± 10	316 ± 14$^{\dagger,\sim}$	35 ± 3†	118 ± 5*	326 ± 14†	39 ± 2†
L-NAME + OT	123 ± 5	341 ± 14	42 ± 3	130 ± 5	334 ± 8	43 ± 2	112 ± 4	325 ± 7	37 ± 1	117 ± 7*	339 ± 13	40 ± 3*
ATO	117 ± 5	350 ± 8	41 ± 2				111 ± 5	325 ± 14	36 ± 2	110 ± 7	319 ± 17	35 ± 3
ANA	121 ± 4	345 ± 11	42 ± 3				102 ± 7	325 ± 12	32 ± 2	107 ± 6	323 ± 18	34 ± 2
L-NAME	124 ± 5	355 ± 12	44 ± 2				108 ± 5	328 ± 16	35 ± 2	111 ± 6	326 ± 11	36 ± 3

SAP (systolic arterial pressure, mmHg), HR (heart rate, beats/min), RPP (rate pressure product, beat min^{-1} mmHg × 10^3). OT (oxytocin), ATO (atosiban), ANA (anantin). Values are presented as the mean ± SEM.

$^{\dagger}P < 0.05$, vs. baseline within the same group.

$^{*}P < 0.05$ vs. control,

$^{\sim}P < 0.05$, vs. OT.

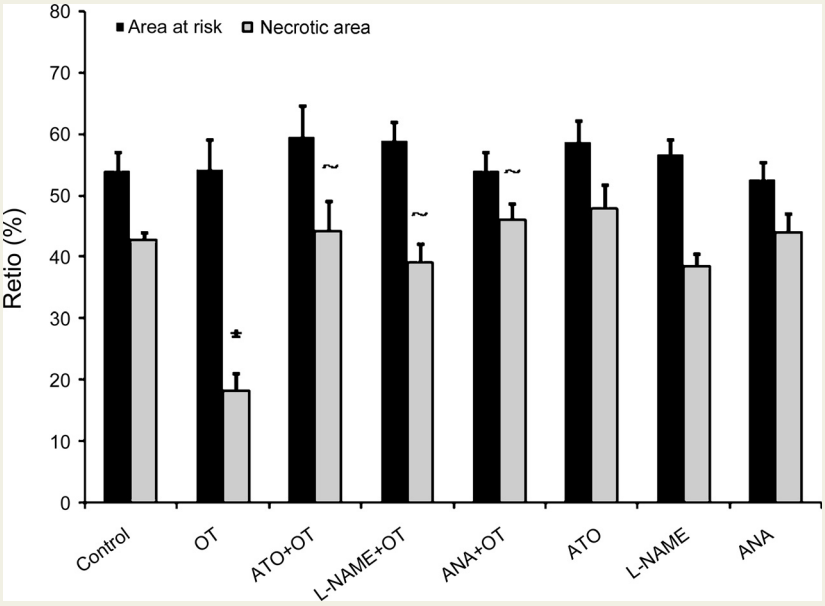

Figure 1 Myocardial area at risk (AAR/LV %) and necrotic area (NA/AAR %). OT (oxytocin), ATO (atosiban), ANA (anantin) and L-NAME. The values are expressed as the mean ± SEM.
*P < 0.05 vs. control, ~P < 0.05 vs. OT.

administration. The level of LDH was significantly higher than control group in L-NAME + OT group (Table 2).

Malonyldialdehyde (MDA) plasma level in OT treated group was significantly reduced compared to control group. When atosiban and L-NAME was administrated before OT, the level of MDA was significantly increased, but the anantin-receiving animals had MDA level similar to those of the OT group. Inhibitor alone had no significant effect on MDA, LDH and CK-MB plasma level compared to I/R group.

Table 2 Plasma levels of LDH, CK-MB and MDA at the end of reperfusion.

Group	LDH (U/L)	CK-MB (U/L)	MDA (nmol/ml)
Control	1836 ± 215	2273.8 ± 238	3.3 ± 0.2
OT	850 ± 75*	1462 ± 138*	2.7 ± 0.15*
ATO + OT	923 ± 154	2604 ± 365~	3.3 ± 0.2~
ANA + OT	1979 ± 145~	2741 ± 220~	2.3 ± 0.15*
L-NAME + OT	2239 ± 160*,~	2523 ± 288~	3.4 ± 0.25~
ATO	1064 ± 109	2522 ± 256	3.1 ± 0.2
ANA	1996 ± 155	2487 ± 277	2.7 ± 0.2
L-NAME	1916 ± 155	2486 ± 276	3.5 ± 0.3

LDH (lactate dehydrogenase), CK-MB (creatine kinase-MB), MDA (malondialdehyde). OT (oxytocin), ATO (atosiban), ANA (anantin). The values are expressed as the mean ± SEM.
*P < 0.05, vs. control.
~P < 0.05, vs. OT group.

Ventricular Arrhythmias During Ischaemia

In the control group, all rats experienced VT, while VF occurred in 80% of the hearts, and the arrhythmias score (AS) averaged 4.7 ± 0.2.

In OT treated group, the total PVC and AS were significantly decreased during occlusion period compared to the control (P < 0.05). In addition, no ventricular arrhythmia (VT and VF) was observed during ischaemia in the OT group.

Oxytocin and ANP receptor blockers in ATO+OT and ANA + OT groups and nitric oxide inhibitor in L-NAME + OT group increased arrhythmias score and abolished the cardioprotective effect of OT on arrhythmias (Figs. 2–4). The number of episodes of VT in L-NAME + OT and duration of VT in ANA + OT group was markedly enhanced compared to ATO + OT group (Fig. 3).

The incidence of VT was 0% in the OT group and 100% in the control, ATO + OT, ANA + OT and L-NAME + OT groups.

Compared with the control rats, OT abolished VF. Administration of atosiban, anantin and L-NAME prior to OT increased the incidence of VF to 33.3% (P > 0.05), 57.2% and 57.1% (P < 0.05) respectively, (vs. to 0% in OT). Moreover, duration of ventricular fibrillation (VF) in ANA + OT group was markedly enhanced compared to control group (Fig. 4). Atosiban, anantin and L-NAME alone had no significant effect on ventricular arrhythmias (Figs. 2–4).

Ventricular Arrhythmias During Reperfusion

The duration of ventricular tachycardia (VT) in the OT group was shorter than the other groups and only two out of six rats

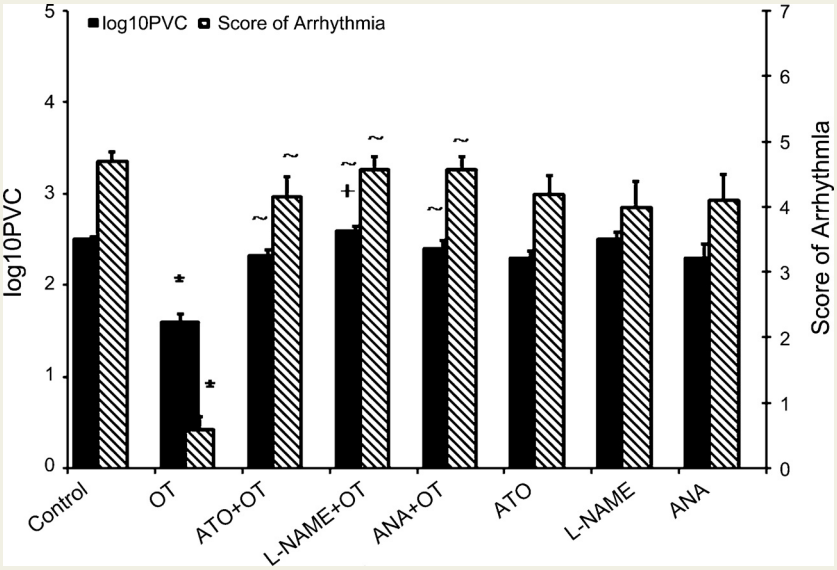

Figure 2 Distribution of the score of arrhythmia and $\log_{10}$ PVC during ischaemia. OT (oxytocin), ATO (atosiban), ANA (anantin). The values are expressed as the mean ± SEM.
*P < 0.05 vs. control, ~P < 0.05 vs. OT, +P < 0.05 vs. ATO + OT.

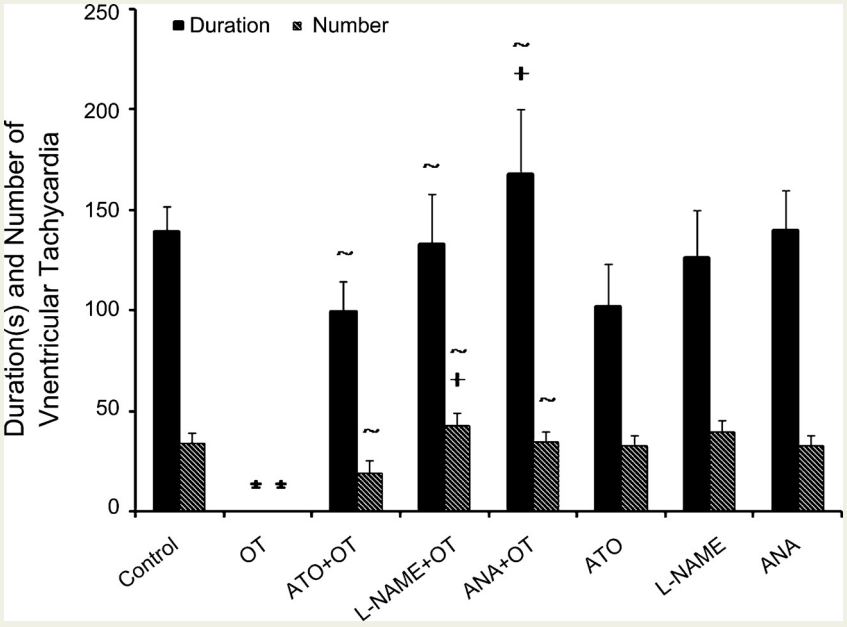

Figure 3 Duration (s) and episode number of VT (ventricular tachycardia) during ischaemia. OT (oxytocin), ATO (atosiban), ANA (anantin). The values are expressed as the mean ± SEM.
*P < 0.05 vs. control, ~P < 0.05 vs. OT, +P < 0.05 vs ATO + OT.

had VT with a mean of 0.4 ± 0.2 episodes. Rats pretreated with OT experienced not only a significantly reduced PVC, but also experienced a significant reduction in the AS (0.8 ± 0.5) compared with the control group.

The incidence of VT was significantly less in OT and ATO + OT groups than in L-NAME + OT group. The atosiban (ATO + OT) group had fewer PVC than the L-NAME + OT and ANA + OT groups. Both anantin and L-NAME administration before OT resulted in a marked increase in the duration of VT (Table 3). Atosiban, L-NAME and anantin by themselves had no significant effect on arrhythmias.

Discussion

In order to explore the mechanisms involved in the OT-induced early preconditioning against ischaemia and

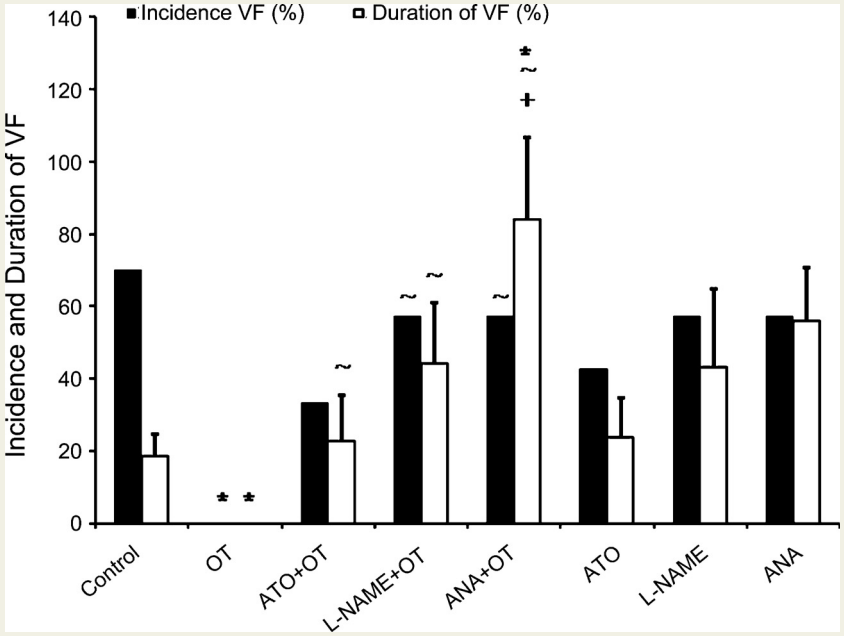

Figure 4 The incidence and duration of ventricular fibrillation (VF) during ischaemia. OT (oxytocin), ATO (atosiban), ANA (anantin). The values are expressed as the mean ± SEM.
*P < 0.05 vs. control, ~P < 0.05 vs. OT, +P < 0.05 vs. ATO + OT.

Table 3 **The severity of arrhythmias during reperfusion.**

Group	Log_{10} PVC	Duration of (s) Arrhythmia(s)		Episode of Arrhythmias		Incidence of (%)		Arrhythmia Score
		VT	VF	VT	VF	VT	VF	
Control	1.3 ± 0.2	6 ± 3	0 ± 0	2 ± 0	0 ± 0			2 ± 0
OT	0.6 ± 0.1*	1.6 ± 1.4	0 ± 0	0.4 ± 0.2	0 ± 0	33.3	0	0.8 ± 0.5*
ATO + OT	0.8 ± 0.3	8.1 ± 4	0 ± 0	0.7 ± 0.3	0 ± 0	50	0	1.5 ± 0.2
ANA + OT	1.7 ± 0.3~	16 ± 9	6.9 ± 6.9	3.4 ± 2	0.4 ± 0.4	71.4	14.3	3 ± 0.3~
L-NAME + OT	1.2 ± 0.1~	15.1 ± 5~	0 ± 0	2 ± 0.6	0 ± 0	100~,+	0	2.1 ± 0.1~
ATO	0.8 ± 0.2	9.4 ± 3	0 ± 0	1 ± 0.4	0 ± 0	71	0	1.7 ± 0.2
ANA	1.5 ± 0.2	11 ± 5	5.7 ± 3.7	3 ± 0.8	0.7 ± 0.5	57	25	2.3 ± 0.5
L-NAME	1.4 ± 0.2	16.3 ± 5	0.9 ± 0.9	2.4 ± 0.7	0.1 ± 0.1	86	14.3	2.4 ± 0.3

PVC (premature ventricular contraction), VT (ventricular tachycardia), VF (ventricular fibrillation). OT (oxytocin), ATO (atosiban), ANA (anantin). Data are presented as mean ± SEM.
*P < 0.05 vs. control,
~P < 0.05 vs. OT.

ventricular arrhythmias in the rat heart in vivo, we investigated the potential role of natriuretic peptide pathways. In the present study, we found that blockade of the ANP receptors by anantin, a competitive NPR-A antagonist [12], prevented the cardioprotective properties of OT, indicating that the ANP pathway plays a prominent role in OT-induced cardiac protection. The involvement of NO in oxytocin reduced lipid peroxidation was also determined.

Although there is evidence suggesting the protective role for ANP against I/R injury in the heart, this is the first study to our knowledge that shows a link between OT and ANP in the regulation of protection on myocardial I/R injury. This is also supported by a recent study which suggests the role of the oxytocin-ANP system in cardiomyogenesis [13]. In a recent study on diuresis and natriuresis effects of OT, ANP pathway involvement was proposed [6]. Similarly, others have demonstrated that OT plays an important role in cardiovascular homeostasis by regulating blood volume via ANP release from cardiac atria [5,6].

In the present study, OT significantly reduced I/R-induced heart damage with lower changes of haemodynamic values than those in control group. In spite of vasodilatory effects of

ANP, blockade of ANP receptor (NPR-A), in ANA + OT group, had no effect on haemodynamic parameters before induced occlusion, but significantly reduced these parameters during I/R. In agreement with our results, Chowdrey et al. [14] and Takata et al. [15] have demonstrated that ANP has no significant influence on haemodynamic variables when it was added before ischaemia.

The plasma level of LDH and CK-MB, associated with heart ischaemia reperfusion injury, were also significantly lower after reperfusion in the OT group than in the control group. Furthermore, OT prevents lipid peroxidation in the ischaemic reperfused myocardium. Malondialdehude (MDA), a biomarker of cardiac oxidative injury, is a product of lipid peroxidation that can be produced by a variety of oxidative damages [16]. Biyikli et al. has been reported that OT reduces oxidant renal injury in pyelonephritic rats by its antioxidant actions [17].

In addition, OT exerts antioxidant effects on vascular smooth muscle cells, aortic ECs, and macrophages through attenuation of NADPH-oxidase-dependent superoxide production [18]**.** The antioxidant ability of OT was also responsible for the renoprotective properties of OT [19].

In our study, unlike increased LDH and CK-MB levels, the plasma level of MDA was not significantly changed by anantin, as compared to OT. Therefore, possibly other signalling pathway(s) than ANP are involved in antioxidative properties of OT. On the other hand, OT receptor activation and NO production have a significant role in this effect of oxytocin. Nitric oxide can function as an in vivo antioxidant [20]; hence, OT, through NO, may function as an effective antioxidant.

OT-induced infarct size reduction was reversed by anantin and L-NAME. These results may imply that infarct size reduction in response to oxytocin mediated by OT/ANP/NO pathway, as it was previously demonstrated that OT leads to the release of ANP, which then stimulates the NO production [9]. In agreement, Okawa et al. demonstrated that NO is involved in the ANP induced infarct size reducing effect through NO-mitochondrial ATP-sensitive K^+ (mK_{ATP}) channel-dependent pathway [21]**.**

However, in the control group, both regional coronary occlusion and its reperfusion induced ventricular arrhythmia, reperfusion of the ischaemic myocardium led to less severity of arrhythmias in all groups under study. The results of the present study showed that OT completely prevented episodes of VF induced both in occlusion and reperfusion period. Oxytocin significantly decreased PVC and lowered the arrhythmias severity index compared with control and prevented VT in occlusion period. Treatment with atosiban abolished OT-reduced arrhythmia, indicating a receptor-mediated effect. Our data also revealed that blockade of ANP and NO pathway(s) abolished this antiarrhythmic effect. This finding demonstrates the role of ANP and NO system on the anti-arrhythmic effects of OT during both ischaemia and reperfusion phases. Although drug (atosiban, anantin and L-NAME) pretreatment prevented OT effects and increased the severity of lethal arrhythmias during ischaemia and reperfusion periods, the major effect was observed in ischaemia phase as significant increase in VT and VF. On the other hand, since during the reperfusion, VF was increased by anantin, we assume that ANP pathway plays an important role in anti-VF effects of OT.

It seems that anti-lipid peroxidation property of OT, functioning as an antioxidant is not the cause of antifibrillation effect, because natriuretic peptide is not involved in this OT action. In contrast to ANP, nitric oxide not only eliminated the anti-lipid peroxidation effect of oxytocin but also decreased the production of fibrillation. Since anti-VF effect of oxytocin appears to be independent of lipid peroxidation, thus, possibly, anti-lipid peroxidation, anti-arrhythmic and anti-ischaemic effects of OT induced by different pathways (or mechanisms).

In addition, the cardioprotective action of ANP in I/R injury was shown to be mediated through the nitric oxide [21], elevation of cGMP levels [22] and by PKC, PKG dependent transduction mechanisms [21,23] followed by mK_{ATP} channel activation. Okawa et al. also demonstrated that preischaemic infusion of synthetic ANP has myoprotective effects against I/R through NO- mK_{ATP} channel-dependent pathway [21]. Then, it is possible that OT effect on mK_{ATP} channels [24] was indirect and comes from the ANP action on this channel. Further studies are required to discriminate the specific role of OT and ANP in mediating K_{ATP} channel activity.

Based on our observation, anantin abolished OT effects through blockade of NPR-A receptor activation, which is coupled to an increase in the intracellular concentration of cGMP. Thus, decreased cGMP generation by inhibition of NPR-A receptor may be involved in abolishing OT preconditioning. However, the literature has proposed an involvement of cGMP as a messenger in multiple physiologic actions of OT [1], but the data obtained in this study do not allow us to conclude the involvement of cGMP-mechanism in the OT- precondition effect.

Additionally, intracardiac OT is essential for basal release of ANP in the heart [6] and OT receptors are associated with the ANP-cGMP and NO-cGMP pathways, which reduce the force and rate of contraction and increase vasodilatation [1]**.**

Since no significant differences were observed between the two groups of ANA+OT and L-NAME+OT in infarct size and ventricular arrhythmia, it is possible that OT exerts cardioprotective effects through OT/ANP/NO pathways. In addition, unlike L-NAME, anantin cannot change the effect of oxytocin in reducing lipid peroxidation. Therefore, the possible mechanism of OT/ANP/NO pathway is not involved in both the anti-ischaemic and anti-lipid peroxidation effects of oxytocin. Hence, based on our findings, it seems that OT exerts cardioprotective effects through OT/ANP/NO and OT/NO pathway independently; however, further investigations are needed.

Conclusion

Although further studies are needed to clarify the contribution of other transduction pathways or mechanisms, our

findings indicate that OT exerts its protective effect through ANP pathway and also via attenuating lipid peroxidation.

Acknowledgments

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