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RISK pathway is involved in oxytocin postconditioning in isolated rat heart

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ABSTRACT

The reperfusion injury salvage kinase (RISK) pathway is a fundamental signal transduction cascade in the cardioprotective mechanism of ischemic postconditioning. In the present study, we examined the cardioprotective role of oxytocin as a postconditioning agent via activation of the RISK pathway (PI3K/Akt and ERK1/2).

Animals were randomly divided into 6 groups. The hearts were subjected under 30 minutes (min) ischemia and 100 min reperfusion. OT was perfused 15 min at the early phase of reperfusion. RISK pathway inhibitors (Wortmannin; an Akt inhibitor, PD98059; an ERK1/2 inhibitor) and Atosiban (an OT receptor antagonist) were applied either alone 10 min before the onset of the ischemia or in the combination with OT during early reperfusion phase. Myocardial infarct size, hemodynamic factors, ventricular arrhythmia, coronary flow and cardiac biochemical marker were measured at the end of reperfusion.

OT postconditioning (OTpost), significantly decreased the infarct size, arrhythmia score, incidence of ventricular fibrillation, Lactate dehydrogenase and it increased coronary flow. The cardioprotective effect of OTpos was abrogated by PI3K/Akt, ERK1/2 inhibitors and Atosiban.

Our data have shown that OTpost can activate RISK pathway mostly via the PI3K/Akt and ERK1/2 signaling cascades during the early phase of reperfusion.

1. Introduction

Cardiac Ischemic postconditioning (Ipost) is an influential cardioprotective phenomenon in which one or more brief episode of ischemia–reperfusion immediately applied after a sustained occlusion of a coronary artery [26,47,50]. Some endogenous peptides (Bradykinin, opioids and adenosine) play an important role for triggering Ischemia cardiac conditioning [27,57]. The major cardioprotective signal transduction cascade of IPC and Ipost includes the reperfusion injury salvage kinase (RISK; PI3K/Akt, ERK1/2) pathway [22]. The RISK pathway has a determinant role in the genesis of the cardioprotective effect of IPC and is thought to converge at the mitochondria where it may interfere with several mediators including: protein tyrosine kinase, mitogen-activated protein kinase, protein kinase C (PKC) and reactive oxygen species (ROS) [41,60]. The modification of the RISK pathway has been known as the factor responsible for increasing tolerance to ischemia/reperfusion (IR) injuries. Thus, finding new endogenous IPC agents is an ideal research target for cardioprotection against IR injuries.

Oxytocin (OT) is a peptide-like hormone related to reproductive phenomena and a potent IPC agent [2–4,53]. OT and OT receptors (OTR) are found in several parts of the ventricle and atria [32]. Recently, it has been shown that OT synthesis in the heart, initiated by chronic exercise training, plays a role in endocrine and

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neuroendocrine regulation of cardiovascular function mediated via both receptor and non-receptor mechanisms [20]. Several previous experimental studies have documented that OT in the high concentrations induces negative inotropic and chronotropic effects [15,20,53]. These results may be related to several direct and indirect effects of OT in the heart [52]. Recent experimental studies have demonstrated that OT has an ability to decrease infarct size in IR injuries [2–5,14,29,53]. In a series of recent studies, Alizadeh et al. have demonstrated the cardioprotective effect of OT as an IPC agent are mediated via mitoK_{ATP}, PKC, nitric oxide (NO) and ROS [2–5]. However, the exact underlying downstream signaling cascade for OT in reperfusion is still under debate. Additionally, previous studies have demonstrated that Ipost and OT share a common signaling pathway to protect the heart during IR injury [7,30,54,58]. However, it remains unclear how OT-induced cardioprotection is mediated via the RISK pathway protein kinases. We hypothesized that OT has a cardioprotective role as an Ipost agent by the activation of some protein kinases involved in the RISK pathway (PI3K/Akt and ERK1/2) during reperfusion.

2. Materials and methods

2.1. Animal care

The experiments were performed on 8–9 week-old male Wistar rats (Pasteur Institute of Iran, Tehran, Iran) ($n = 42$) with a body weight range of 250–280 g. All rats were maintained in animal house under standardized conditions with a 12-h light/dark cycle, 20–24 °C ambient temperature, and 45–55% humidity, with free access to rat-food and water. All procedures used in this study are conformed to the rules and principles of the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) were approved by the local bioethics committee at the deputy of research and technology, Golestan University of Medical Sciences, Golestan, Iran.

2.2. Drugs

Wortmannin (Wort; an PI3k/Akt inhibitor) and PD98059 (PD; an ERK1/2 inhibitor) were purchased from "Santa Cruz Biotech, Dallas, Texas, USA" Evans blue, Atosiban (Ato; an OTR antagonist) and 2,3,5-triphenyl-tetrazolium chloride (TTC) were purchased from "Merck, Kirkland, Quebec, Canada" and Oxytocin was purchased from Aburaihan Tehran, Iran. All compounds were water soluble.

2.3. Isolated hearts

All surgical procedures have been previously explained [36]. Briefly, animals were intraperitoneally (IP) anesthetized with sodium pentobarbital (50 mg/kg) and anti-coagulated with heparin (200 IU/kg, IP). The aorta was cannulated and the hearts were rapidly excised and mounted on Langendorff perfusion system at a constant pressure of 75 ± 3 mmHg (dependent on coronary flow) [1,18,23,56,61] with oxygenated (95% O_2, 5% CO_2, 37 °C) Krebs-Henseleit buffer (pH 7.35–7.45) as previously described [7,36,37].

2.4. Experimental protocol

Animals were randomly divided into 6 groups ($n = 7$ per group) as demonstrated in Fig. 1. The hearts were perfused for an initial 30 minutes (min) as the stabilization period (baseline) and 30 min of regional ischemia followed by 100 min of reperfusion period. Ischemia induced by the left anterior descending (LAD) artery occlusion using silk string (6–0 mm). The following protocols were performed: (1) IR, Ischemia reperfusion (rats received no treatment); (2) Ischemic postconditioning (Ipost), it was achieved by 3 episodes of 10 s ischemia and 10 s reperfusion at the onset of the reperfusion phase [39,45]; (3) OT postconditioning (OTpost), OT was applied through the first 15 min of the reperfusion phase; (4) Ipost + Ato (0.1 μM); (5) OTpost + Wort (400 nM); (6) OTpost + PD (400 nM). All the inhibitors and antagonist were applied 10 min before the onset of the regional ischemia till 15 min of reperfusion phase [42,64]. All the inhibitors and OT were perfused together during the first 15 min of the reperfusion phase [30,59,63].

2.5. Hemodynamic parameters determination

Left ventricular pressure was measured through a latex water-filled balloon inserted into the left ventricle and connected to a pressure transducer (Pressure Transducer, Bridge Amp AD Instruments, Australia). The left ventricular end diastolic pressure (LVEDP) was set by the balloon inflation (5–10 mmHg) until the optimal value of the LV systolic pressure is obtained. Thus, left ventricular developed pressure (LVDP) is defined as the differences between LV systolic pressure minus LV diastolic pressure. We also measured the maximal rates of pressure development and fall (+dP/dt max and −dP/dt max) as indexes of contraction and relaxation, the rate-pressure product (RPP) as an indicator of cardiac function (RPP = HR × LVDP ÷ 1000). All these variables were monitored by Power Lab software (Power Lab 8/30 AD Instruments, Australia). Coronary flow (CF) was measured at the end of the reperfusion period.

2.6. Infarct size determination

At the end of the reperfusion period, the LAD was occluded by a silk string and Evans blue 1% was perfused into the aorta for staining various parts of the heart. After freezing at −20 °C for 24 h, the hearts were sliced into 0.2-mm sections and stained with TTC (2,3,5-triphenyl-tetrazolium chloride) 1% at 37 °C for 20 min. For the best contrast results, sections were then incubated in 10% formalin for 48 h. The infarct size was calculated by a computerized planimetry technique using Photoshop ME7 software as described previously [2–4,53]. The areas of the normal ventricle (blue), the area at risk (AAR, red) and the area of infarct (IS, pale) were determined by counting the number of pixels occupying each area. The total area at risk was expressed as a percentage of the left ventricle (AAR/LV%). Infarct size was then expressed as a percentage of the area at risk (IS/AAR%) [37].

2.7. Biochemical analysis

To measure the activity of lactate dehydrogenase (LDH), coronary effluent was collected during baseline and at the end of reperfusion. The activity of LDH was analyzed using commercial kits (Pars Azmoon, Iran) by using the Mindray BS-200 Chemistry Analyzer.

2.8. Assessment of ventricular arrhythmias

Ventricular arrhythmias were evaluated in accordance with the Lambeth Conventions [11]. In this regard, three forms of ventricular arrhythmias were analyzed as follows: ventricular premature beats (VPBs) was identified by counting the premature ventricular complex (PVC), bigeminy and salvo; ventricular tachycardia (VT) was defined as four or more serial ectopic beats; and ventricular fibrillation (VF) was characterized as an undetectable QRS complex (see Fig. S1 Supplementary data in the online version at DOI: http://dx.doi.org/10.1016/j.peptides.2016.10.001) [37]. The incidence and duration of arrhythmias were used to identify arrhythmia severity according to the following scoring system: (0) ≤10 VPBs; (1) ≥10 VPBs; (2) 1–5 episodes of VT; (3) ≥5 episodes of VT and/or VF

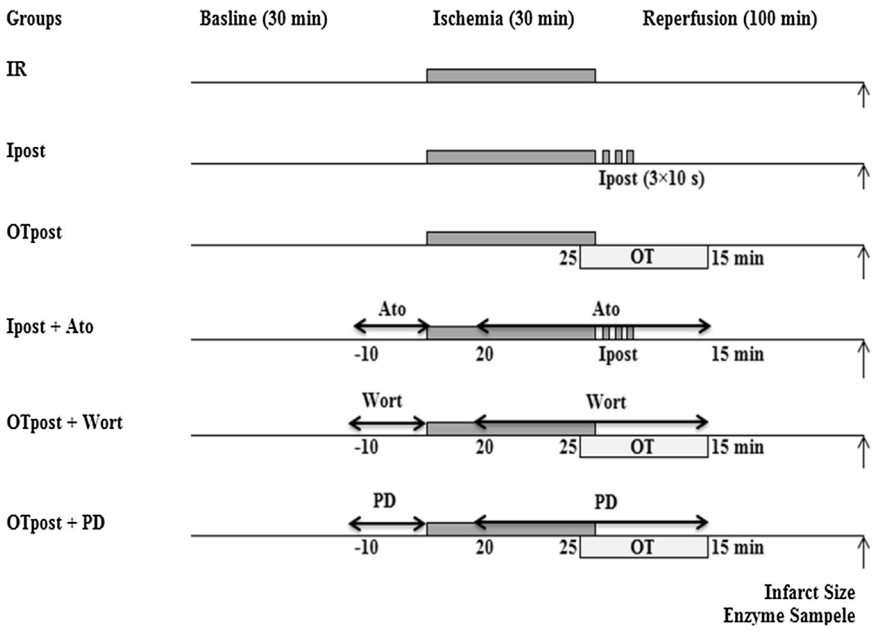

Fig. 1. Experimental protocols. All experimental groups were first perfused for 30 min on langendorff apparatus to allow the isolated hearts to stabilize. The hearts were then divided into different groups. All groups were subjected to 30 min of regional ischemia followed by 100 min reperfusion. IR; Ischemia reperfusion, Ipost; Ischemic postconditioning (3 × 10 s ischemia and reperfusion at the onset of reperfusion period), OTpost; Oxytocin (0.01 μM) was applied at the onset of reperfusion, Ato; Atosiban an OT receptor antagonist (0.1 μM), Wort; Wortmanin a PI3K/AKT inhibitor (400 nM), PD; PD98059 (2′-Amino-3′-methoxyflavone) an ERK1/2 inhibitor (400 nM).

(provided VT and VF had a total combined duration <40 s); (4) 2–5 episodes of VF (provided VT and VF had a total combined duration <80 s); (5) ≥5 episodes of VF (provided VT and VF had a combined duration <160 s); (6) VT or VF or both (total combined duration <300 s); and (7) VT or VF or both (total combined duration >300 s) [11].

2.9. Statistical assessment

Data were analyzed by Graph-Pad Prism 5 software (7825 Fay Avenue, CA, USA) and expressed as means ± SEM. Differences in the infarct size and LDH between the groups were evaluated by one-way analysis of variance (ANOVA) and Tukey's post hoc tests. Arrhythmia scores were analyzed with Kruskal-Wallis tests and the incidences of VF were compared by Fisher's exact tests. *P* values < 0.05 were considered significant.

3. Results

3.1. Infarct size

The protective role of OT as a postconditioning agent has been investigated in the presence of RISK kinase inhibitors as shown in Fig. 2. The results revealed that Wort and PD abolished the cardioprotective role of OTpost in the reduction of IS. Also the cardioprotective role of Ipost was abolished in the presence of Ato ($p < 0.05$, Fig. 2).

3.2. Coronary flow

OTpost and Ipost significantly increased CF versus IR values. CF significantly decreased in the presence of RISK kinase inhibitors (Wort and PD) and Ato respectively ($p < 0.05$, Fig. 3).

3.3. Biochemical analysis

Decreases in LDH levels were observed in the OTpost and Ipost groups. The addition of Ato during the Ipost cycles, increased LDH levels ($p < 0.05$). Also applying the RISK kinase inhibitors in the presence of OTpost significantly increased LDH levels (Fig. 4).

3.4. Arrhythmia severity

OTpost and Ipost decreased ventricular arrhythmia score and incidence of VF, whereas applying the RISK kinase inhibitors in the presence of OTpost and Ato during the Ipost cycles, increased the severity of arrhythmia and incidences of VF ($p < 0.05$, Fig. 5).

3.5. Hemodynamic properties

A preliminary experiment showed the stability of isolated heart during a 180-min period (see Table S1 Supplementary data in the online version at DOI: http://dx.doi.org/10.1016/j.peptides.2016.10.001). LV hemodynamic properties of isolated rat hearts were assessed in all groups (Figs. 6 and 7 and Table 1).

4. Discussion

The current study demonstrated that cardioprotective effects of OT as a postconditioning agent was abrogated by applying Ato and RISK pathway inhibitors. Thus, we prove that OT as a postconditioning agent, protects myocardium by activating prosurvival kinase PI3K/Akt and ERK1/2.

In the present study, we have used an isolated perfused rat heart model to determine the OT signaling cascades. Sevetral experimental studies have shown controversial cardioprotective role of OT in IR models [2,19,49,51]. Recently, Gonzalez-Reyes et al. have shown that OT has a optimium effect on cardiomyocytes viability in a simulated IR model when administered at the onset of reperfusion, whereas OT was ineffective as an IPC agent [19]. Alternatively, several studies have demonstrated OT reduce myocardial injury as a conditioning agent in various models including in-vivo, in-vitro and in a co-culture of mesenchymal cells and cardiomyocytes [2,3,7,31,49,51]. Consistent with these findings, our results demonstrated that OT as a postconditioning agent has cardioprotective effects when it was administered at the onset of reperfusion.

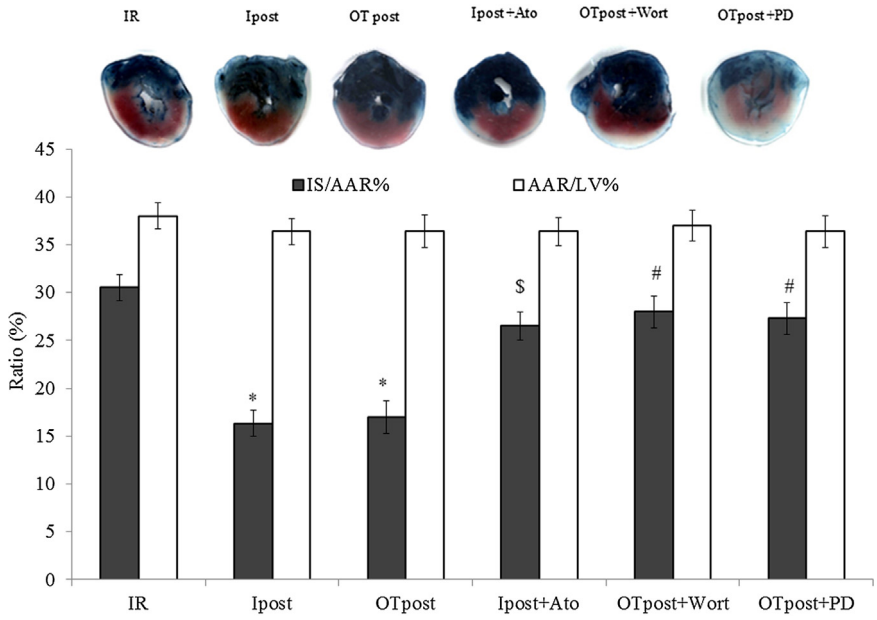

Fig. 2. Representative photographs of TTC (2,3,5-triphenyl-tetrazolium chloride) stained rat heart sections and statistical data of myocardial infarct size (IS/AAR%) and area at risk (AAR/LV%) in isolated perfused rat hearts subjected to IR, Ipost, OTpost (0.01 μM), Ipost + Ato, OTpost + Wort and OTpost + PD. Data are presented as means ± SEM and expressed in percentage. $^{*}P < 0.05$ vs. IR, $^{\$}P < 0.05$ vs. Ipost and $^{\#}P < 0.05$ vs. OTpost.

Table 1
Time dependent recording effect of oxytocin Postconditioning on functional parameters of the isolated rat heart.

Variable	Groups	Pre-Ischemia	Rep 5 min	Rep 45 min	Rep 100 min
LV systolic pressure (mmHg)	IR	122 ± 18.2	104 ± 17.4	90 ± 14.9	91 ± 15.9
	Ipost	125 ± 17.2	119 ± 12.7	120 ± 13.7	117 ± 13.5
	Ipost + Ato	128 ± 18.3	128 ± 12.3	121 ± 10.2	116 ± 15.3
	OTpost	125 ± 17.2	113 ± 11.2	119 ± 10	116 ± 19.8
	OTpost + Wort	112 ± 19.1	92 ± 14.2	104 ± 21.2	90 ± 10.3
	OTpost + PD	126 ± 18.7	108 ± 15.3	101 ± 19.7	87 ± 10.8
LV diastolic pressure (mmHg)	IR	7 ± 2.6	23 ± 4.5	20 ± 3.4	23 ± 3.7
	Ipost	9 ± 1.5	19 ± 4.9	21 ± 5.2	24 ± 6.8
	Ipost + Ato	6 ± 3.7	21 ± 5.9	27 ± 6.5	30 ± 8.5
	OTpost	6 ± 1.8	29 ± 4.6	25 ± 6.7	24 ± 6.5
	OTpost + Wort	7 ± 2.7	41 ± 9.2	50 ± 8.4*	54 ± 4.5*
	OTpost + PD	6 ± 4.8	45 ± 9.3	42 ± 6.7	44 ± 5.9*
LVDP (mmHg)	IR	115 ± 8.4	81 ± 9.8	70 ± 8.3	68 ± 9.2
	Ipost	116 ± 6.8	100 ± 7.8	99 ± 10.4	93 ± 8.7
	Ipost + Ato	122 ± 8.2	107 ± 11.3	94 ± 12.1	86 ± 10.4
	OTpost	119 ± 7.9	84 ± 13.1	94 ± 11.6	92 ± 9.3
	OTpost + Wort	105 ± 7.9	51 ± 14.1	54 ± 10.6*	36 ± 7.4*
	OTpost + PD	120 ± 11.4	63 ± 14.4	59 ± 10.4*	43 ± 8.7*

IR, Ischemic reperfusion; Ipost, Ischemic postconditioning; Ato, Atosiban; OTpost, Oxytocin (0.01 μm) was applied at the onset of reperfusion; Wort, Wortmanin (PI3K/Akt inhibitor); PD, PD98059 (ERK inhibitor). Data are means ± SEM.
LV, Left ventricle; LVDP, Left ventricular developed pressure (LV systolic pressure minus LV diastolic pressure).
* $p < 0.05$ vs. OTpost values.

To prove the cardioprotective role of OT as a postconditioning agent in the present study, Ato was perfused during the early phase of reperfusion. Our results demonstrated that the cardioprotective effect of Ipost was abolished by Ato. Likewise, OT has shown protective effects after applying it during the early reperfusion phase. Furthermore, our results have shown that OTpost had anti-infarctand anti-arrhythmic effects. In the present study, we did not determine the endogenous secretion of OT in the IR model. However, previous studies have shown that OT is synthesized and released by the heart and OTR are found in several parts of the ventricle and atria [31–33]. Additionally, the endogenous production of OT in the heart during myocardial stress, such as ischemia, heart failure, chronic exercise and acute stress has demonstrated in several studies [20,21,33,48,49]. Taken together, endogenously released OT may play a fundamental role in the mechanism of infarct-sparing effect of Ipost via the activation of OTR [7,30,51]. Moreover, previous studies have documented that OT acts on its cardiac receptors to decrease ROS generation and inhibit mitochondrial permeability transition pore (mPTP) onset [2,5]. Thus, our results further indicate a regulatory role of endogenously released OT during reperfusion in the mechanisms of the cardioprotective effects of Ipost.

In the present study, we demonstrated that OT provoked vasodilation, whereas ATO and both RISK inhibitors induced vasoconstriction in the coronary vessels. Several studies have been demonstrated that the effect of OT on the coronary vasculature is contradictory [6,34,44,46]. Several experimental studies have provided evidences for a possible direct vasorelaxation and vasoconstrictor response to OT at low and high concentration, respectively [44,46,52]. Oxytocin induces vasodilatation by stim-

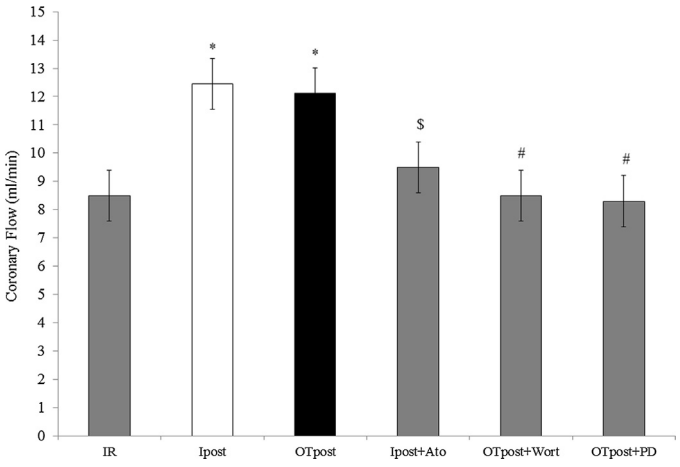

Fig. 3. Changes in coronary flow in isolated rat heart. Effects of IR, Ipost, OTpst, Ipost + Ato, OTpost + Wort and OTpost + PD groups on the Coronary flow (CF) recovery at the end of reperfusion. Data are presented as means ± SEM and expressed in percentage of baseline values. * $P < 0.05$ vs. IR, $^{\$}P < 0.05$ vs. Ipost, and $^{\#}P < 0.05$ vs. OTpost values.

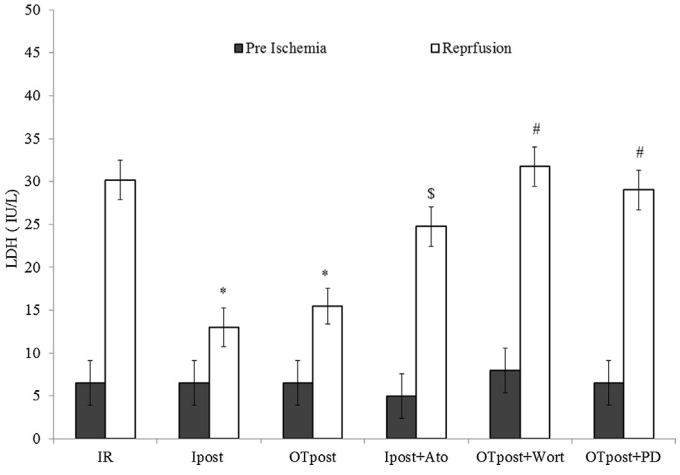

Fig. 4. The activity of LDH in coronary effluent at the pre-ischemia and the end of reperfusion in IR, Ipost, OTpst, Ipost + Ato, OTpost + Wort and OTpost + PD groups. Data are presented as mean ± SEM. $^{*}P < 0.05$ vs. IR values, $^{\$}P < 0.05$ vs. Ipost, $^{\#}P < 0.05$ vs. OTpost.

ulating NO release in the endothelial cells, whereas OTR in smooth muscle cells are thought to mediate vasoconstriction [34]. Therefore, in the present study, the stimulation of OTR by OT will produce vasodilatation, which in turn increased production of nitric oxide and activation of RISK pathway components.

In the present study, OTpost significantly reduced LDH release in the isolated rat hearts subjected to an ischemia–reperfusion cycle. Elevated levels of LDH have been considered as a specific biomarker of cardiomyocyte oxidative stress, apoptosis and tissue damage [12,48]. The reduction in infarct size with immediate Ipost was accompanied by a decrease in LDH activity. On the other hand, the infarct size-limiting effect and reduction of LDH level of OTpost was markedly reduced in the presence of RISK pathway inhibitors. Moreover, Previous study demonstrated that exposure to various stressors conditions can elevate LDH levels, which reversed by OT as IPC agent [29,48]. Therefore, as the mitochondria involved in the oxidative stress lead to more permeability transition ATP opening and progressively lead to impaired hemodynamic function of the heart (stunning) [2].

In this study, we speculated that the OT cardioprotective effects are mediated through the RISK pathway during the reperfusion phase. Thus, to determine the postconditioning role of OT, we applied RISK pathway inhibitors at the onset of reperfusion. Our findings demonstrated that these inhibitors abrogated anti-infarctand anti-arrhythmic effects of OTpost.

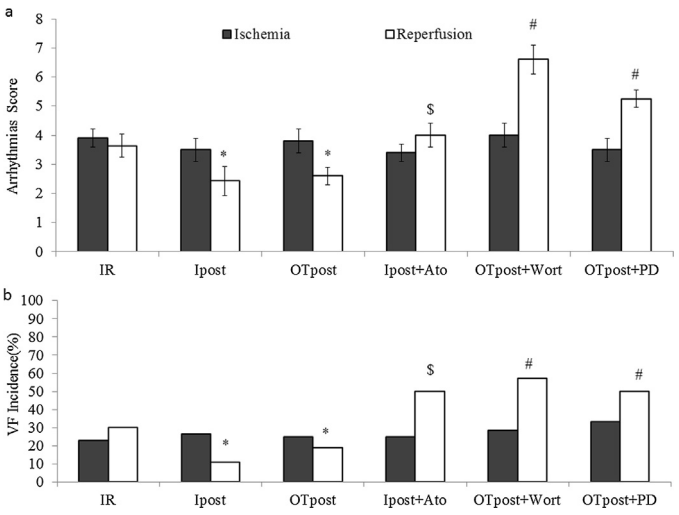

Fig. 5. Arrhythmias score and Incidence of ventricular fibrillation (VF) calculation of the ischemia and the reperfusion period in isolated rat heart. (a) Effect of IR, Ipost, OTpst, Ipost + Ato, OTpost + Wort and OTpost + PD groups on the arrhythmias score. Scale range is 0–7. Data are presented as mean ± SEM. (b) Effect of IR, Ipost, Ipost + Ato, OTpost + Wort, OTpost + PD groups on the incidence of VF. Data are presented as percentage of incidence. $^{*}P < 0.05$ vs. IR, $^{\$}P < 0.05$ vs. Ipost, $^{\#}P < 0.05$ vs. OTpost.

It is well recognized by several experimental studies that RISK pathway contributes to the infarct reduction effects of the Ipost phenomenon [10,24,58]. In fact, during early reperfusion phase, the activation of PI3K/Akt, ERK1/2 and GSK-3β kinases by several natural peptides including: adenosine, opioids and OT leads to the inhibition of the mPTP onsets and induces the activation of Akt and ERK1/2 [17,19,28,43,62]. Consistent with previous studies, our results have confirmed that the cardioprotective benefits of OT are dependent on the activation of PI3K/Akt and ERK1/2 at the early phase of reperfusion. We propose that OT triggers postconditioning by activating certain protein kinases in the RISK pathway. More recently, it has been shown that the cardioprotective effect of OT is triggered by production of low levels of ROS to activate PI3K/Akt and ERK1/2 and inhibits apoptosis [19,55].

In contrast to Gonzalez-Reyes, our data and other studies have clearly established the cardioprotective effect of OT as an IPC agent [4,19]. They have used H9c2 cells and an ischemic buffer to simulate an IR model to explain these controversial results [19]. However, they did not consider the direct effect of OT on hemodynamic factors and heart rate. Additionally, isolated whole hearts differ from cardiomyocyte cultures in both the 3D architecture and myocardial blood supply [13]. The changes in cellular architecture and gap junctions are necessary in the cardioprotective mechanism of cardiac IPC [16]. However, the mechanisms of reperfusion in isolated cardiomyocytes are not the same as in the whole heart, in which the cardiomyocytes apply a lot force on each others, which causes substantial late and early enzyme release, huge sarcolemmal disruptions and a secondary influx of Ca^{2+} ions into the damaged cells [13]. In addition, several previous studies have demonstrated that the expression and function of connexin 43 increases after IR injury [25,35,38]. Therefore, the difference between the two methods may be related to changes in phosphorylation and expression of connexin 43 and cardiac 3D structures.

The activation of the PI3K/Akt pathway during early reperfusion phase is the cardioprotective downstream mechanism of OT to limit both apoptosis and cell damage. On the other hand, several

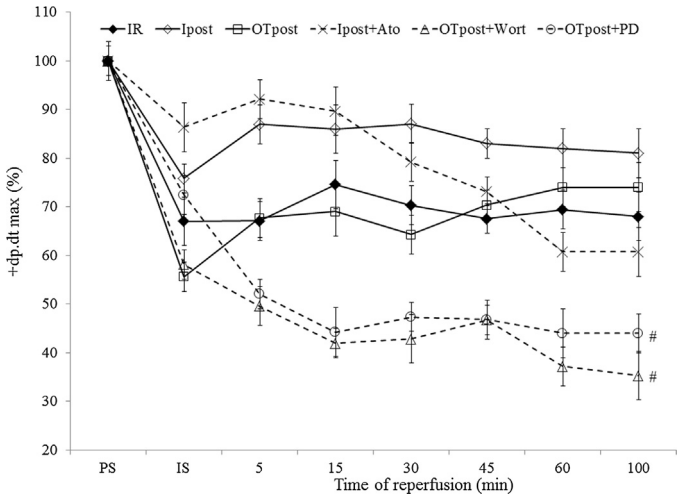

Fig. 6. Effect of OTpost on time course recovery of post-ischemic changes of +dp/dt max in the isolated rat heart. Data are presented as% of pre-ischemia (PS) values. $^{\#}P < 0.05$ vs. OTpost.

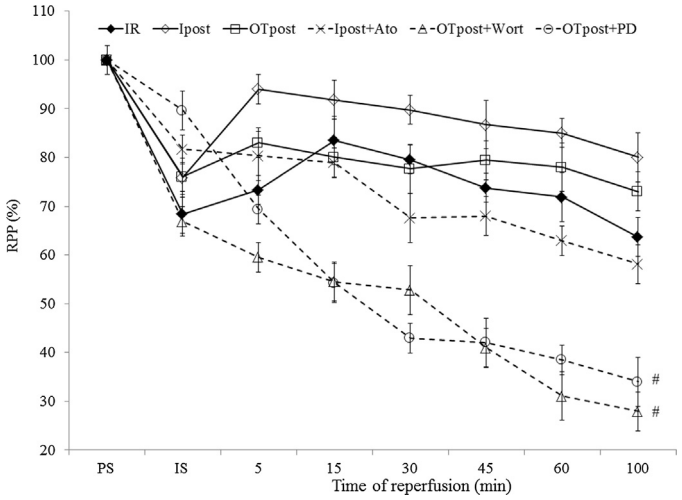

Fig. 7. Effect of OTpost on Time course recovery of Post-ischemic changes of rate pressure product (RPP) in the isolated rat heart. Data are presented as % of pre-ischemia (PS) values. $^{\#}P < 0.05$ vs. OTpost.

mechanisms have been proposed to explain the cardioprotective mechanism of OT [2,3]. Previous study demonstrated that repeated stress exposure resulted in activation of anti-apoptotic Akt kinase pathway and this leds to an elevation in HSP-90 and p53 proteins. The results further indicated a regulatory role of OTR in the control of molecular mechanisms underlying responses of the rat heart to stress [8]. OT-induced angiogenic, anti-apoptotic and cardiac anti-remodeling functions, which occurs by increasing the expression of genes that play a role in response to IR injury [24]. In a series of studies, Alizadeh et al. established the anti-apoptotic role of OT in an in-vivo model of IR injury in rat heart [2–5]. Likewise, Kobayashi et al. demonstrated that the administration of OT (10 mg/kg, subcutaneously) during the early reperfusion phase significantly reduces IS via the up-regulation of Bcl-2 (anti-apoptotic) and the activation of Akt and Erk1/2 as well as eNOS [40]. In the present study, we didn't assay apoptosis and further studies are needed to explore the specific mechanisms concerning the cardioprotective effect of OTpost in cardiomyocytes. Taken together, OT plays a crucial cardioprotective role by the pleiotropic mechanisms that include the stimulation of the RISK signaling cascade for inhibiting apoptosis during reperfusion.

4.1. Limitations

The limitations of this study relate to the use of non-specific inhibitors such as Wort, which inhibits the PI-3 kinase and not Akt directly. The use of such inhibitors can only indicate that a particular signaling cascade is blocked, but cannot determine the exact downstream protein kinase involved. To establish the endogenous role of OT during IR, we will need to design a separate study and determine the endogenous release of OT within the reperfusion phase by radioimmunoassay techniques. However, it must be stated that Ato is a non-selective OTR antagonist and also blocks vasopressin receptors [8]. Moreover, we did not explore the changes in expression and protein activity of Akt and ERK1/2 during reperfusion. Further studies for identifying phosphorylation of pro-survival kinase signaling cascade may provide greater specificity to the findings of the present study.

In the present study, we found evidence which suggesting OT has a postconditioning role. Thus, we can propose a potential beneficial role of OT in hospitalized patients with acute coronary syndrome. Accumulating evidences points to different cardiovascular beneficial effects of OT. The administration of OT could induce a systemic cardiovascular effect including: vasodilatation [9], decreased BP [20] and negative inotrophic and chronotrophic effects [53]. Therefore the administration of OT as a cardioprotctive IPC agent must be with caution and after evaluation of the OT safety as conditioning agent in several clinical trials.

5. Conclusion

The results of the present study clearly indicate that OT administration at the early phase of reperfusion triggers protective signaling cascades in the isolated rat heart exposed to IR injury in accordance with the RISK pathway. Based on the results obtained from the use of specific inhibitors, we can conclude that in isolated heart undergoing IR, OT afford cardioprotection by activating Akt and ERK1/2. Therefore, we suggest that in the clinical setting, OT might be a good candidate as a postconditioning agent for management of patients with acute myocardial infarction (AMI).

Conflict of interest

The authors have not any Conflict of Interest.

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