

Oxytocin protects rat heart against ischemia–reperfusion injury via pathway involving mitochondrial ATP-dependent potassium channel

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ABSTRACT

Cardiac preconditioning represents the most potent and consistently reproducible method of rescuing heart tissue from undergoing irreversible ischemic damage. One of the major goals of the current cardiovascular research is to identify a reliable cardioprotective intervention that can salvage ischemic myocardium. The aim of the present study is to evaluate the oxytocin (OT)-induced cardioprotection and the signaling pathway involved with mitochondrial ATP-dependent potassium (mitoKATP) channel in the anesthetized rat heart. Animals were divided into six groups ($n=6$): (1) IR; hearts were subjected to 25 min ischemia and 120 min reperfusion, (2) OT; oxytocin was administered (0.03 $\mu\text{g}/\text{kg}$ i.p.) 25 min prior to ischemia, (3) ATO+OT; atosiban (ATO) was used as an OT-selective receptor antagonist (1.5 $\mu\text{g}/\text{kg}$ i.p.) 10 min prior to OT administration, (4) ATO; atosiban was used 35 min prior to ischemia, (5) 5HD+OT; 5-hydroxydecanoic acid (5HD) was used as a specific inhibitor of mitoKATP channel (10 mg/kg i.v.) 10 min prior to OT administration, (6) 5HD; 5HD was used 35 min prior to ischemia. Then infarct size, ventricular arrhythmia and creatine kinase-MB isoenzyme (CK-MB) plasma level were measured. Hemodynamic parameters were recorded throughout the experiment. OT administration significantly decreased infarct size, CK-MB plasma level, severity and incidence of ventricular arrhythmia as compared to IR group. Administration of atosiban and 5HD abolished the cardiopreconditioning effect of OT. This study demonstrates that cardioprotective effects of OT are mediated through opening the mitoKATP channels.

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1. Introduction

One of the major goals of cardiovascular research is to identify a reliable cardioprotection intervention that can salvage ischemic myocardium. Cardiac preconditioning represents the most potent and consistently reproducible method of rescuing heart tissue from undergoing irreversible ischemic damage. Unfortunately, the clinical value of ischemic preconditioning (IPC) itself is limited. None of the several identified pharmacologic agents that appear to limit reperfusion injury is available for clinical use [36].

Oxytocin (OT) has been considered to be a cardiovascular hormone. It is produced and released by the heart and large vessels. Systemic administration of OT has significant effects on cardiovascular functions [7,13,14]. It has been shown that OT protects kidney and liver tissues against IR injuries [2,32]. Ondrejčáková et

al. reported that OT has protective effects on ischemia–reperfusion-induced myocardial injury in isolated rat heart [25]. Interestingly, our previous experimental study confirmed the protective effects of OT on myocardial injury of the ischemic reperfused heart in the anesthetized rat [9]. We also showed endogenous OT involvement in cardioprotective effects of IPC (submitted).

Some studies showed OT role on protein kinase C (PKC) activation in cardiomyocytes [1,8]. It has been reported that PKC activation may promote mitochondrial ATP-dependent potassium (mitoKATP) channels opening in rat heart [3,12]. The role of mitoKATP channels activators in cardioprotection against ischemia–reperfusion (IR) injury has widely been discussed [6,21]. Activation of mitoKATP channels can lead to cardiomyocytes survival during ischemia. 5-Hydroxydecanoic acid (5HD), a specific inhibitor of mitoKATP channels, can revert this protective effect [18]. A number of studies have depicted mitoKATP channels as an end-effector of cardiac preconditioning [18,4,26,31,23]. The present study was designed to evaluate the possible role of the mitoKATP channel in the cardioprotective effects of oxytocin in the anesthetized rat.

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2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Weighing 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 [9,11]. 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 H₂O).

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) 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. Coronary artery occlusion and reperfusion were confirmed by epicardial cyanosis and hyperemia, respectively.

2.3. Experimental protocol

The heart of all animals was subjected to 25 min ischemia and 120 min reperfusion (Fig. 1). Rats were divided into six groups (*n*=6): (1) IR; hearts were subjected to 25 min ischemia and 120 min reperfusion, (2) OT; oxytocin was administered (0.03 µg/kg i.p.) 25 min prior to ischemia, (3) ATO+OT; atosiban (ATO) was used as an OT-selective receptor antagonist (1.5 µg/kg i.p.) 10 min prior to OT administration, (4) ATO; atosiban was used 35 min prior to ischemia, (5) 5HD+OT; 5HD was used as a specific inhibitor of mitoKATP channel (10 mg/kg i.v.) 10 min prior to OT administration, and (6) 5HD; 5HD was used 35 min prior to ischemia.

2.4. 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.

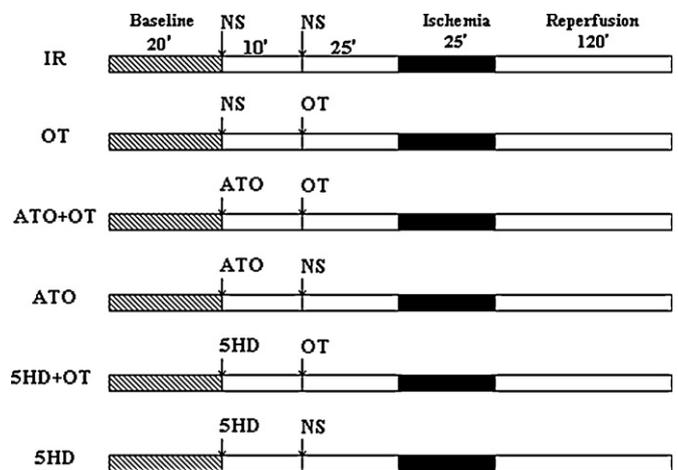


Fig. 1. Illustration of the experimental protocols. Hearts in all groups were subjected to 25 min of ischemia followed by 120 min reperfusion. IR = ischemia–reperfusion; NS = normal saline; OT = oxytocin; 5HD = 5-hydroxydecanoic acid; ATO = atosiban.

2.5. 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.

2.6. Assessment of ventricular arrhythmias

Ischemia-induced ventricular arrhythmias were counted during of occlusion period and determined in accordance with the Lambeth Conventions [34]. 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 (Fig. 2). The incidence, time of occurrence and duration of arrhythmias were used to identify arrhythmias severity according to the following scoring system [29]; 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, 9: fatal VF starting <1 min after occlusion.

2.7. 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 activity was analyzed using ELECSYS System (ELECSYS 2010, Roche, Germany) and commercial kits (Pars Azmoon, Iran).

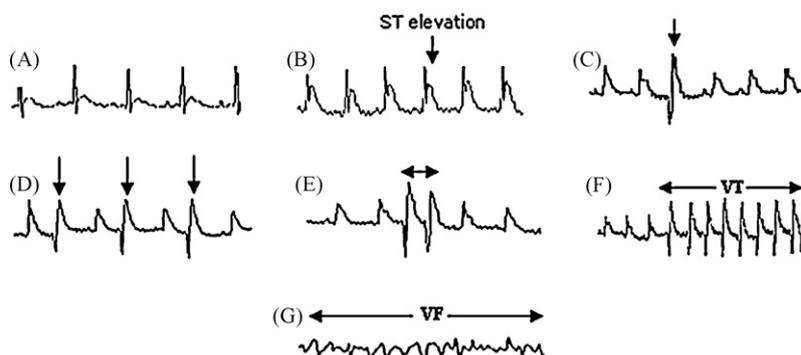


Fig. 2. Electrocardiogram recording. (A) During baseline; (B) during coronary artery occlusion; (C) ventricular ectopic beat (VEB); (D) bigeminy; (E) couplet; (F) ventricular tachycardia (VT); (G) ventricular fibrillation (VF).

2.8. Materials

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

2.9. Statistical analysis

Statistical analysis of hemodynamic data within and between groups was performed with two-way analysis of variance. Differences in infarct size and CK-MB plasma level were determined by one-way analysis of variance. Groups were individually compared using Dunnett'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 \pm SEM. 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 measurements

AAR was not different among groups. Infarct size was decreased significantly in OT group compared to IR ($22 \pm 1.5\%$ vs. $45 \pm 2.2\%$ in IR group, $P < 0.05$). Administration of atosiban and 5HD in ATO+OT and 5HD+OT groups increased infarct size to $42 \pm 1.8\%$ and $43 \pm 1.5\%$, respectively in comparison with OT group ($P < 0.05$). Atosiban and 5HD alone had no significant effect on infarct size (Fig. 3).

Table 1
Hemodynamic parameters.

Groups	n	Baseline			Ischemia 25'			Reperfusion 120'		
		MBP	HR	RPP	MBP	HR	RPP	MBP	HR	RPP
IR	6	126 \pm 6	347 \pm 11	44057 \pm 2204	114 \pm 5	333 \pm 14	42398 \pm 1537	105 \pm 5	331 \pm 16	41925 \pm 1698
OT	6	118 \pm 4	354 \pm 8	44845 \pm 2532	112 \pm 3	343 \pm 12	42164 \pm 1992	104 \pm 3	343 \pm 11	39133 \pm 1707
ATO+OT	6	121 \pm 5	341 \pm 13	43117 \pm 2620	106 \pm 4	327 \pm 12	39317 \pm 1712	104 \pm 5	328 \pm 13	38707 \pm 1675
ATO	6	109 \pm 6	335 \pm 14	41390 \pm 2169	99 \pm 3	305 \pm 9	36407 \pm 1117	95 \pm 5	296 \pm 16	34076 \pm 2203
5HD+OT	6	114 \pm 5	349 \pm 12	42516 \pm 2065	106 \pm 4	336 \pm 14	39670 \pm 1547	101 \pm 4	332 \pm 12	38820 \pm 1608
5HD	6	117 \pm 4	338 \pm 9	41043 \pm 1780	109 \pm 5	335 \pm 10	37652 \pm 1842	98 \pm 5	325 \pm 11	37444 \pm 2230

Data are presented as mean \pm SEM. n = number of animals in each group; MAP = mean arterial pressure (mm Hg); HR = heart rate (beats/min), RPP = rate pressure product (beats/min mm Hg); OT = oxytocin; ATO = atosiban; IR = ischemia–reperfusion; 5HD = 5-hydroxydecanoic acid.

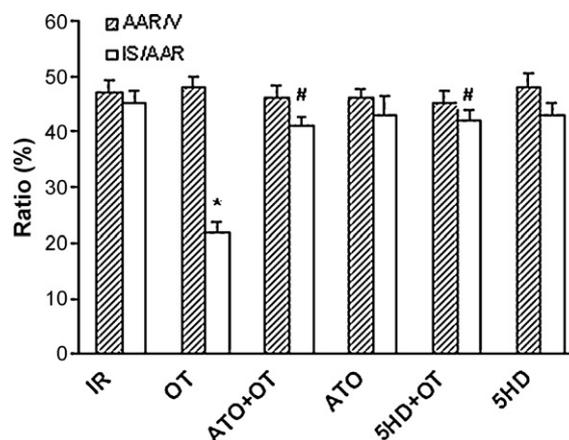


Fig. 3. Myocardial area at risk (AAR/V%) and infarct size (IS/AAR%) in IR, OT, ATO+OT, ATO, 5HD+OT and 5HD groups. Data are presented as mean \pm SEM. * $P < 0.05$ vs. IR group. # $P < 0.05$ vs. OT group. OT = oxytocin; ATO = atosiban; IR = ischemia–reperfusion; 5HD = 5-hydroxydecanoic acid.

3.3. Ventricular arrhythmias during ischemia

3.3.1. Severity of arrhythmias

Administration of OT prior to ischemia significantly declined ventricular arrhythmias severity compared to IR group (0.7 ± 0.3 vs. 3.7 ± 0.2 in IR group, $P < 0.05$). Administration of atosiban and 5HD in ATO+OT and 5HD+OT groups was intensified severity of arrhythmias compared to OT group (3.6 ± 0.3 and 3.3 ± 0.3 , respectively, $P < 0.05$). Atosiban and 5HD alone had no significant effect on arrhythmias severity (Fig. 4).

3.3.2. Incidences of VT and VF

In IR group, all animals were experienced VT, while VF occurred in 67% of hearts. Administration of OT attenuated VT and VF incidence to 33% and 17%, respectively ($P < 0.05$). In comparison with OT group, administration of atosiban and 5HD restored the incidence

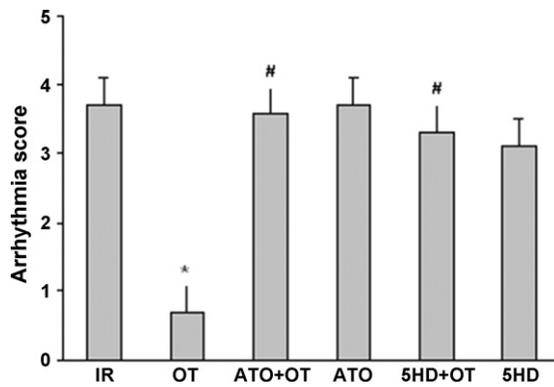


Fig. 4. Distribution of the arrhythmia score during 25 min ischemia in IR, OT, ATO+OT, ATO, 5HD+OT and 5HD groups. Data are presented as mean ± SEM. * $P < 0.05$ vs. IR group. # $P < 0.05$ vs. OT group. OT = oxytocin; ATO = atosiban; IR = ischemia–reperfusion; 5HD = 5-hydroxydecanoic acid.

of VT to 100% and 100% and VF to 51% and 67% in ATO+OT and 5HD+OT groups, respectively ($P < 0.05$). Atosiban and 5HD alone had no significant effect on VT and VF incidences (Fig. 5).

3.4. Biochemical analysis

OT administration significantly decreased CK-MB plasma level in comparison with IR group at the end of reperfusion period ($P < 0.05$). Atosiban and 5HD administration prior to OT significantly increased CK-MB plasma level ($P < 0.05$). Atosiban and 5HD alone had no significant effect on CK-MB plasma level (Fig. 6).

4. Discussion

In the present study, the cardioprotective effects of oxytocin-induced hormonal preconditioning against IR injury have been demonstrated. OT administration before ischemia could protect the myocardium. Oxytocin significantly decreased infarct size, CK-MB level, severity and incidence of ventricular arrhythmia as compared to IR group. These effects were abolished by atosiban, a selective OT-receptor antagonist and 5HD, a mitoKATP channel blocker. These findings suggest mitoKATP channel involvement in early phase protection of hormonal preconditioning afforded by oxytocin via activation of its cardiac specific receptors.

The role of mitoKATP channels in hormone- and drug-induced cardioprotection is well established. Garlid et al. reported the first evidence for mitoKATP channel role in cardioprotection [4]. They

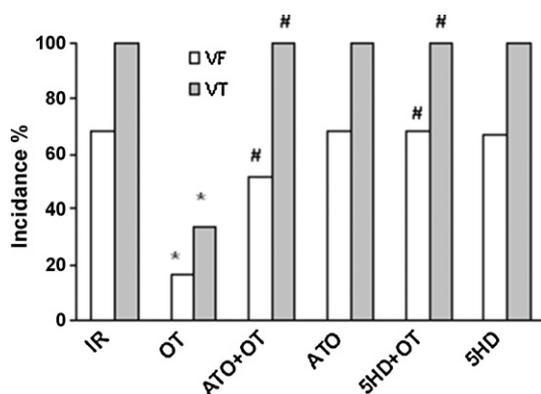


Fig. 5. The incidence of ventricular fibrillation (VF) and ventricular tachycardia (VT) during 25 min ischemia in IR, OT, ATO+OT, ATO, 5HD+OT and 5HD groups. Data are presented as mean ± SEM. * $P < 0.05$ vs. IR group; # $P < 0.05$ vs. OT group. OT = oxytocin; ATO = atosiban; IR = ischemia–reperfusion; 5HD = 5-hydroxydecanoic acid.

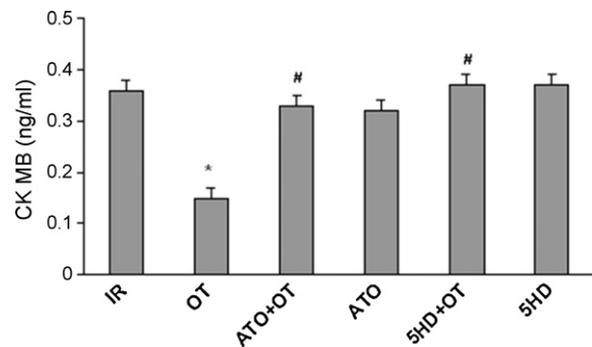


Fig. 6. The effect of oxytocin, atosiban and 5HD on creatine kinase-MB (CK-MB) isoenzyme plasma level at the end of reperfusion in IR, OT, ATO+OT, ATO, 5HD+OT and 5HD groups. Data are presented as mean ± SEM. * $P < 0.05$ vs. IR group; # $P < 0.05$ vs. OT group. OT = oxytocin; ATO = atosiban; IR = ischemia–reperfusion; 5HD = 5-hydroxydecanoic acid.

showed that lipid bilayers were 1000–2000 times more sensitive to specific mitoKATP channel openers than its sarcolemal ones. A number of studies have depicted mitoKATP channels as an end-effector of cardiac preconditioning [4,18,26,31,23]. These channels are activated by a decreased ATP/ADP ratio in hypoxic/ischemic conditions. MitoKATP-channel activation may shorten the action potential duration, reduce cellular calcium overload and preserve myocardial viability in ischemic situations [35,33,28]. MitoKATP channels opening followed by a mitochondrial membrane depolarization causes mitochondrial potential dissipation and reduces the force for Ca^{+2} uptake. It can preserve mitochondrial function during IR [19,22]. Mitochondrial matrix volume may also increase while membrane potential remains nearly stable. This would be favorable for ATP generation due to the reduction in driving force and speculated to preserve mitochondrial function during IR [15].

It has been reported that administration of OT induces cardioprotective effects on ischemia–reperfusion injury in isolated rat heart [25]. Exogenous administration of OT has been shown to have cardiac protection against IR injury in our previous study [9]. We have also depicted that the cardioprotective effects of IPC can be induced by endogenous OT (Submitted). In the present experiment, 5HD abolished the cardioprotective effects of OT which implies the contribution of mitoKATP channel opening in hormonal preconditioning. Changes in the ischemic area and infarct size in unconscious rats could influence the incidence of lethal ventricular arrhythmias following pre-treatment with mitoKATP blockers [33]. In our experiment, infarct size, the incidence and severity of arrhythmias were changed in the same direction in all groups. Therefore, the size of infarct area may have a key role in antiarrhythmic effect of OT. Interestingly, 5HD pretreatment abolished the beneficial effects of OT on arrhythmia which implies the possible role of mitoKATP channel activation in these effects. We also showed that 5HD administration could not induce significant changes on hemodynamic parameters. This observation suggests that the effects of OT on myocardium are not related to alteration in hemodynamic parameters. So the in vivo effects of OT on the ischemic myocardium are likely to depend upon direct cytoprotection at the cellular level.

The exact mechanism leading to mitoKATP-channel opening remains unclear. Brief ischemia, bradykinin, PKC activators, nitric oxide (NO) and pharmacological agents have been identified as mitoKATP channel activators [5,10,16,17,20]. In the present study, one possible mechanism would be NO release as a channel opener. Our previous study showed that role of NO in the cardioprotection induced by OT in the anesthetized rat (unpublished). Nitric oxide synthase (NOS) activity in the heart is stimulated by some pharmacological agents. The relation between NO and the mitoKATP

channel modulation was examined in the various studies [16,37]. Sasaki et al. showed that NO itself modulates the opening of the mitoKATP channel [30]. Oldenburg et al. have also showed that the release of reactive oxygen species and subsequent NO production activate this channel [24]. OT may also stimulate NO release from liver cells and cardiomyocytes during ischemia–reperfusion in rat [2,27]. A recent report suggests that the protective effects of OT on ischemia–reperfusion injury may be caused by the NO release [2]. Thus NO release may have a key role in mitoKATP channel activation through OT receptors. Finally, our results suggest that the opening of mitoKATP channels is involved in the signal transduction of the early phase protection of hormonal preconditioning afforded by OT.

5. Conclusion

In conclusion, the present study demonstrates that the cardioprotection effects of OT are mediated through activation of the mitoKATP channels.

Acknowledgment

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