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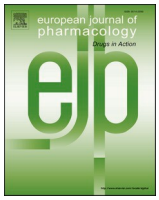

Role of oxytocin and c-Myc pathway in cardiac remodeling in neonatal rats undergoing cardiac apical resection

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ABSTRACT

Oxytocin (OT) is a nonapeptide hormone that can improve cardiomyocyte proliferation, suggesting a potential heart regeneration function. Here, we investigated the role of oxytocin and the c-Myc pathway in cardiac remodeling in neonatal rats undergoing cardiac apical resection. We have utilized a knockout of oxytocin receptor (OTR) with OTR-shRNA. A neonatal rat model of cardiac resection (≈10%–15%) was first established. The protein levels of OTR and c-Myc and the expression of cyclin d1 and c-Myc genes were then evaluated in the cardiac tissues at 1, 7, and 21 days after cardiac resection. We also analyzed the proliferation of cardiomyocytes through α-actinin, BrdU, and ki-67 markers. At last, the hemodynamic and electrophysiologic functions were evaluated eight weeks after cardiac resection. At 21 days, the regeneration of cardiomyocytes was repaired among rats in the control and resection groups, while OTR-shRNA groups were failed to improve. Inhibition of OTR failed cardiac regeneration and reduced the number of proliferating cardiomyocytes. The c-Myc protein was significantly reduced in the OTR-shRNA injection hearts. Moreover, we have severely found a depressed heart function in the OTR-shRNA injection animals. These observations revealed that the OT must improve cardiac remodeling in neonatal rat hearts by regulating the c-Myc pathway.

1. Introduction

Former studies have shown that the heart muscle contains a collection of cardiac stem cells with a significantly increased proliferation rate and function during heart damage (Heallen et al., 2019). Recently, researchers have shown that rodent infants' heart injuries can be repaired in the early days of life. The presence and formation of endogenous cardiac stem cells around the infarcted areas could be crucial in repairing damage and restoring cardiac function (Barile et al., 2007). Porrello et al. (2011) showed that the hearts could be repaired within 21 days of birth after 10–15% damage to neonatal rat's hearts. Their results showed that a large part of this repairing process results from the increasing proliferation of mature cardiomyocytes present at the site of injury and increasing proliferation, migration, and differentiation of cardiac stem cells into the cardiac cell lines (Porrello et al., 2011; Zhao et al., 2020). An improved function of cardiac stem cells is due to factors such as raised paracrine/autocrine functions, cardiomyocyte divisions, and cardiac transcription factors. Earlier studies represented that the expression level of oxytocin receptor (OTR) in the heart could significantly increase in the early days of mammalian life, indicating an essential role in controlling oxytocin pathways in the development and function of the heart in the first days of birth. The function of oxytocin (OT) and the induction of different signaling pathways by OTR can play a central role in enhancing the proliferation and differentiation of cardiac stem cells (Zhao et al., 2020). Thus, it seems that OTR can protect the cells from apoptosis and improve myocardial function after

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infarction (Alizadeh et al., 2011; Alizadeh and Mirzabeglo, 2013). Moreover, OTR was significantly increased in the first seven days of gastrulation in the primary myocardium of rodents. Pournajafi-Nazarloo et al., (2007) revealed that the treatment with OT could considerably increase the OTR level in neonatal myocardium within 21 days after birth. Furthermore, they showed that during 21 days after birth, the OTR level could significantly decrease, indicating the possible role of the OTR and its signaling pathways in the repair process of neonatal hearts (Pournajafi-Nazarloo et al., 2007).

Additionally, the regulation of myocytes' metabolism, cell viability, cardiac vasodilation, and promoting the commitment and migration of cardiomyocytes can be the central biological mentioned effects of OTR on cardiomyocytes (Gutkowska and Jankowski, 2009). OT seems to be the main factor for indicating regeneration into the injured myocardium based on arrays of developmental biology observations (Jankowski et al., 2012; Noiseux et al., 2012). During the generation of cardiomyocytes, OT through the G-protein activation can enhance cell differentiation through PI3K/Akt, Ras/Raf1, and MEK5 signaling pathways (Gutkowska and Jankowski, 2012). Increasing angiogenesis and anti-fibrotic function of OTR has been demonstrated through the NO signaling pathway (Gutkowska and Jankowski, 2012). In this setting, the activation of c-Myc protein into the cardiomyocytes is critical for promoting the proliferation and differentiation of cardiomyocytes. Wang et al. (2020) observed that the flowing process of neonatal heart regeneration could promote the proliferation of cardiomyocytes by regulating c-Myc signaling pathways (Wang et al., 2020). Regarding the expression pattern of OTR in mammalian neonatal myocardium, it seems that there are significant relationships between the high levels of OTR in the early days of neonatal life and mammalian cardiac repair. Therefore, we aimed at the role of oxytocin and the c-Myc pathway in cardiac remodeling in neonatal rats undergoing cardiac apical resection.

2. Materials and methods

2.1. Materials

Anti-c-Myc antibody (ab17355), Anti-BrdU antibody (ab152095), Anti-Alpha Actinin antibody (ab90776), Anti-Ki67 antibody (ab833), and Anti-oxytocin Receptor antibody (ab217212) were purchased from the Abcam (USA). Oxytocin Receptor ELISA Kit (MBS765381) was purchased from the MyBioSource (USA). Triazole Reagent (T9424) was purchased from Sigma (USA). RevertAid First Strand cDNA Synthesis Kit (K1622) and Real-Time PCR kit were purchased from Thermo Fisher Scientific.

2.2. Ethical approval

A review board committee approved the experimental procedures and care protocols of Tehran University of Medical Sciences (NO: IR.TUMS.REC.1394.1869).

2.3. Lentivirus containing OTR gene

The short hairpin RNA (shRNA) sequence was designed using the BLOCK-iT RNAi Designer Invitrogen (CA, Carlsbad, Invitrogen). The BLAST site was systematically used to confirm the sequences. The shRNA's spliced downstream of the U6 promotor using the pENTR/U6 plasmid (Invitrogen, Gateway Cloning Technology) was cloned into lentivirus (adeno-associated viral vectors) as described recently (Barrett et al., 2013).

2.4. Animal groups

Forty-eight neonatal rats (one day old, purchased from Iran Pasteur Institute) were divided into four groups: (1) the control group; containing 12 neonatal rats without intervention, (2) cardiac resection group; containing 12 neonatal rats with cardiac resection, (3) OTR-shRNA group; containing 12 neonatal rats with OTR blocking, and (4) Resection-OTR-shRNA group; containing 12 neonatal rats with cardiac resection together with OTR blocking.

2.5. Blocking of cardiac-OTR in neonatal hearts

Hypothermia was used to induce anesthesia. Upon access to the thoracic space and excision of the pericardium, 10 μl of the plasmid solution of the OTR-shRNA gene was added by Hamilton's syringe in the lower left ventricle. The thoracic was sutured using suture adhesive, and the animals were placed in a warm environment to recover (Pournajafi-Nazarloo et al., 2007).

2.6. Cardiac resection procedure

Hypothermia was used to induce anesthesia. After access to the thoracic space and removal of the pericardium, approximately 10%–15% of the left ventricular apex was removed using a micro-surgery method. The chest was then sutured using suture adhesive, and the animals were placed in a warm environment (Heallen et al., 2013).

All animals were conducted within the Weatherall report's international guidelines and the national guidelines of the Institutional Animal Care and Use Committee (IACUC) of Tehran University of Medical Sciences.

2.7. Evaluation of cardiac-OTR expression

The hearts were examined for cardiac-OTR protein levels at 1, 7, and 21 days after cardiac resection or OTR-shRNA injection. The left ventricle of the euthanized animals was isolated and frozen in liquid nitrogen. The frizzed tissues were then lysed by a lysis buffer at room temperature for 30 min. The supernatants were then centrifuged at 2000 g for 10 min, and the cardiac-OTR protein level was measured by the ELISA Reader at 350 nm.

2.8. Evaluation of cell proliferation

The left ventricles were isolated and placed in 10% formalin. After preparing the paraffin-embedded blocks, the slices were prepared in 3–5 μm size. The slices were incubated on days 1, 7, and 21 after cardiac resection or OTR-shRNA injection and processed by anti-α-actinin, anti-BrdU, and anti-Ki-67 antibodies. The immunostaining was performed for BrdU, Ki-67, and α-actinin according to the kit manufacturer's instructions. The sections were first deparaffinized and hydrated. They have then undergone an antigen retrieval process [with 10 mM citrate buffer (pH = 6) for 15 min at 98 °C] and followed by incubation with 3% H2O2 solution to inhibit endogenous peroxidase activity. The samples were then stained with the primary monoclonal antibodies of BrdU, Ki-67, and α-actinin proteins at a concentration of 1/100 at 4 °C overnight. The samples were then incubated for 1 h at 37 °C with an HRP-conjugated secondary antibody. All samples were counterstained with Hematoxylin. The slides were studied using the OLYMPUS–BX51 microscope, and digital images were prepared using the OLYMPUS-DP12 camera. The semi-quantitative standard technique of H-score was used based on the following formula to interpret results: H-score = Pi (i + 1), where i is the intensity of dyeability (high dyeability = 3, medium dyeability = 2, weak dyeability = 1, lack of dyeability = 0), and Pi denotes the percentage of stained cells.

2.9. Evaluation of c-Myc protein expression by Western blotting

Western blotting was used to evaluate the c-Myc expression at 21 days after cardiac resection or OTR-shRNA injection. The tissues were lysed with the RIPA buffer and then centrifuged at 10000 g and 4 °C for 15 min. The samples were then prepared for loading on the SDS-PAGE

gel electrophoresis. After electrophoresis, the gels were transferred to cellulose acetate paper, blocked with milk for 1 h, and incubated with a primary antibody for 24 h. They were then washed and incubated with a secondary conjugated antibody for 4 h. At last, they were identified by the ELC kit, and Imaging J software was used to quantify the bands.

2.10. Expression of c-Myc and Cyclin D1 genes

Expression of c-Myc and Cyclin D1 genes was done at 21 days after cardiac resection or OTR-shRNA injection. The total RNA was extracted from the tissues according to the Triazole protocol. The spectrophotometry was then used at 260 and 280 nm optical absorption to confirm the extracted RNA. Besides, cDNA was synthesized using a cDNA Synthesis kit, and about 1.5 μl of the synthetic cDNA was then used for the RT-PCR technique (Khori et al., 2018). GAPDH gene was used as an internal control. The sequences of primers are given in Table 1.

2.11. Evaluation of isolated heart function after OTR-shRNA injection

The surgical procedure was described in the previous studies (Alizadeh et al., 2011; Heallen et al., 2013). Animals were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p). Hearts were heparinized (500 IU, i.p) and quickly placed in the Krebs-Henseleit at 4 °C. Then, the hearts were immediately placed on Langendorff's system. Perfusion was performed by 80 mm Hg pressure with Krebs-Henseleit Buffer with the following composition in NaCl 118, KCl 4, MgCl2 1.2, NaHCO3 25, NaH2PO4 2H2O 1.2, CaCl2 1.9, and dextrose 11.1, and O2 (95%), CO2 (5%), and pH = 7.4 at 37 °C (Khori et al., 2012).

2.12. Stimulus protocols

The excitation protocols were performed using the Software (RIAN DARMAN CO, AV NODE PACK, Tehran, Iran) and an electric actuator (USA, FL, Sarasota, World Precision Instruments). Extracellular signals were recorded from the left ventricular epicardial surface with single silver electrodes (100 μm, AMI Co., Lexington, CA, USA). Electrocardiogram (ECG) and monophasic electrophoretic (MAPs) signals were amplified by a preamplifier (World Precision Instruments, ISODAM 8, USA, FL, Sarasota, Inc). All data were analyzed by online (AV-node-pack software) and offline applications (NSW, Bella Vista, AD Instruments, and Power Lab 7Lab Diagram, Australia). Online signal detection and measurement were performed using the software (RIAN DARMAN CO, AV NODE PACK, Tehran, Iran) (Khori et al., 2012).

A silver electrode was used to record monophasic action potential (MAP) from the right ventricular epicardial surface using a differential amplifier (USA, FL, Sarasota, World Precision Instruments). A bipolar platinum-iridium stimulation electrode (250 mm, AMI Co.) was inserted into the septum (Khori et al., 2012). The hearts were stimulated with a 2 mm monophasic pulse at constant diastolic threshold values (USA, FL, Sarasota, World Precision Instruments). The following parameters were measured: Restitution Action Potential Duration (APD), which refers to the correlation between APD and previous diastolic distance (DI). DIs were determined as the difference in S1 (first stimulation) and S2 (second stimulation) binding stimulation intervals and APD 90 and were then calculated at steady state S1–S2 (Fig. 1).

2.13. Electrical properties of the isolated rat hearts

In the standard terminology, APD 90 are the times from the onset of a depolarization until the cell repolarization to 90% of the full action potential (AP) amplitude. We used the specific drawing method to construct the APD restitution curve. Standard APD restitution acts on the right ventricle by the stimulation (S1) and then an additional stimulator (S2) and repetition with a shorter 30. This procedure was repeated for five different basal cycle lengths (BCL) 400 to 170 ms (400, 300, 250, 220, and 170 ms). Then, APD 90 was designed as previous DI functions, and restitution curves were adjusted using the following single function. Ventricular Effective Refractory Period (VERP) was estimated with an additional standard stimulus protocol (S2–S1) in different BCLs 400 to 170 ms. ERP is defined as the longest cycle length and the time of onset of the S1-and S2-MAP signals measured, and the delay of the successive stimuli was measured (Fig. 1).

2.14. Hemodynamic properties of the isolated hearts

Data were recorded by the PowerLab Software (AD Instruments, Sydney, Australia) to measure electrical and mechanical parameters. A water balloon connected to a pressure transducer was inserted into the left ventricle to obtain diastolic and systolic pressures between 5 and 10 mm Hg. Hemodynamic factors, including the systolic pressure, diastolic pressure, heart rate (HR), left ventricular diastolic pressure (LVDP), maximum and minimum pressures (dP/dt max+ and dP/dt min-), were shown as the indicators of contraction. Moreover, LVDP was calculated as an index of cardiac contraction function from the formula; LVDP = LV systolic pressure - LV diastolic pressure. The rate pressure product (RPP) was calculated by cardiac functions (RPP = HR × LVDP).

2.15. Statistical analysis

All results are presented as Mean ± S.D. All data were tested for normality before applying the statistical tests. Differences between the two groups were tested by the Student's t-test with Graph Pad Prism5 Software. Comparisons among multiple groups were made by one-way analysis of variance with the Scheffe contrasts. By using a computer, Marquardt's technique was used to perform non-linear curve fitting. $P \leq 0.05$ was considered significant.

3. Results

3.1. Evaluation of cardiac-OTR protein level

OTR protein level in cardiac tissues was examined using a specific oxytocin antibody (Oxytocin Receptor ELISA Kit ABIN Cat. No 6017841). The sensitivity of this test was 0.156–10 ng/ml. The results revealed that cardiac-OTR protein level was significantly decreased in OTR-shRNA groups' hearts on the 7th and 21st days after OTR-shRNA injection (Table 2). Interestingly, the cardiac-OTR protein level was significantly decreased in the OTR-shRNA injection groups compared to the control and resection groups. However, its expression was dramatically reduced in all hearts of the control, Resection, OTR-shRNA, and Resection-OTR-shRNA groups on the 21st day compared to the 7th day (Table 2).

Table 1
The primers used to evaluate gene expression in the present study.

Genes	Direct primer	Reverse primer	Product size (bp)
c-Myc	5′- CCCAGCGAACGTGATGATAAT-3′	5′- GCTTCCTCGGTTGGATATAGGAT -3′	241
Cyclin D1	5′- CTCCGTATCTTACTTCAAGTGCG -3′	5′- CTTCTCGGCAGTCAAGGGAA -3′	250
GAPDH	5′- CATCACTGCCACCCAGAAGACTG -3′	5′- ATGCCAGTGAGCTTCCCGTTCAG -3′	231

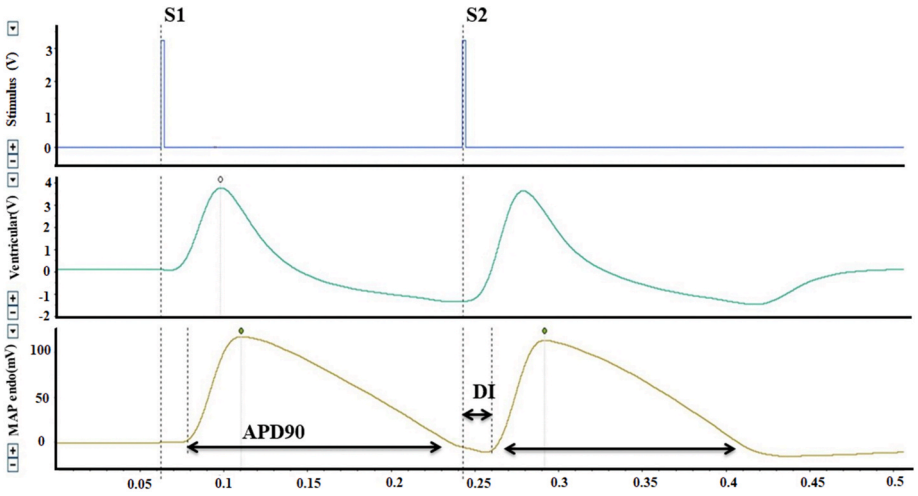

Fig. 1. A plot of the monophasic action potential duration 90 in neonatal rat hearts. First electrical stimulus (S1) during the 400 ms cycle and an additional stimulus (S2) at scheduled intervals, the plot shows the diastolic interval (DI) and MAP period length measurement in 90% of ventricular depolarization (MAPD90). Right ventricular stimulation, electrocardiogram (ECG), and ventricular monophasic action potential (MAP).

Table 2
The cardiac-OTR protein levels in neonatal rat hearts after OTR-shRNA injection and cardiac resection.

Groups	Days		
	1	7	21
Control	7.28 ± 0.5	6.65 ± 0.3	$^{\#}$4.55 ± 0.3
Resection	6.88 ± 0.9	6.42 ± 1.4	$^{\#}$4.94 ± 1.2
OTR-shRNA	7.02 ± 0.7	$^{*,\$}$3.22 ± 0.2	$^{\#,\$}$1.2 ± 0.1
Resection-OTR-shRNA	7.0 ± 0.8	*,†3.13 ± 0.1	$^{\#,\dagger}$1.25 ± 0.1

The results showed that although the expression changes of cardiac-OTR protein were not significant between Control and Resection groups, its expression was significantly decreased in the OTR-shRNA groups.
*P < 0.05 compared to 1 day.
$^{\#}$P < 0.05 compared to 7 days.
$^{\$}$ P < 0.05 compared to the control group.
†P < 0.05 compared to the Resection group.

3.2. The cell proliferation markers after OTR-shRNA injection

Table 3 shows the expression of ki-67, BrdU, and α-actinin proteins at days 7 and 21 in control, Resection, OTR-shRNA, Resection-OTR-shRNA groups after OTR-shRNA injection or cardiac resection. The results showed that, although the expression changes of ki-67, BrdU, and α-actinin proteins were not significant between Control and Resection groups, their expression was significantly decreased in the OTR-shRNA injection groups at the 7th and 21st days (P < 0.05) (Table 3).

Table 3
The expression of cell proliferation proteins in neonatal rat hearts after OTR-shRNA injection and cardiac resection.

Groups	7 day			21 day		
	Marhers					
	Ki-67	BrdU	α-actinin	Ki-67	BrdU	α-actinin
Control	4.4 ± 0.3	4.2 ± 0.5	4.1 ± 0.3	4.7 ± 0.3	4.5 ± 0.2	4.3 ± 0.4
Resection	5.2 ± 0.5	5.3 ± 0.6	5.2 ± 0.5	*5.7 ± 0.7	*6.7 ± 0.4	*6.1 ± 0.6
OTR-shRNA	*2.2 ± 0.3	*2.6 ± 0.4	*2.6 ± 0.5	*2.6 ± 0.3	*2.8 ± 0.3	*2.9 ± 0.4
Resection-OTR-shRNA	$^{\#}$1.8 ± 0.2	$^{\#}$2.1 ± 0.1	$^{\#}$1.9 ± 0.2	$^{\#}$2.1 ± 0.1	$^{\#}$2.3 ± 0.2	$^{\#}$2.3 ± 0.3

*P < 0.05 compared to the control group.
$^{\#}$P < 0.05 compared to the Resection group.

3.3. c-MYC protein expression in cardiac tissues

Western blotting was used to evaluate the c-Myc protein expression on the 21st day after OTR-shRNA injection and cardiac resection. The results showed that although the expression changes of c-Myc protein were not significant between Control and Resection groups, its expression was significantly decreased in the OTR-shRNA injection groups (P < 0.05) (Fig. 2).

3.4. The expression of cyclin D1 and c-Myc genes

The expression of cyclin D1 and c-MYC genes was done on the 21st day after OTR-shRNA injection and cardiac resection. The results showed that the cyclin D1 and c-Myc genes' expression in the OTR-shRNA injection group's hearts was significantly different from the control and Resection groups (P < 0.05, Fig. 3). Moreover, their expression was significantly decreased in the OTR-shRNA injection groups (P < 0.05, Fig. 3).

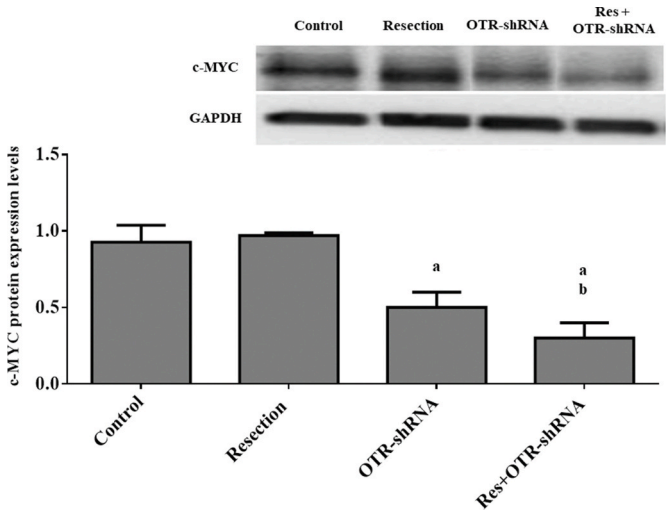

Fig. 2. The expression of c-Myc protein in the effects of the OTR-shRNA injection on neonatal rat hearts. ^{a} P < 0.05 compared to the control group. ^{b} P < 0.05 compared to the OTRshRNA group.

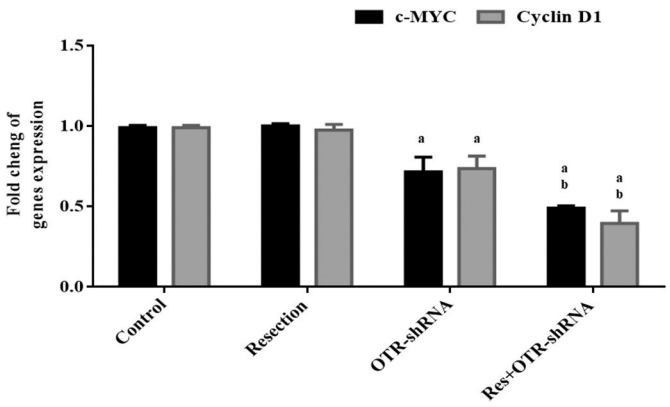

Fig. 3. The expression of cyclin D1 and c-MYC genes in the effects of the OTR-shRNA injection on neonatal rat hearts. [a] $P < 0.05$ compared to the control group. [b] $P < 0.05$ compared to OTRshRNA group.

3.5. The effects of OTR-shRNA injection on the hemodynamic and electrophysiologic parameters in isolated rat hearts

In the stimulated hearts, the duration of the action potential in the OTR-shRNA group was increased more than the control group (Figs. 4 and 5). In the long DI, the recovery curve of the Resection-OTR-shRNA group shifted upward to the left compared to the control group; whereas, in the short DI, it caused a segment to flatten, and the slope of curves have been removed ($P < 0.05$) (Fig. 6).

Except for End Diastolic Pressure (EDP), the rest of the hemodynamic parameters, including HR, LVDP, RPP, MAX dP/dt, and contractility, were reduced OTR-shRNA injection groups (Table 4). Moreover, the electrophysiologic parameters, including QT interval, QTc, Tpeak-Tend Interval, and ST, increased in the OTR-shRNA injection groups (Table 5).

4. Discussion

We have shown some developed heart problems in the left ventricle with OTR depletion two months after birth in neonatal rats. In these animals, the cardiac function was unusually decreased; hence, it seems that this model could mimic the potential effects of OTR depletion in adult animals. We hypothesize that the combined impact of hearts' mechanical and electrical instability is essential for the onset of arrhythmias. Although future studies are needed to demonstrate the specific effects of OT on different cells and tissues, our study showed that

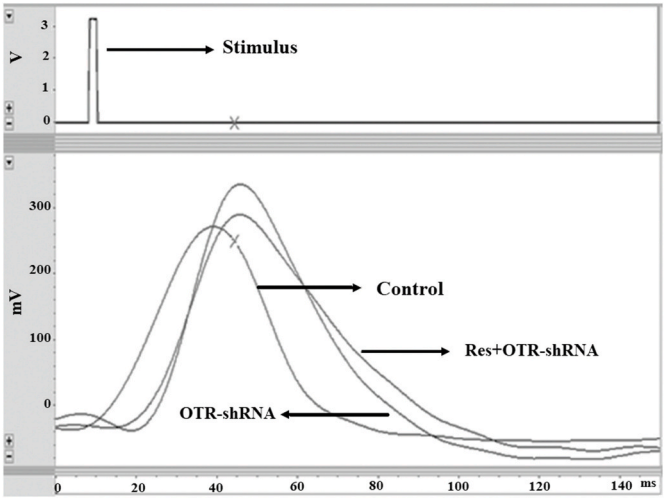

Fig. 4. The monophasic action potential (MAP) from the right ventricular epicardial in the effects of the OTR-shRNA injection on neonatal rat hearts.

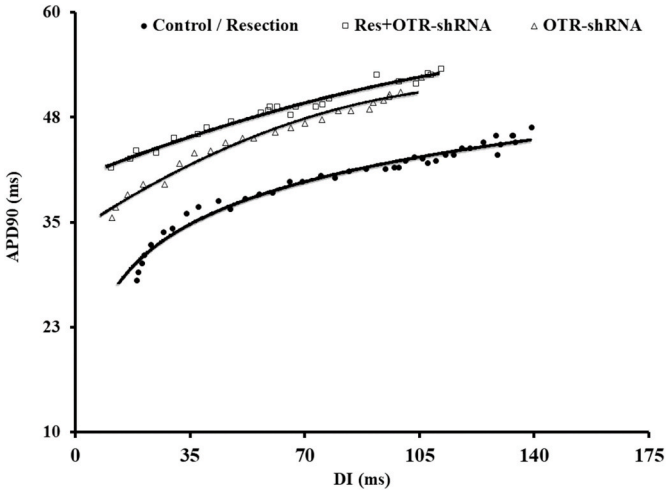

Fig. 5. The standard restitution curve [APD90 compared to diastolic intervals (DI)] in the effects of the OTR-shRNA injection on neonatal rat hearts.

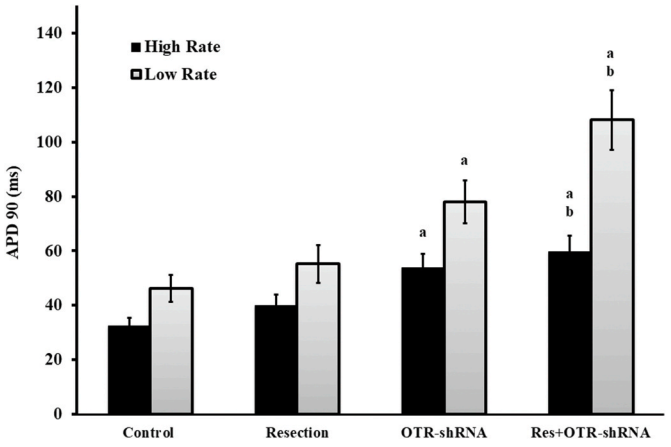

Fig. 6. The action potential length in the effects of the OTR-shRNA injection on neonatal rat hearts. [a] $P < 0.05$ compared to the control group. [b] $P < 0.05$ compared to the cardiac resection group.

Table 4
The hemodynamic parameters in the effects of the OTR-shRNA injection on isolated rat hearts.

Groups	Index					
	EDP	LVDP	HR	Max dp/dt	contractility	RPP
Control	8.5 ± 1.1	85 ± 15	249 ± 13	2231 ± 423	37.4 ± 1.3	21165 ± 698
Resection	9.1 ± 1.1	86 ± 14	235 ± 14	2188 ± 524	33.2 ± 1.6	20210 ± 734
OTR-shRNA	25.2 ± 1.2*	79 ± 13*	220 ± 5.6*	1950 ± 252*	29.1 ± 2.1*	17380 ± 531*
Resection-OTR-shRNA	38.1 ± 1.6#	66 ± 16#	154 ± 11#	1390 ± 169#	20.1 ± 1.1#	10164 ± 537#

EDP: End-diastolic pressure, **LVDP:** left ventricle diastolic pressure, **HR:** heart rate, **dP/dtmax+:** maximal pressure growth rate, **RPP:** product production rate. Data presented as Mean ± S.D.
*$P < 0.05$ compared to the control group.
#$P < 0.05$ compared to the Resection group.

Table 5
Electrophysiological parameters in the effects of the OTR-shRNA injection on isolated rat hearts.

Group	Index			
	QT Interval (s)	QTc (s)	Tpeak-Tend Interval (s)	ST (V)
Control	0.06 ± 0.001	0.1 ± 0.002	0.01 ± 0.001	0.02 ± 0.001
Resection	0.07 ± 0.002	0.12 ± 0.002	0.01 ± 0.002	0.02 ± 0.002
OTR-shRNA	0.09 ± 0.003*	0.17 ± 0.001*	0.04 ± 0.001*	0.08 ± 0.006*
Resection-OTR-shRNA	0.12 ± 0.003#	0.19 ± 0.001#	0.07 ± 0.001#	0.12 ± 0.006#

QT interval: the interval between Q to T, **QTc:** modified QT (Bazett's formula), **Tpeak-Tend interval:** the interval between T to peak-T to end, **ST elevation:** the distance between S and T.
*P < 0.05 compared to the control group.
#P < 0.05 compared to the Resection group.

the OTR deficiency could reduce cardiac cell genetic responses.

Our IHC observations on the BrdU, ki-67, α-actinin were shown a significant decrease in their protein levels from 7 days after cardiac resection in the Resection-OTR-shRNA rats (Muskhelishvili et al., 2003). Like other G protein-coupled receptor ligands, OT can act via PI3K/Akt activation and projection into downstream kinases (Cattaneo et al., 2008). Indeed, the PI3K/Akt and ERK1/2 pathways are vital transducers of anti-apoptotic signals in stimulated-OT cardiac tissues. These pathways are involved in cardiomyocyte functions, including proliferation, differentiation, motility, survival, and intracellular trafficking (Cattaneo et al., 2008). Different strategies such as c-Myc activation through the Wnt signaling pathway have been shown to successfully regenerate and maintain neonatal hurt hearts. In cell growth and proliferation, c-Myc is a downstream transduction pathway for the signaling pathway to generate positive or negative regulatory genes. The c-Myc transcription factor can be bounded to several molecules to regulate the transcriptional proliferation of genes involved in cell growth and apoptosis. It is also one of the most frequently mutated genes in proliferation and tumors. Here, our study suggests that OT can represent a present physiological inducer of cardiomyocyte proliferation. Its systemic delivery can counteract the defects in the regeneration and maintenance of neonatal harmed hearts.

Moreover, the inhibition of OTR was led to changes in the hemodynamic and electrical remodeling parameters in the OTR-shRNA injection groups. One interesting finding of the present study is the length of the APD associated with scattering in the cardiac ventricular tissues, resulting in recurrent arrhythmia (Delanian et al., 2003). Here, we propose that OTR-shRNA injection may cause an APD prolongation due to electrophysiological abnormalities and structural changes (Delanian et al., 2003). In the present study, we suggest that the OTR-shRNA injection may induce the APD flattening, possibly by the electrophysiological abnormality and structural remodeling. Moreover, the restitution hypothesis states that there is a direct relationship between APD and diastolic interval. The restitution curve's maximum slope can describe the magnitude of the APD shortening with changes in the diastolic interval. Some studies have shown the possibility of the electrical alternance and spiral break up even with the APD restitution slope <1 in the ischemic and remodeling tissues (Cherry and Fenton, 2004; Taggart et al., 1996). Tissue remodeling involves abnormal electrical function, apoptosis, and death. These events require the development of APD and ERP processes, electrical instability, and cardiac arrhythmias (Taggart et al., 1996). As a result, the effects of OTR-shRNA injection on the electrical instabilities and myocardial structural remodeling may contribute to its fundamental mechanism to induce electro-structural remodeling. Therefore, the increase in APD and ERP is probably related to electrical instability. Ventricular remodeling has been confirmed as a distinct process resulting from the Resection-OTR-shRNA hearts, which can cause cardiac remodeling (Porrello et al., 2011) (Bei et al., 2015).

5. Conclusion

Our results showed that OT is a physiological factor that can induce cardiac cell proliferation and cardiac remodeling; therefore, OT can partially alleviate cardiac tissue regeneration defects. It appears that the positive effects of OT on cardiac tissue homeostasis are practical, and OT and OTR agonists may potentially serve as functional and practical molecules against the deterioration of cardiac tissues.

Author contributions

Vahid Khori: study conception and design, data analysis, and manuscript preparation. **Fatemeh Mohammad Zadeh:** sample processing, manuscript revision. **Bahareh Tavakoli-Far:** sample processing, manuscript revision. **Ali Mohammad Alizadeh:** study conception and design, manuscript revision. **Solmaz Khalighfard:** sample collection, sample processing, and data analysis. **Maziar Ghandian Zanjan and Maryam Gharghi:** data analysis and manuscript revision. **Saeed Khodayari and Hamid Khodayari:** study conception and design. **Pedram Keshavarz:** manuscript preparation.

Ethical approval

According to the Declaration of Helsinki (DOH) guidelines, all experiments and procedures were performed, and its later amendments or comparable ethical standards. A review board committee approved the experimental procedures and the animal use and care protocols of the Tehran University of Medical Sciences (TUMS).

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Declaration of competing interest

The manuscript authors have no conflicts of interest to declare and are responsible for the paper's content.

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