Octreotide (somatostatin analog) attenuates cardiac ischemia/reperfusion injury via activating nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling pathway in rat model of hyperthyroidism

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Abstract

Background: Hyperthyroidism is known to increase the risk of ischemic heart diseases. Octreotide has been reported to attenuate ischemia/reperfusion (I/R) injury. Whether it is useful when ischemic heart disease is accompanied with co-morbidities like hyperthyroidism needs more clarifying. So, this study aimed to explore the effect of octreotide on cardiac I/R injury in hyperthyroid rats and to clarify if Nrf2 activation is involved in this effect. Forty adult female Wistar rats were subdivided into control (euthyroid) (n = 10) and hyperthyroid (n = 30) groups. Rats in hyperthyroid group received l-thyroxine (12 mg/L) in drinking water for 35 days, then were randomly divided into three equal subgroups (n = 10): hyperthyroid control positive group, hyperthyroid octreotide treated group, and hyperthyroid octreotide + Nrf2 inhibitor (brusatol) treated group. Isolated hearts were submitted to I/R and evaluated for cardiac hemodynamics and infarct size. Serum T3 and T4, coronary efflux lactate dehydrogenase (LDH) and creatine kinase-myoglobin binding (CK-MB) and cardiac tissue malondialdehyde (MDA) were estimated. Nrf2-regulated gene expressions of HO-1, SOD, GPx, and catalase were assessed.

Results: Octreotide administration to hyperthyroid rats improved baseline and post-ischemic recovery of cardiac hemodynamics, decreased the high coronary efflux LDH and CK-MB and tissue MDA, reduced infarction size, and upregulated the decreased antioxidative enzymes HO-1, SOD, GPx, and catalase mRNA expressions in the hyperthyroid I/R rat hearts. The Nrf2 inhibitor brusatol reversed the cardioprotective effect of octreotide in hyperthyroid I/R rat hearts.

Conclusion: Octreotide can reduce oxidative stress to effectively alleviate I/R injury in the hyperthyroid rat hearts through upregulation of Nrf2-dependent antioxidative signaling pathways.

Keywords: Hyperthyroidism, Myocardial ischemia/reperfusion injury, Nrf2, Octreotide, Oxidative stress

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Background

Myocardial ischemia is caused by impaired coronary flow and insufficient oxygen supply that leads to myocardial injury and necrosis [1]. Improving blood supply, known as reperfusion, is considered the most effective therapeutic maneuver to rescue ischemic myocardium [2]. Although reperfusion effectively reduces the mortality of cardiac cells, early restoration of blood flow leads to overproduction of free radicals like reactive oxygen species (ROS), which may further lead to cardiac tissue injury [3] accompanied by activation of cardioprotective mechanisms, an effect called hormesis [4]. These mechanisms include production of important antioxidative molecules as catalase, superoxide dismutases (SOD), glutathione, glutathione S-transferases, glutathione peroxidases (GPx), heme oxygenases (HO), thioredoxin reductases, and thioredoxins that can be regulated by the transcription factor nuclear factor (erythroid 2-related) factor 2 (Nrf2) [5]. Nrf2 is a transcription factor that binds to antioxidant response elements (AREs) in target genes and plays a decisive role in the coordinated induction of genes encoding many stress-responsive and cytoprotective enzymes and related proteins [6]. Consequently, activation of Nrf2 could be considered as a therapeutic option in cardiovascular diseases [7].

The cardiac functions are known to be regulated by thyroid hormones (TH) that have cardiac inotropic, chronotropic, lusitropic, and dromotropic properties [8]. Thyroid dysfunction has been related to high risk of cardiovascular morbidity and mortality. It was reported that tachycardia, cardiac hypertrophy, and heart failure were frequently demonstrated in hyperthyroidism [9]. The high levels of TH increase the cardiac susceptibility to ischemia/reperfusion (I/R) injury with elevated apoptotic rate of hypertrophied cardiomyocytes [10]. Such deleterious effects are attributed to increased rates of ATP consumption and overproduction of ROS encountered in hyperthyroidism [11]. It was reported that thyroid diseases are more common in women than in men, typically in their third to the fifth decade of life [12]. Moreover, it has been confirmed in mouse, rat, rabbit, and dog animal models that females exhibit a cardioprotective phenotype during their reproductive life. However, it was found that rat estrous cycle does not influence I/R injury either in vivo or ex vivo rat models [13].

Somatostatin (SS) is growth hormone inhibitory peptide that is produced by neuroendocrine and immune cells in response to ions, nutrients, neuropeptides, thyroid and steroid hormones, growth factors, and cytokines. Somatostatin receptors were found to be expressed in the rat heart and aortic myocytes [14]. Octreotide, a potent analog of somatostatin, was reported to have an antioxidant effect and positive effects on I/R injury in several organ rat models. It has protective effect against intestinal [15], pancreatic [16], and ovarian [17] I/R injury in rats. Moreover, octreotide can mimic the cardioprotective action of ischemic preconditioning against myocardial infarction [18]. However, assessment of its role in cardiac I/R injury in the presence of disease conditions such as hyperthyroidism needs to be investigated.

The current study aimed to explore the effect of octreotide on cardiac I/R injury in hyperthyroid female rats and to clarify if Nrf2 activation is involved in this effect.

Methods

Experimental animals

A total number of 40 adult female Wistar rats aged 12–15 weeks weighing 180–220 g were used as experimental animals in the current study and were purchased from animal house of the Faculty of Veterinary Medicine. The animals were housed in standard cages (five rats/cage). They were maintained under controlled room temperature (24–26 °C) and humidity (50–60%) with 12-h light and 12-h dark cycle and were fed on a standard diet with free access to water. All experimental procedures and protocols were following the guide for the care and use of laboratory animals (8th edition, National Academies Press) and have been reviewed and approved by Zagazig University institutional animal care unit committee (ZU-IACUC; Sharkia; Egypt) with approval number: ZU-IACUC/3/F/38/2019.

Experimental protocol

Following acclimatization for 1 week, rats were randomly divided into two groups: control (C) euthyroid group (n = 10) and hyperthyroid (HT) (n = 30) groups. Rats in hyperthyroid group received L-thyroxine (synthetic form of T4) (Eltroxin tablet, aspen, Egypt) (12 mg/L) in drinking water for 35 days [19]. After assessment of hyperthyroidism, the hyperthyroid rats were randomly divided into three equal subgroups (n = 10): hyperthyroid control positive (HTC) group, hyperthyroid octreotide treated (HT+OCT) group, and hyperthyroid octreotide + Nrf2 inhibitor brusatol treated (HT+BRU+OCT) group.

The rats in HT+OCT group received subcutaneous injections of octreotide (Sandostatin Amp. Novartis Co. Egypt) (35 µg/kg, in saline) 30 min prior to I/R [17]. The rats in HT+BRU+OCT group received intraperitoneal injection of brusatol (0.4 mg/kg, in dimethyl sulfoxide [DMSO], BioVision, Inc., USA) 10 min prior to octreotide injection [20], and the concentration of the vehicle was maintained at 0.5% (v/v). Rats of both control and HT groups received subcutaneous saline, 30 min prior to I/R, and intraperitoneal DMSO, 10 min prior to saline, while rats of HT+OCT group received intraperitoneal DMSO, 10 min prior to octreotide injections.
Assessment of induction of hyperthyroidism
At the end of hyperthyroidism induction phase, blood samples were obtained from tail veins and allowed to clot for 2 h at room temperature before centrifugation for 20 min, then examined for levels of 3,5,3’ triiodothyronine (T3) and thyroxine (T4) using commercial ELISA Kit (MyBioSource, Inc., USA) by colorimetric method according to the manufacturer’s instructions.

Preparation of I/R model in the isolated rat heart
Forty-eight hours following the last T4 dose and assessment of hyperthyroidism, the rats were sacrificed after 12 h of fasting under anesthesia (chloral hydrate) inhalation. The heart was rapidly excised to be retrogradely perfused on a Langendorff apparatus with modified Krebs-Henseleit buffer (pH 7.4, 37 °C) containing the following (in mM): NaCl 118.0, NaHCO3 25.0, CaCl2 1.25, KCl 4.7, MgSO4 1.2, KH2PO4 1.2, and glucose 11.0, equilibrated with 95% O2 + 5% CO2. Left ventricular pressure (LVP) was measured with a pressure transducer connected via a catheter to a latex balloon placed in the left ventricle through the left atrium. The balloon was filled with water to realize left ventricular end diastolic pressure (LVEDP) of about 5 mmHg. After 20 min of equilibration, the hearts were exposed to 30 min of global ischemia followed by 120 min of reperfusion. Left ventricular end diastolic pressure (LVEDP), LV maximal derivative of pressure (max. dP/dt), and LV minimal derivative of pressure (min. dP/dt) (±dp/dt) were recorded by using Power Lab 4/30 (ML 888, AD Instruments, Australia). All recordings were digitized and analyzed using the program Lab Chart Pro software (version 7.3.7, AD Instruments, Australia). All measures were recorded at the end of baseline period and 30 min, 60 min, and 120 min of reperfusion period.

Determination of infarct size
At the end of reperfusion, hearts were removed from the perfusion apparatus. Ventricular sections were sliced from the apex to base into 1.5 to 3 mm circumferential slices, incubated 20 min in 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) in phosphate buffer (pH 7.4) at 37 °C, then immersed in 10% formalin for 2 h to identify viable myocardium as red stained, while necrotic (infracted) tissue remains unstained (pale gray). TTC-stained ventricle slices were placed between two glass slides and photographed with a digital camera (Canon WB250F). Infarct size was measured by using Fiji Image J (1.51n; National Institute of Health; NIH, Bethesda, MD, USA) and expressed as a percentage of the total ventricular area [21].

Determination of cardiac enzymatic markers
Coronary efflux (the reperfusion fluid) was collected for 10 min of reperfusion and spectrophotometrically assayed for lactate dehydrogenase (LDH) by rat ELISA kits (Vitro Scient, Egypt) and creatine kinase-myoglobin binding (CK-MB) by rat ELISA Kits (Pointe Scientifc, Inc., USA).

Determination of cardiac tissue lipid peroxidation
The hearts’ tissue were frozen and stored at ~ 80 °C until analysis of malondialdehyde (MDA) in cardiac homogenates using commercially available ELISA kits (Bio diagnostic, Egypt) for monitoring lipid peroxidation spectrophotometrically.

Real-time reverse transcription polymerase chain reaction (RT-PCR) for the relative quantification of Nrf2-regulated gene expressions of HO-1, SOD, GPx, and catalase
Total RNA was extracted from homogenized heart specimens using the ribozol RNA extraction reagent (Amresco, Solon, Cleveland, OH, USA) as per the manufacturer’s instructions. cDNAs were synthesized using the SensiFAST TM cDNA synthesis kit (Bioline, London, UK). Real-time PCR was performed using 10 μl of SYBER Green PCR Mix (SensiFAST™ SYBER Lo-ROX Kit, Bioline, London, UK). The SYBER green data were analyzed with a relative quantification to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the reference gene. The sets of primers used were provided by Sigma Aldrich (Sigma-Aldrich, Germany) (Table 1). The relative gene expression ratio is calculated from the real-time PCR using the 2−ΔΔCt method [22].

Statistical analysis
The results are presented as descriptive statistics (mean ± standard deviation). Statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 25 (SPSS, Inc., IBM Company, Chicago, IL, USA). The normal distribution of data from each group was confirmed using the Kolmogorov-Smirnov normality test. Since the test indicated that variables followed normal distribution, comparisons among the experimental groups were analyzed by one-way analysis of variance (ANOVA) followed by least significance differences (LSD) test to evaluate statistical difference between two groups. Independent samples t test was used to compare the two groups. P value < 0.05 was considered to be statistically significant.

Results
Development of the rat model of hyperthyroidism
Serum level of T3 was (28.9 ± 4.9) and (236.3 ± 14.4) ng/dL and T4 was (5.2 ± 0.86) and (14.4 ± 1.3) μg/dL in the control and HT groups, respectively.
There was a significant increase in serum levels of T3 and T4 in HT group when compared by control one \( p < 0.001 \) (Fig. 1).

**Effect of octreotide on left ventricular function**

Baseline cardiac hemodynamics: LVDP and ±dp/dt were significantly lower in the hyperthyroid group than control \( p < 0.001 \) while LVEDP show insignificant difference between all groups \( F = 0.897, P = 0.452 \). Octreotide administration leads to significant increase in LVDP and ±dp/dt \( p = 0.002, p < 0.001, p < 0.001 \) respectively in the HT+OCT group when compared with the HTC group, and this effect was abolished by brusatol leading to significant decrease in LVDP and ±dp/dt values in the HT+BRU+OCT \( p = 0.018, p = 0.016, p < 0.001 \) respectively in comparison to the HT+OCT group.

Induction of cardiac ischemia led to a state of ischemic contracture or stone heart, and the ±dp/dt reached zero levels in all experimental groups.

After ischemia, hyperthyroid group showed a significant increase in LVEDP (post ischemic contracture), compared to control \( p < 0.001 \), Octreotide significantly reduced the LVEDP during reperfusion phase in the HT+OCT group when compared with the HTC group \( p < 0.001 \); in addition, brusatol significantly abolish the octreotide action on LVEDP during reperfusion phase in the HT+BRU+OCT group compared to the HT+OCT group \( p < 0.001 \).

Post-ischemic ±dp/dt and LVDP decreased significantly in the HTC group, compared with the control group \( p < 0.001 \). Octreotide significantly improved the ±dp/dt and LVDP in reperfusion phase in the HT+OCT group when compared with the HTC group \( p < 0.001 \), and this action is abolished by brusatol leading to significant decrease in LVDP and ±dp/dt values in the HT+BRU+OCT group \( p < 0.001 \) in comparison to the HT+OCT group (Table 2).

**Effect of octreotide on infarct size**

Infarction size as % of the total area was 40.6 ± 4.8, 56.6 ± 4.35, 46.9 ± 5.5, and 53.8 ± 5.4 in the control, HTC, HT+OCT, and HT+BRU+OCT groups, respectively.

Infarction size was increased by induction of hyperthyroidism; HTC \( p < 0.001 \), HT+OCT \( p = 0.008 \), and HT+BRU+OCT \( p < 0.001 \) in comparison to control rats. Octreotide administration leads to significant decrease in infarction size \( p < 0.001 \) in the HT+OCT group when compared with the HTC group, and this effect was abolished by brusatol \( p = 0.004 \) in comparison to the HT+OCT group (Fig. 2).

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**Table 1** Primer sequences

| Gene | Forward | Reverse |
|------|---------|---------|
| HO-1 | 5′-CGTGCAGAGAATTCTGAGTTC-3′ | 5′-AGACGCTTTACGTAGTGCTG-3′ |
| SOD  | 5′-ATGGGACAATACTACAAAGGC-3′ | 5′-TCATCTGTTTCGTTGAC-3′ |
| GPx  | 5′-CACAGTCACCAGTGATGCC-3′ | 5′-AGTTGGGCTGAACCCACC-3′ |
| Catalase | 5′-GTCCGATTCCACAGTGC-3′ | 5′-CGCTGAACAAAGTAACCTG-3′ |
| GAPDH | 5′-CCATCAACGACCCCTTCATT-3′ | 5′-GACCAGCTCCCTCTTCCAG-3′ |

HO-1 heme-oxygenase-1, SOD superoxide dismutase, GPx glutathione peroxidase, GAPDH glyceraldehyde-3-phosphate dehydrogenase

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Fig. 1 Evaluation of serum levels of thyroid hormones. a T3, b T4. n = 10 rats in the control group and 30 rats in hyperthyroid. Data are represented as mean ± standard deviation. *Differs significantly from the control group with \( p < 0.05 \)
Effect of octreotide on post-ischemic cardiac enzymes release

Post-ischemic coronary efflux LDH was (230.6 ± 12.3), (289.9 ± 13.9), (245.9 ± 16.6), and (283.5 ± 12.7) IU/L and CK-MB was (617 ± 17.2), (687 ± 10.2), (653 ± 13.1), and (684 ± 15.3) IU/L in control, HTC, HT+OCT, and HT+BRU+OCT groups, respectively.

Coronary efflux LDH and CK-MB was elevated by induction of hyperthyroidism: HTC (p < 0.001), HT+OCT (p = 0.019; p < 0.001), and HT+BRU+OCT (p < 0.001) in comparison to the control group. Octreotide administration leads to significant decrease in their level (p < 0.001) in the HT+OCT group when compared with the HTC group, and this effect was abolished by brusatol in the HT+BRU+OCT group (p < 0.001) in comparison to the HT+OCT group (Fig. 3).

Effect of octreotide on cardiac tissue MDA

Cardiac tissue MDA level was higher in hyperthyroid rats: HTC (p < 0.001), HT+OCT (p = 0.02), and HT+BRU+OCT (p < 0.001) in comparison to the control group. Octreotide administration leads to significant decrease in MDA level (p < 0.001) in the HT+OCT group when compared with the HTC group, and this effect was abolished by brusatol in the HT+BRU+OCT group (p < 0.001) in comparison to the HT+OCT group (Fig. 4).

Effect of octreotide on Nrf2 antioxidative-regulated gene mRNA expressions

Nrf2 antioxidative gene HO-1, SOD, GPx, and catalase mRNA expressions were decreased by induction of hyperthyroidism (p < 0.001) in comparison to the control group. Octreotide administration improved this action leading to significant increase in HO-1, SOD, GPx, and catalase mRNA expressions (p < 0.001) in the HT+OCT group when compared with the HTC group, and this effect was abolished by brusatol in the HT+BRU+OCT group (p < 0.001) in comparison to the HT+OCT group (Fig. 5).

Table 2 Effect of octreotide on left ventricular hemodynamic parameters in hyperthyroid rat hearts exposed to ischemia/reperfusion

|                          | Baseline | Reperfusion (min) time-point |
|--------------------------|----------|-----------------------------|
|                          |          | 30  | 60  | 120 |
| LVEDP (mmHg)             |          |     |     |     |
| Control                  | 8.5 ± 1.01 | 33.8 ± 2.3 | 31.2 ± 3.2 | 29.2 ± 2.7 |
| HTC                      | 9.3 ± 1.4 | 43.5 ± 3.6* | 41.7 ± 2.9* | 38.9 ± 3* |
| HT+OCT                   | 8.7 ± 1.5 | 35.3 ± 1.7* | 32 ± 3.7* | 30.8 ± 3.1* |
| HT+BRU+OCT               | 9.5 ± 1.98 | 41.4 ± 2.5* | 40.1 ± 2.7* | 37.7 ± 4.9* |
| LVDP (% of baseline)     |          |     |     |     |
| Control                  | 100 (93.2 ± 8.4 mmHg) | 62.1 ± 2.8 | 50.9 ± 3.4 | 37.2 ± 3.2 |
| HTC                      | 100 (72.2 ± 8.8 mmHg)* | 54.2 ± 3.5* | 43.7 ± 3.1* | 29.5 ± 3.3* |
| HT+OCT                   | 100 (84.2 ± 6.01 mmHg)*$ | 63.1 ± 2.8* | 52.3 ± 3.5* | 35.6 ± 4.1* |
| HT+BRU+OCT               | 100 (75.4 ± 8.3 mmHg)*$ | 53.1 ± 3.3* | 42.1 ± 4.1* | 28.2 ± 3.8* |
| Max. dP/dt (% of baseline)|          |     |     |     |
| Control                  | 100 (2794 ± 121 mmHg/s) | 60.3 ± 3.8 | 49.1 ± 4.8 | 36.6 ± 3.6 |
| HTC                      | 100 (2457 ± 102 mmHg/s)* | 53.8 ± 4.1* | 42.1 ± 4.4* | 28.5 ± 3.6* |
| HT+OCT                   | 100 (2672 ± 160 mmHg/s)*$ | 62.1 ± 2.6* | 51.1 ± 2.3* | 37.8 ± 4.9* |
| HT+BRU+OCT               | 100 (2531 ± 106 mmHg/s)*$ | 52.1 ± 3.2* | 41.4 ± 3.2* | 29.6 ± 2.9* |
| Min. dP/dt (% of baseline)|          |     |     |     |
| Control                  | 100 (2025 ± 199 mmHg/s) | 59.6 ± 3.5 | 47.9 ± 4.4 | 38.2 ± 4.7 |
| HTC                      | 100 (1401 ± 117 mmHg/s)* | 52.4 ± 3.9* | 39.7 ± 3.9* | 28.5 ± 3.6* |
| HT+OCT                   | 100 (1788 ± 124 mmHg/s)*$ | 60.1 ± 3.1* | 48.1 ± 4.9* | 36.8 ± 4.6* |
| HT+BRU+OCT               | 100 (1336 ± 116 mmHg/s)*$ | 50.9 ± 4.4* | 38.4 ± 2.9* | 27.9 ± 3.7* |

n = 10 in each group. Data are represented as mean ± SD

LVEDP left ventricular end diastolic pressure, LVDP left ventricular developed pressure, max. dP/dt LV maximal derivative of pressure, min. dP/dt LV minimal derivative of pressure

Significance (P < 0.05): *significant when compared with the control group, $significant when compared with HTC groups, *significant when compared with HT+OCT.
Discussion

In the current work, our findings indicate that hyperthyroidism increased injury induced by I/R in female rat heart. Treatment with octreotide attenuated myocardial injury and improved cardiac function in the I/R heart of the hyperthyroid female rats. Activation of the Nrf2 antioxidative function was involved in this action of octreotide as evidenced by reduction in the cardio-protective effect of octreotide by Nrf2 inhibitor, brusatol.

In the present study, adult female rats were given L-thyroxine in drinking water showing hyperthyroidism as evidenced by high T3 and T4 levels compared to control groups. Long-term T4 administration increased serum levels of T4, and serum T3 levels were also increased as T4 can be converted into T3 by deiodinases type 1 and 2 in target tissues [23]. As the incidence of most thyroid diseases is higher in women than men (ratio 8:1) [24], the current study was designed to use female rats.
Fig. 4 Effect of octreotide on cardiac tissue MDA in rat hearts exposed to ischemia for 30 min and reperfusion for 120 min. \( n = 10 \) rats in each group. Data are represented as mean ± standard deviation. Significance \((P < 0.05)\): *significant when compared with the control group, $significant when compared with HTC group, $significant when compared with HT+OCT.

Fig. 5 Effect of octreotide on Nrf2-regulated gene expressions of HO-1 (a), SOD (b), GPx (c), and catalase (d) in rat hearts exposed to ischemia for 30 min and reperfusion for 120 min. Data are represented as mean ± standard deviation. Significance \((P < 0.05)\): *significant when compared with the control group, $significant when compared with HTC group, $significant when compared with HT+OCT.
Regarding ventricular function, baseline values of LVDP and $\pm$dp/dt were lower in the hyperthyroid rats and revealed a decreased recovery of LVDP and $\pm$dp/dt following I/R with increase in LVEDP (post-ischemic contracture) indicating more susceptibility to I/R injury. These findings are supported by findings of other researchers [11, 19] who demonstrated that LVDP recovery was significantly impaired due to persistently increased LVEDP levels throughout the reperfusion period with marked reduction in contractility and relaxation velocities in thyrotoxic rat hearts. In contrast, it was noticed that there was an improvement in the cardiac hemodynamic in the hyperthyroid male mouse heart after I/R injury [25]. Moreover, it was found that hyperthyroidism provides cardio-protective effects following I/R in male rats [26, 27].

Our finding concerned increased LDH and CK-MB levels in first 10 min of reperfusion and increased infarct size in hyperthyroid rats indicates higher susceptibility of the hyperthyroid rat heart to I/R injury. This could be attributed to higher oxidative stress activity in hyperthyroid rats that could oxidize membrane lipids as evidenced in the current work by elevated cardiac tissue MDA levels in hyperthyroid rats. This increased oxidative stress is associated with decrease in endogenous antioxidative defenses indicated by decreased antioxidative enzymes HO-1, SOD, GPx, and catalase mRNA expressions in the hyperthyroid I/R heart.

Oxidative stress occurs when there is an imbalance between pro-oxidants and antioxidants, which occurs when antioxidant defenses cannot neutralize oxidants [28]. Excess thyroid hormones increases energy demand and mitochondrial activity that surge the ROS production [29]. Moreover, TH could increase ROS by direct actions of mitochondrial activity as T3 receptors have been described to present in mitochondria [30].

Supporting our results, it was found that catalase, SOD2, and GPX1 mRNA expressions were downregulated in hyperthyroid rat hearts after I/R injury [19]. In addition, clinical studies described diminished antioxidant enzymes expression in hyperthyroid patients that could be reestablished by antithyroid drugs [31].

Octreotide, a potent somatostatin analog, was reported to have an antioxidant effect and had promising actions against intestinal [15], hepatic [32], and ovarian [17] I/R injury in rats. Moreover, it was reported that octreotide may have a protective action against I/R injury in hyperthyroid male rats [33].

The data obtained from the current study revealed that octreotide administration to hyperthyroid rats 30 min prior to I/R improved baseline and post-ischemic recovery of cardiac function in hyperthyroid rats that accompanied by reduction of myocardial injury, as indicated by decreased coronary efflux LDH and CK-MB and reduced infarction size. Moreover, octreotide reduced the lipid peroxidation product MDA and upregulated the decreased antioxidative enzymes HO-1, SOD, GPx, and catalase mRNA expressions in the hyperthyroid I/R heart suggesting that the cardio protective action of octreotide could be due its antioxidant properties.

Mechanisms of octreotide action on I/R injury in different organ models were discussed in several studies that highlighted the antioxidant properties of octreotide. It was reported that octreotide decreased retinal MDA in a retina I/R model [34]. Moreover, tissue MDA and myeloperoxidase activity were decreased by octreotide treatment in rat model of acute increased intra-abdominal pressure-induced I/R injury [35]. Similarly, favorable effects of octreotide against intestinal I/R injury were found that are based on HO-1 induction [15].

It was postulated that Nrf2 could upregulate expression of antioxidative enzymes including HO-1, SOD, and GPx and catalase to activate protective mechanisms against oxidative injury [36]. Abnormalities in Nrf2 and Nrf2-regulated genes have been associated with cardiovascular diseases [37]. Moreover, previous studies revealed that octreotide i.p. immediately after experimental ischemic stroke in rats could activate the Nrf2 signaling pathway that upregulated HO-1 and downregulated NF-kB expressions an important nuclear transcription factor that regulates the genes of a vast number of inflammatory mediators [38].

Consequently, whether octreotide could diminish I/R injury in the hyperthyroid heart through activation of Nrf2-linked antioxidative pathway was investigated in the current study, and we found that the Nrf2 inhibitor brusatol reversed the cardioprotective effect of octreotide in hyperthyroid I/R hearts. These results suggested that octreotide reduces oxidative stress in the hyperthyroid I/R heart at least, partly via modulations of the Nrf2 antioxidative pathway. However, the underlying mechanism that mediates the activation of Nrf2 by octreotide in the hyperthyroid I/R rat heart needs to be clarified.

Conclusion
In conclusion, octreotide, a potent analog of somatostatin, can reduce oxidative stress to effectively alleviate I/R injury in the hyperthyroid rat hearts through upregulation of Nrf2-dependent antioxidative signaling pathway. Future studies to elucidate the exact mechanism of octreotide-dependent activation of Nrf2 are recommended.

**Abbreviations**
- ANOVA: Analysis of variance
- CK-MB: Creatine kinase-myoglobin binding
- dp/dt: LV derivative of pressure
- GPx: Glutathione peroxidases
- HO: Heme oxygenases
- I/R: Ischemia/reperfusion
- LDH: Lactate dehydrogenase
- MDA: Malondialdehyde
- Nrf2: Nuclear factor (erythroid-derived 2)-like 2
- ROS: Reactive oxygen species
- SOD: Superoxide dismutases
- T3: 3,5,3'-Triiodothyronine
- T4: Thyroxine
- TH: Thyroid hormones
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Authors’ contributions
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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
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27. Iliopoulos I, Mourouzis I, Lambrou GI, Iliopoulos D, Koutsouris DD, Pantos C (2018) Time-dependent and independent effects of thyroid hormone administration following myocardial infarction in rats. Mol Med Rep 18(1): 864–876. https://doi.org/10.3892/mmr.2018.9008 PMID: 29767239; PMCID: PMC6059718

28. Marullo LF, Magalhães RA, Lemes RPG, de Almeida Filho TP, de Castro MF, Maia Filho PA, Cunha TOV, Quiñodo AR, Fontenele EGP, Sun G, Martins MRA (2018) Levotiroxine replacement improves oxidative status in primary hypothyroidism. Front Endocrinol (Lausanne) 9:655. https://doi.org/10.3389/fendo.2018.00655 PMID: 30,467,493

29. Murphy MP (2009) How mitochondria produce reactive oxygen species. Biochem J 417(1):1–13. https://doi.org/10.1042/BJ20081386 PMID: 19,061,483

30. Wrutniak-Cabello C, Casas F, Cabello G (2017) Mitochondrial T3 receptor and targets. Mol Cell Endocrinol 458:112–120. https://doi.org/10.1016/j.mce.2017.01.054 PMID: 28,167,126

31. Lassoued S, Mseddi M, Mnif F, Abid M, Guermazi F, Masmoudi H, El Feki A, Attia H (2010) A comparative study of the oxidative profile in Graves’ disease, Hashimoto’s thyroiditis, and papillary thyroid cancer. Biol Trace Elem Res 138(1–3):107–115. https://doi.org/10.1007/s12011-010-8625-1 PMID: 20,204,550

32. Yang J, Sun H, Guan R, Liu W, Xia Y, Zhao J, Liu J (2014) Hepatocellular protein profiles after hepatic ischemia/reperfusion injury with or without octreotide preconditioning in a rabbit model. Transplant Proc 46(10):3282–3288. https://doi.org/10.1016/j.transproceed.2014.06.071 PMID: 25,498,038

33. Morsy WE (2018) Effects of somatostatin analogue (octreotide) on ischemia—reperfusion injury in adult hyperthyroid albino rats. Al-Azhar Med J 47(4):669–688. https://doi.org/10.12816/0053053

34. Wang J, Sun Z, Shen J, Wu D, Liu F, Yang R, Ji S, Ji A, Li Y (2015, 2015) Octreotide protects the mouse retina against ischemic reperfusion injury through regulation of antioxidation and activation of NF-kB. Oxid Med Cell Longev 970156. https://doi.org/10.1155/2015/970156 PMID: 26,175,842

35. Kaçmaz A, Polat A, User Y, Tilik M, Ozkan S, Senler G (2004) Octreotide improves reperfusion-induced oxidative injury in acute abdominal hypertension in rats. J Gastrointest Surg 8(1):113–119. https://doi.org/10.1016/j.gassur.2003.09.026 PMID: 14,746,843

36. Lu MC, Ji JA, Jiang ZY, You QD (2016) The Keap1-Nrf2-ARE pathway as a potential preventive and therapeutic target: An Update. Med Res Rev 36(5): 924–963. https://doi.org/10.1002/med.21396 PMID: 27,192,495

37. Ndisang JF (2017) Synergistic interaction between heme oxygenase (HO) and nuclear-factor E2-related factor-2 (Nrf2) against oxidative stress in cardiovascular related diseases. Curr Pharm Des 23(10):1465–1470. https://doi.org/10.2174/1381612823666170113153818 PMID: 28,088,909

38. Chen L, Wang L, Zhang X, Cui L, Xing Y, Dong L, Liu Z, Li Y, Zhang X, Wang C, Bai X, Zhang J, Zhang L, Zhao X (2012) The protection by octreotide against experimental ischemic stroke: up-regulated transcription factor Nrf2, HO-1 and down-regulated NF-kB expression. Brain Res 1475:80–87. https://doi.org/10.1016/j.brainres.2012.07.052 MID: 22885292

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