

EFFECTS OF SOMATOSTATIN ANALOGUE (OCTREOTIDE) ON ISCHEMIA–REPERFUSION INJURY IN ADULT HYPERTHYROID ALBINO RATS

By

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ABSTRACT

Background: Thyroid dysfunction is associated with increased cardiovascular morbidity and mortality. Hyperthyroidism increases the susceptibility of the heart to ischemia reperfusion injury. Moreover, myocardial ischemia can be further worsened by increased cardiac workload and oxygen demand elicited by the excess thyroid hormones.

Objectives: Investigating the cardiac dysfunction induced by ischemia-reperfusion (IR) in rats exposed to excess thyroid hormones and role of somatostatin analogue against ischemia/reperfusion injury in hyperthyroid rats.

Patients and Methods: This study was performed on 24 adult male albino rats of a local strain weighing 140-200 g, allocated into three equal groups: **Group I** (*Control IR group*): Euthyroid control group exposed to ischemia-reperfusion. Hearts of this group were isolated and perfused in a Langendorff apparatus. Isolated hearts were subjected to 15 min of stabilization, 20 min of zero-flow global ischemia and 30 min of reperfusion. **Group II** (*Hyperthyroid IR group*) were subjected to the same protocol of ischemia reperfusion. **Group III** (*Hyperthyroid IR-OCT group*): Hyperthyroid rats were supplemented with the somatostatin analogue, octreotide (OCT), 35 µg/kg subcutaneously, 20 minutes before sacrifice, followed by in-vitro study of cardiac responses to ischemia reperfusion. Blood samples were collected for measurement of plasma free T3, T4 and TSH. Coronary efflux lactate dehydrogenase levels were measured. Also, cardiac tissue malondialdehyde (MDA) levels and tumor necrosis factor alpha (TNF-α) levels were estimated.

Results: Hyperthyroid (IR) rats revealed significant elevation in plasma free T3 and T4 with concomitant decrease in TSH levels. A significant reduction in final BW and BW gain were observed with higher absolute weights of left ventricle (LV) and cardiac index compared to the euthyroid controls. Systolic blood pressure (SBP) and mean arterial pressure (MAP) significantly elevated in non-treated hyperthyroid rats with a significant increase in their baseline chronotropic and inotropic activity compared to euthyroid control rats. But after ischemia those rats exhibited significantly higher degree of bradycardia (ΔHR) and % change in heart rate (HR) compared to control rats. Moreover, significantly deteriorated post-ischemic recovery of inotropic parameters and significantly higher Δ changes of these parameters were observed compared to euthyroid controls. These changes were accompanied by significant elevation in coronary efflux LDH levels and both cardiac tissue MDA and TNFα. Meanwhile, octreotide administration to hyperthyroid rats showed improvement of their baseline chronotropic and inotropic parameters as evidenced by the significantly reduced degree of bradycardia as well as significantly lower Δ change of PT and PT/LV parameters compared to non-treated hyperthyroid rats. Such findings could be ascribed partly to the reduction of

oxidative and inflammatory responses to IR as determined by the significantly decreased cardiac tissue MDA and TNF α respectively.

Conclusion: Hyperthyroid hearts are less tolerant to ischemia/reperfusion injury with higher susceptibility to oxidative stress as well as overproduction of pro-inflammatory mediators. Thus, pharmacological targets that defend oxidative challenge and confer anti-inflammatory properties, namely octreotide (somatostatin analogue) are considered to have potential therapeutic effect and may protect against myocardial ischemic damage in hyperthyroidism.

Key words: Ischemia reperfusion injury, hyperthyroid, oxidative stress, inflammation, somatostatin, octreotide.

INTRODUCTION

Thyroid hormones are known to play crucial roles in the regulation of cardiovascular functions. Cardiac inotropic, chronotropic properties as well as vascular resistance are directly affected by thyroid hormones levels (*Seara et al., 2018*).

Hyperthyroidism is associated with tachyarrhythmias, cardiac hypertrophy and heart failure with increased cardiovascular morbidity and mortality (*Rodondi et al., 2008*). The high levels of thyroid hormones increase the susceptibility of the heart to ischemia/reperfusion (IR) injury, elevate apoptotic rate of hypertrophied cardiomyocytes, and increase heart failure (*Wang et al., 2010*). Such deleterious effects are attributed to increased rate of ATP consumption, redox imbalance and overproduction of ROS encountered in hyperthyroidism (*Zaman et al., 2014*).

Moreover, myocardial ischemia can be further worsened by increased cardiac workload and oxygen demand elicited by the excess thyroid hormones (*Pantos et al., 2011* and *Seara et al., 2018*).

Somatostatin (SS) is a peptide, known predominantly for its inhibitory action on growth hormone, however, SS is localized in the nerve fibers within muscle bundles

of both the atria and ventricles with a surprising high level of somatostatin receptors (SSTR) expressed in the rat heart with different distribution of the receptor subtypes in cardiac fibroblasts and cardiomyocytes (*Widiapradja et al., 2017*).

Octreotide, a synthetic analogue of somatostatin can mimic ischemic preconditioning (PC) to provide cardioprotection against myocardial infarction through activation of protein kinase C, tyrosine kinase C, and mitochondrial ATP-sensitive potassium channels (*Wang et al., 2005*).

Although thyrotoxic hearts are more vulnerable to IR damage, up to our knowledge, only a recent study performed by *Seara et al. (2018)* has issued the progression of ischemia reperfusion injury in rat hearts exposed to supraphysiological concentration of thyroid hormones .

In addition, most experimental data on somatostatin analogue has focused on the “non-diseased” myocardium (*Wang et al., 2005*) or other ischemic organ damage such as hepatic ischemia reperfusion injury (*Yang et al., 2014*). Yet, no available studies in the literature have accessed its cardioprotective role against IR injury in the presence of disease conditions such as hyperthyroidism .

The present study was designed to investigate the cardiac dysfunction induced by ischemia-reperfusion in rats exposed to excess thyroid hormones and through more light on the potential role of somatostatin analogue against ischemia/reperfusion injury in hyperthyroid rats.

MATERIALS AND METHODS

Experimental animals:

The present study was performed on 24 adult male albino rats of local strain weighing 140-200 g. Rats were purchased from the Egyptian Organization for Biological Products and Vaccines (VACSERA) and were kept in the Animal House, Physiology Department, Faculty of Medicine, Ain Shams University under standard conditions of boarding and feeding with free water access. Animals were kept in their cages, (30cm width x 50cm length x 20 cm height, 4 rats/cage), well ventilated, and in normal day/night cycle. Ethical approval for the study was given by the Research Ethics Committee, Faculty of Medicine, Ain Shams University (FMASU, REC, Cairo, Egypt). The study period was 8 weeks during which rats were allocated into three equal groups:

Group I (Control IR group):

Euthyroid control group exposed to ischemia-reperfusion. Hearts of this group were isolated and perfused in a Langendorff preparation. Isolated hearts were subjected to 15 min of stabilization, 20 min of zero-flow global ischemia and 30 min of reperfusion.

Group II (Hyperthyroid IR group):

Hyperthyroid rats were subjected to

the same protocol of ischemia reperfusion.

Group III (Hyperthyroid IR-OCT group):

Hyperthyroid rats were supplemented with the somatostatin analogue, octreotide (OCT), 35 µg/kg subcutaneously, 20 minutes before sacrifice, followed by in-vitro study of cardiac responses to ischemia reperfusion.

Induction of Hyperthyroidism:

Rats in the experimental hyperthyroid groups (groups II&III) were given intraperitoneal injection of L-thyroxine (Sigma chemicals Co., St. Louis, MO), in a dose of 10 µg /100g body weight, daily for 8 weeks (*Jiang et al., 2001*). L-thyroxine was dissolved in a minimum amount of NaOH, and then the concentration was adjusted to 2×10^{-3} M by dilution with normal saline solution (*Komeda et al., 1977*). Final pH was adjusted at 7. Euthyroid control rats (group I) were injected intraperitoneally with an equivalent volume of saline diluted-NaOH solution (the solvent for L-thyroxine) daily for 8 weeks.

Administration of Octreotide (Somatostatin Analogue):

It was given to group III in the form of sandostatin purchased from Novartis Pharm Co., in a dose of 35 µg/kg subcutaneously, 20 minutes prior to IR (*Wang et al., 2005*).

Measurement of Arterial Blood Pressure:

Arterial blood pressure (systolic, diastolic and mean) was performed the day before scarification using the non-invasive small animal tail blood pressure system (NIBP200A, Biopac systems Inc., USA).

Experimental Procedures:

On the day of sacrifice, overnight fasted rats were weighed, and then injected intraperitoneally with 5000 IU/Kg B.W heparin sodium (Nile Company, Egypt). Fifteen minutes later, the rats were anesthetized with intraperitoneal injection of thiopental sodium (Sandoz, Austria), in a dose of 40 mg/kg B.W. A midline abdominal incision was made to expose and cannulate the abdominal aorta. Blood samples were taken from the abdominal aorta and collected in heparinized plastic tube which was then centrifuged at 4000 rpm for 10 minutes to separate plasma. The plasma was then pipetted into clean storage tubes and stored at -20°C for later determination of free T3, T4, TSH.

Isolated Heart Study:

Isolated hearts were immediately transferred to a constant pressure Langendorff apparatus. A baseline recording was obtained at a speed of 50mm/sec to determine the baseline beating rate (HR), peak tension (PT), time to peak tension (TPT) and half relaxation time ($\frac{1}{2}$ RT). Myocardial flow rate (MFR) was determined by collecting the fluid passing out of the heart in a glass beaker for 3 minutes (*Ayobe and Tarazi, 1983*).

Total global ischemia was induced by stopping of the perfusion fluid delivered to the heart by a clamp for 20 minutes. Cardiac responses (HR, PT, TPT & $\frac{1}{2}$ RT) and MFR were recorded during 5, 15 and 30 minutes of reperfusion following 20 minutes of total global ischemia. The values of different cardiac responses were calculated from the recordings as follows:

The heart rate (HR, beats per minute) was calculated by the formula:

HR: 3000 was divided by the distance in (mm) between two successive peaks of tension.

For irregular HR: 15000 was divided by the distance between 5 successive peaks.

The peak tension (PT, g), which is the amplitude of the recorded contraction force, was measured in mm and equivalent force in g was obtained from calibration curve. The peak tension was calculated per left ventricular weight (PT/LV, g/100mg).

The myocardial flow rate (MFR, ml/min) was calculated per minute. MFR was, also, calculated per left ventricular weight (MFR/LV, ml/min/100mg).

Determination of Cardiac Weights:

Following heart perfusion, hearts were washed with normal saline, dried by filter paper and cleaned from fat and fibrous tissue. They were wrapped in parafilm and frozen at -80°C. Cardiac chamber weights (atria, right ventricle and left ventricle) and whole heart weight were expressed as absolute value in (mg) as well as relative values (absolute weight/ body weight ratio, cardiac indices) in mg/g.

Biochemical Analysis:

- **Plasma Free T3, T4 and TSH Levels** were carried out by electrochemiluminescence immunoassay "ECLIA" techniques, using kits supplied by Roche Diagnostics Switzerland (*Fisher, 1996*).
- **Cardiac Tissue Malondialdehyde (MDA) Levels** were measured according to the technique of (*Ohkawa*

et al., 1979). This was performed using kits supplied by Bio-diagnostic, Egypt.

- **Tumor Necrosis Factor Alpha (TNF- α) Levels** in Cardiac Tissue were determined via a commercial rat quantitative ELISA kit (R&D systems Co., USA) (*Tartaglia and Goeddel, 1992*).

- **Coronary Efflux Lactate Dehydrogenase Levels:** Coronary efflux was collected during 30 minutes of reperfusion and was used for the measurement of lactate dehydrogenase (LDH) activity in IU/L spectrophotometrically (LDH UV Fluid, Rolf Greiner Biochemica, Germany).

LDH release was expressed per gram of tissue and was used as an index of myocardial injury (*Astles et al.*, 1994).

Statistical Analysis:

Statistics were done using statistical package for the social sciences (SPSS) program (SPSS Inc., version 20). All data were expressed as mean \pm standard error of mean (SEM). Statistical significance for data was determined using a one-way analysis of variance (ANOVA) with post-hoc test, LSD (least significant difference) and student's "t" test for paired data. The level of significance was accepted when $P \leq 0.05$ (*Armitage and Berry, 1987*).

RESULTS

Arterial Blood Pressure Changes (Table 1): Concerning SBP and MAP, non-treated hyperthyroid (IR) rats showed significant elevation of these parameters compared to control rats ($P < 0.001$). Moreover, the hyperthyroid (IR)-OCT rats exhibited significant reduction in their

SBP and MAP compared to non-treated hyperthyroid (IR) rats ($P < 0.001$) and being none significantly changed compared to control group. The DBP did not show any significant difference among the three studied groups.

Table (1): Mean \pm SEM values of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) in the three studied groups

| Parameters \ Groups | Control (IR) group (8) | Hyperthyroid (IR) group (8) | Hyperthyroid (IR)-OCT group (8) |
|---------------------|------------------------|-----------------------------|---------------------------------|
| SBP (mmHg) | 124 \pm 4.18 | 161 \pm 6.50 ^a | 120 \pm 3.71 ^b |
| DBP (mmHg) | 74 \pm 2.44 | 72 \pm 1.55 | 77 \pm 2.60 |
| MAP (mmHg) | 100 \pm 3.84 | 127 \pm 2.98 ^a | 103 \pm 4.33 ^b |

a: Significance from control group (IR) calculated by LSD.

b: Significance from hyperthyroid (IR) group calculated by LSD.

The number of observations was given in parentheses.

Body Weight (B.W) and Body Weight Gain (Table 2): Initial BW was not significantly changed among all rat groups. The final BW in the control group was significantly increased compared to the initial values ($P<0.01$), while the final BW in both hyperthyroid groups did not

significantly change compared to their initial values. On the other hand, final BW and BW gain significantly reduced in both the hyperthyroid (IR) and hyperthyroid (IR)-OCT groups compared to control group ($P<0.01$ for both).

Table (2): Mean \pm SEM values of initial body weight (g), final body weight (g) and body weight gain (%) in the three studied groups

| Parameters \ Groups | Control (IR) group (8) | Hyperthyroid (IR) group (8) | Hyperthyroid (IR)-OCT group (8) |
|-------------------------|------------------------|-----------------------------|---------------------------------|
| Initial body weight (g) | 142.50 \pm 5.74 | 154.25 \pm 6.11 | 150 \pm 2.67 |
| Final body weight (g) | 167.50 \pm 3.89* | 139.12 \pm 4.37 a | 141.87 \pm 6.74 a |
| Body weight gain (%) | 18.87 \pm 4.59 | 8.62 \pm 4.94 a | 5 \pm 4.84 a |

*: Significance from initial values calculated by Student's t- test for paired data.

a: Significance from control group (IR) calculated by LSD.

The number of observations was given in parentheses.

Chronotropic activity (Table 3): The present study elucidated that non treated hyperthyroid rats showed significant tachycardia as those rats exhibited significant increase in baseline pre-ischemia HR values compared to the euthyroid control rats ($P<0.001$).

After ischemia, a significant bradycardic response was evident during at all reperfusion values compared to their pre-ischemic values ($P<0.001$ for all, except in hyperthyroid (IR) group at 5 minutes reperfusion ($P <0.01$). Moreover, the degree of bradycardia (Δ HR) after ischemia reperfusion was significantly higher in non-treated hyperthyroid (IR) group compared to euthyroid control group ($P<0.001$). Also, the percentage change in HR observed in non-treated

hyperthyroid (IR) rats was significantly higher compared to control rats (70 \pm 1.95 versus 57 \pm 1.79, $P<0.001$).

However, the baseline pre-ischemic HR values significantly decreased in the octreotide treated hyperthyroid group compared to the non-treated hyperthyroid group ($P<0.001$). A significant decrease in HR at all reperfusion values compared to their initial pre-ischemic value ($P<0.001$), with significantly reduced degree of bradycardia as evident by the lowest Δ HR in this group. The percentage of HR change significantly reduced in the hyperthyroid (IR)-OCT rats compared to non-treated hyperthyroid (IR) rats (55 \pm 1.33 versus 70 \pm 1.95 $P<0.001$) (Figure 1).

Table (3): Mean \pm SEM values of baseline heart rate (bpm) and effect of 30 minutes of ischemia-reperfusion of isolated hearts from the three studied groups

| Parameters Groups | Baseline value (bpm) | Reperfusion | | | |
|-----------------------------|-----------------------------|-------------------------------|------------------------------|------------------------------|------------------------------|
| | | 5 min (bpm) | 15 min (bpm) | 30 min (bpm) | Δ Change (bpm) |
| Control (IR) group | 229 \pm 3.54 | 126 \pm 5.24* | 103 \pm 2.6* | 98 \pm 5* | 131 \pm 3.83 |
| Hyperthyroid (IR) group | 276 \pm 7.36 ^a | 203 \pm 7.06 ^{a*} | 172 \pm 7.58 ^{a*} | 82 \pm 3.66 ^{a*} | 194 \pm 10.03 ^a |
| Hyperthyroid (IR)-OCT group | 235 \pm 3.83 ^b | 175 \pm 5.65 ^{ab*} | 170 \pm 3.97 ^{a*} | 105 \pm 2.94 ^{b*} | 130 \pm 4.39 ^b |

*: Significance from pre-ischemia baseline value calculated by Student`s t- test for paired data .

a: Significance from control (IR) group, calculated by LSD.

b: Significance from hyperthyroid (IR), calculated by LSD.

Inotropic activity (Table 4): Results of inotropic parameters showed significant increase in pre-ischemic baseline PT, PT/LV values ($P < 0.01$) as well as significant prolongation in baseline TPT values ($P < 0.001$) in non-treated hyperthyroid rats compared to their corresponding values in euthyroid control rats).

After ischemia, non-treated hyperthyroid (IR) group exhibited a significant reduction of PT and PT/LV, prolongation in TPT as well as $\frac{1}{2}$ RT at 15 minute reperfusion values compared to their initial baseline pre-ischemic values ($P < 0.05$, $P < 0.001$ and $P < 0.01$ respectively). The same findings were also found at 30 minute reperfusion values ($P < 0.001$ for all). Moreover, $\frac{1}{2}$ RT of non-treated hyperthyroid rats didn't show any significant difference either in their baseline values or all post-ischemic reperfusion values compared to euthyroid control rats. The deteriorated inotropic recovery of hyperthyroid rats was also evident by the significantly increased Δ PT and Δ PT/LV ($P < 0.01$) and percentage change in PT and PT/LV (74.16 \pm 2.8) compared to euthyroid control group (59 \pm 7.01, $P < 0.05$).

On the other hand, the octreotide - treated hyperthyroid rats showed improvement of their baseline inotropic parameters (PT, PT/LV and TPT) compared to non-treated group as demonstrated by the significant reduction in PT, PT/LV and TPT ($P < 0.01$ for all).

After ischemia, the octreotide - treated hyperthyroid group revealed significant reduction in PT and PT/LV ($P < 0.01$, $P < 0.01$ and $P < 0.001$ respectively) accompanied by a significant prolongation in $\frac{1}{2}$ RT ($P < 0.001$, $P < 0.01$ and $P < 0.001$ respectively) at all recorded reperfusion values and a significant prolongation of TPT ($P < 0.05$ and $P < 0.001$) only at 15 and 30 minute reperfusion values compared to their initial baseline pre-ischemic values. Moreover, a significant low Δ change of PT and PT/LV parameters was observed in octreotide - treated hyperthyroid rats compared to non-treated hyperthyroid group ($P < 0.05$). In addition, the percentage change in TPT significantly reduced in octreotide - treated hyperthyroid rats (55.9 \pm 8.8) compared to non-treated hyperthyroid rats, and being non-significant from the euthyroid controls (84.7 \pm 10.9, $P < 0.05$) (Figure 1).

Table (4): Mean \pm SEM of baseline values of Peak developed tension (PT, g), PT per left ventricular weight (PT/LV, g/100 mg), half relaxation time ($\frac{1}{2}$ RT, msec) and time to peak tension (TPT, msec) and effect of 30 minutes ischemia-reperfusion of hearts isolated from the three studied groups

| Parameters Groups | | Baseline value | Reperfusion | | | Δ Change |
|-------------------------------|-------------------------|-------------------------------|---------------------------------|--------------------------------|--------------------|-------------------------------|
| | | | 5 min | 15 min | 30 min | |
| Control (IR) group | PT (g) | 6.14 \pm 0.36 | 4.98 \pm 0.47* | 3.85 \pm 0.24* | 2.46 \pm 0.339* | 3.67 \pm 0.51 |
| | PT/LV (g/100 mg) | 1.59 \pm 0.09 | 1.16 \pm 0.08* | 0.71 \pm 0.1* | 0.64 \pm 0.1* | 0.95 \pm 0.13 |
| | TPT (msec) | 47.06 \pm 1.57 | 58.97 \pm 1.84* | 67.92 \pm 3.6* | 74.98 \pm 4.19* | 27.92 \pm 3.95 |
| | $\frac{1}{2}$ RT (msec) | 71.76 \pm 3.45 | 77.6 \pm 3.19 | 89.32 \pm 2.54* | 108.52 \pm 3.25* | 36.76 \pm 3.2 |
| Hyperthyroid (IR) group | PT (g) | 8.58 \pm 0.67 ^a | 7.55 \pm 0.85 ^a | 5.54 \pm 0.93 ^{a*} | 2.16 \pm 0.23* | 6.42 \pm 0.58 ^a |
| | PT/LV (g/100 mg) | 2.09 \pm 0.16 ^a | 1.71 \pm 0.17 ^a | 0.91 \pm 0.09* | 0.53 \pm 0.05* | 1.56 \pm 0.14 ^a |
| | TPT (msec) | 37.38 \pm 1.66 ^a | 40.9 \pm 1.69 ^a | 53.98 \pm 3.39 ^{a*} | 68.68 \pm 4.4* | 31.31 \pm 3.89 |
| | $\frac{1}{2}$ RT (msec) | 70.58 \pm 2.23 | 72.56 \pm 2.53 | 92.75 \pm 5.21* | 107.06 \pm 2.97* | 36.48 \pm 3.96 |
| Hyperthyroid (IR) – OCT group | PT(g) | 6.51 \pm 0.29 ^b | 4.52 \pm 0.28 ^{b*} | 4.51 \pm 0.26* | 2.04 \pm 0.19* | 4.48 \pm 0.29 ^b |
| | PT /LV (g/100 mg) | 1.59 \pm 0.08 ^b | 1.1 \pm 0.12 ^{b*} | 0.75 \pm 0.09* | 0.5 \pm 0.05* | 1.09 \pm 0.08 ^b |
| | TPT (msec) | 45.06 \pm 1.46 ^a | 46.81 \pm 1.38 ^{ab} | 55.69 \pm 2.68 ^{a*} | 69.94 \pm 3.87* | 24.87 \pm 3.75 ^b |
| | $\frac{1}{2}$ RT (msec) | 68 \pm 2.26 | 91.12 \pm 4.32 ^{ab*} | 92.75 \pm 5.21* | 103.13 \pm 2.48* | 35.13 \pm 2.39 |

*: Significance from pre-ischemia baseline value calculated by Student`s t- test for paired.

a: Significance from control (IR) group, calculated by LSD data.

b: Significance from hyperthyroid (IR), calculated by LSD.

Myocardial flow rate (Table 5): The baseline values of MFR and MFR/LV and all values of reperfusion showed non-significant differences in the non-treated hyperthyroid (IR) group compared to control group. However, the octreotide treated group revealed significant increase in MFR and MFR/LV at 30 minute

reperfusion value compared to non-treated hyperthyroid (IR) group (P<0.05).

All groups demonstrated non-significant change in the MFR and MFR/LV at 5 minute reperfusion and a significant reduction at 30 minute reperfusion compared to their respective

pre-ischemia baseline values ($P < 0.01$ in control and hyperthyroid (IR)-OCT groups and $P < 0.001$ in non-treated hyperthyroid (IR) group). Moreover, the changes of MFR and MFR/LV from baseline to post-ischemic values (Δ MFR

& Δ MFR/LV) as well as the percentage change of MFR and MFR/LV were non-significantly changed in both hyperthyroid groups (46 ± 1.2 & 32 ± 5.36 respectively) compared to control group (36 ± 7.19) (Figure 1).

Table (5): Mean \pm SEM values of baseline myocardial flow rate (MFR, ml/min) and MFR per left ventricular weight (ml/min/100mg), and effect of 30 minutes ischemia-reperfusion of hearts isolated from the three studied groups

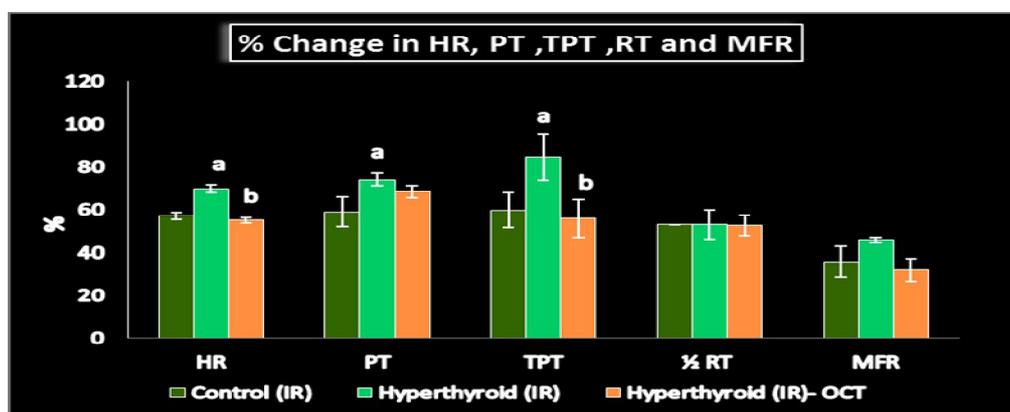
| Parameters Groups | | Baseline value | Reperfusion | | | Δ Change |
|------------------------------------|--------------|-----------------|-----------------|--|--------------------------------|-----------------|
| | | | 5 min | 15 min | 30min | |
| <i>Control (IR) group</i> | MFR | 5.11 \pm 0.43 | 5.30 \pm 0.54 | 4.65 \pm 0.51 | 3.21 \pm 0.40* | 1.9 \pm 0.44 |
| | MFR /LV mass | 1.32 \pm 0.1 | 0.16 \pm 0.13 | 0.9 \pm 0.09* | 0.84 \pm 0.1* | 0.48 \pm 0.1 |
| <i>Hyperthyroid (IR) group</i> | MFR | 4.95 \pm 0.56 | 4.87 \pm 0.64 | 5.05 \pm 0.63 | 2.71 \pm 0.33* | 2.23 \pm 0.24 |
| | MFR /LV mass | 1.21 \pm 0.13 | 0.98 \pm 0.08 | 0.81 \pm 0.1 | 0.66 \pm 0.08* | 0.55 \pm 0.05 |
| <i>Hyperthyroid (IR)-OCT group</i> | MFR | 6.03 \pm 0.46 | 6.07 \pm 0.37 | 4.18 \pm 0.31* | 4.01 \pm 0.28 ^b * | 2.01 \pm 0.47 |
| | MFR /LV mass | 1.47 \pm 0.11 | 1.26 \pm 0.12 | 1.22 \pm 0.1 ^a ^b | 0.98 \pm 0.06 ^b * | 0.49 \pm 0.11 |

*: Significance from pre-ischemia baseline value calculated by Student's t- test for paired data.

a: Significance from control (IR) group, calculated by LSD.

b: Significance from hyperthyroid (IR), calculated by LSD.

Figure (1): Percentage change value of heart rate (bpm), peak developed tension (PT, g), time to peak tension (TPT, msec), half relaxation time ($\frac{1}{2}$ RT, msec) and myocardial flow rate (MFR, ml/min), following ischemia-reperfusion of hearts isolated from the three studied groups



a: Significance from control (IR) group

b: Significance from hyperthyroid (IR)

Absolute Cardiac Weights and Cardiac Indices (Table 6): All rat groups exhibited non-significant changes regarding their whole heart (WH) while WH/BW in both hyperthyroid groups showed significant increase compared to the control group ($P < 0.001$ for both). The octreotide treated hyperthyroid (IR) group revealed no significant change compared to non-treated hyperthyroid (IR) group.

Regarding the absolute weights of left ventricle (LV) and cardiac index (LV/BW), they were significantly higher in both the hyperthyroid (IR) and hyperthyroid (IR)-OCT groups compared to control group ($P < 0.01$ & $P < 0.05$ respectively). Meanwhile, the hyperthyroid (IR)-OCT group showed no significant change compared to non-treated hyperthyroid (IR) group.

Table (6): Mean \pm SEM values of body weight (g), and absolute cardiac weights (mg) and cardiac indices of whole heart (WH) (mg/g), left ventricle (LV) (mg/g) in the three studied groups

| Groups Parameters | Control (IR) group (8) | Hyperthyroid (IR) group (8) | Hyperthyroid (IR)-OCT group (8) |
|-------------------|------------------------|--------------------------------|---------------------------------|
| Body weight (g) | 167.50 \pm 3.89 | 139.12 \pm 4.37 ^a | 141.87 \pm 6.74 ^a |
| WH (mg) | 680.78 \pm 18.93 | 660.82 \pm 16.99 | 655.18 \pm 12.29 |
| WH/BW (mg/g) | 3.25 \pm 0.18 | 4.78 \pm 0.21 ^a | 4.70 \pm 0.28 ^a |
| LV (mg) | 385.96 \pm 6.0 | 410.0 \pm 3.71 ^a | 409.82 \pm 3.2 ^a |
| LV/WH (mg/mg) | 0.57 \pm 0.02 | 0.62 \pm 0.01 ^a | 0.63 \pm 0.01 ^a |

a: Significance from control group (IR) calculated by LSD at $P < 0.05$.

The number of observations was given in parentheses

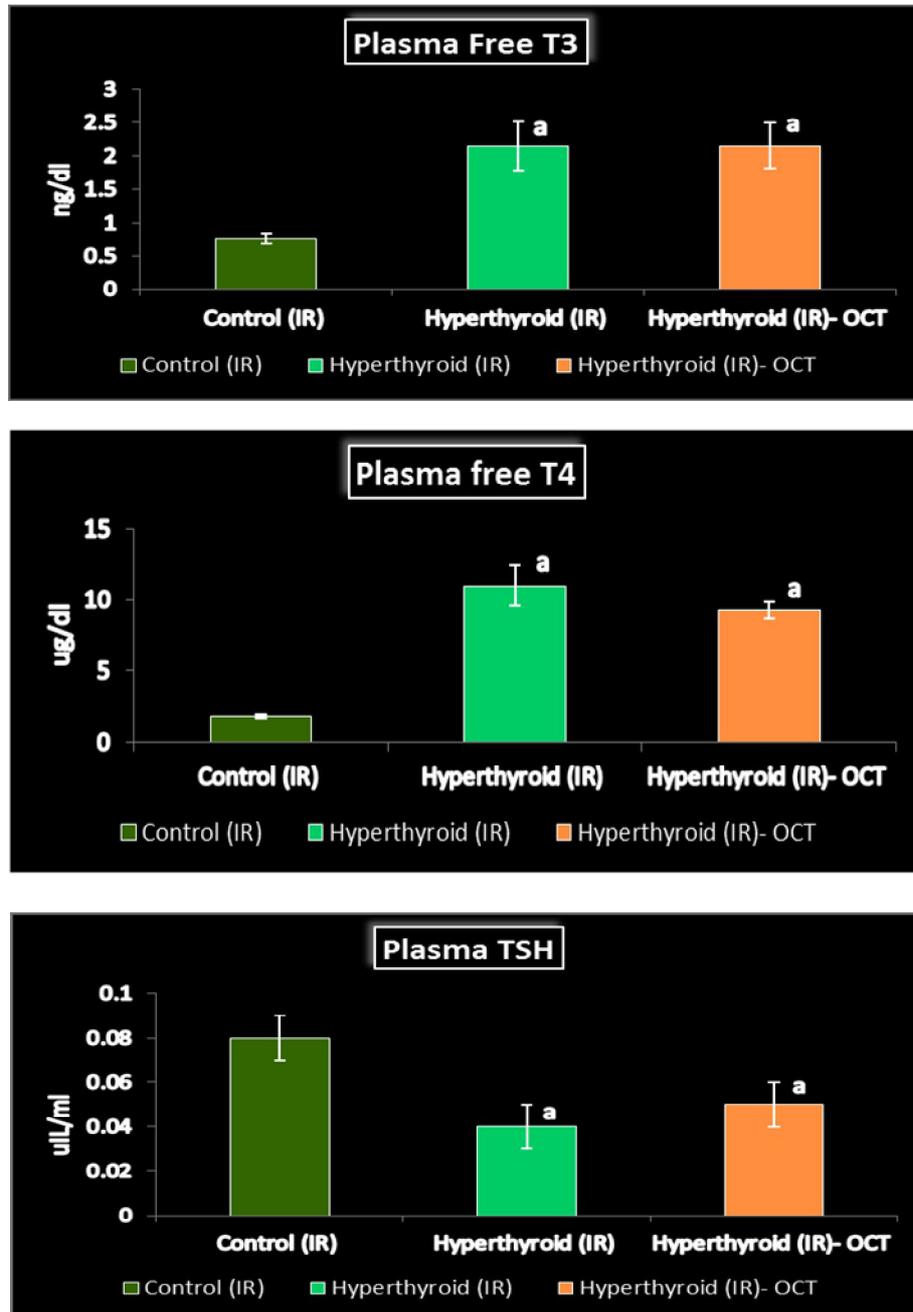
Plasma free T3, T4 and TSH levels (figure 2): Regarding the hormonal profile, plasma free T3 levels were significantly increased in the hyperthyroid groups; (2.14 \pm 0.036 & 2.15 \pm 0.034 respectively) compared to the control group (0.76 \pm 0.08, $P < 0.01$). Also, a significant increase was observed in plasma free T4 (10.97 \pm 1.44 & 9.27 \pm 0.59 respectively) compared to the control group (1.78 \pm 0.13 $P < 0.001$), accompanied by a significant decrease in plasma TSH level (0.04 \pm 0.01 and 0.05 \pm 0.01 versus 0.08 \pm 0.01, $P < 0.001$). These findings confirmed the successful induction of the hyperthyroid state in these groups.

Post-ischemic tumor necrosis factor α (TNF α) and malondialdehyde (MDA) in cardiac tissue (figure 3a&b): The present study showed that both cardiac tissue MDA as well as TNF α were significantly higher in hyperthyroid (IR) group compared to control group (19.72 \pm 1.04 versus

13.56 \pm 1.39 and 12.03 \pm 0.70 versus 7.84 \pm 0.68, $P < 0.01$ & $P < 0.001$ respectively). On the other hand, both parameters were significantly reduced in octreotide treated hyperthyroid (IR) group (11.79 \pm 0.62 & 8.28 \pm 0.52) compared to untreated hyperthyroid (IR) group (19.72 \pm 1.04 & 12.03 \pm 0.7 respectively, $P < 0.001$) and non-significantly different from control group.

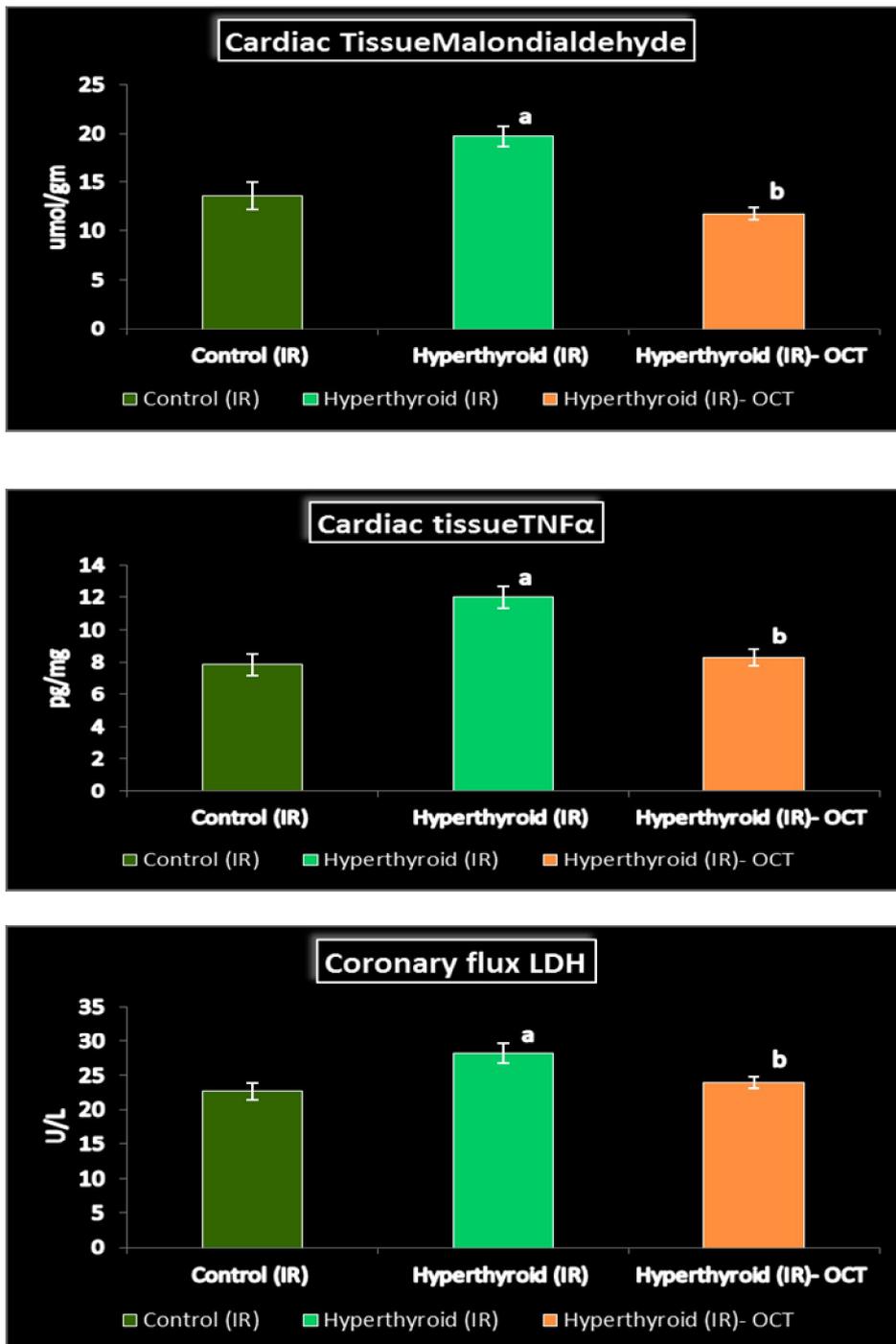
Post-ischemic coronary flux lactate dehydrogenase (LDH) (figure 3c): The hyperthyroid (IR) rat group revealed significant elevation in LDH level compared to control group (28.21 \pm 1.43 versus 22.65 \pm 1.23, $P < 0.01$). However, LDH level was significantly reduced in octreotide - treated hyperthyroid (IR) group compared to untreated hyperthyroid (IR) group (24 \pm 0.90 versus 28.21 \pm 1.43, $P < 0.05$) and non-significantly different from control group.

Figure (2): Free plasma T3 (ng/dl), T4 μ g/dl and TSH (μ IU/ ml) levels in the three studied groups.



a: Significance from control group (IR) calculated by LSD.

Figure (3): A) Cardiac tissue malondialdehyde (MDA, $\mu\text{mol/gm}$), B) tumor necrosis factor α (TNF α , pg/ml) levels and C) lactate dehydrogenase (LDH, U/L) levels in coronary flux in the three studied groups



a: Significance from control group (IR) calculated by LSD.

b: Significance from hyperthyroid(IR)-OCT group calculated by LSD.

DISCUSSION

The finding of the present study elucidated that final body weight, body weight gain were significantly decreased in rats rendered hyperthyroid. This could possibly be explained by increasing β -adrenoreceptor number on the membranes of adipocytes as result of excess thyroid hormones with up-regulation of the catecholamine - stimulated lipolysis (Obregon, 2008). Moreover, (Kim, 2008) observed increased energy expenditure and basal metabolic rate (BMR) in hyperthyroidism which could have added to the weight loss in the hyperthyroid groups.

The observed elevation in free plasma T3, T4 together with the subsequent decrease in TSH, in addition to reduced body weight in hyperthyroid groups confirmed the successful induction of the hyperthyroid state .

In fact, hyperthyroid rats in this study exhibited significantly increased WH, WH/BW, LV and LV/WH in comparison to euthyroid control rats, suggesting development of cardiac hypertrophy, in particular left ventricular hypertrophy. Myocardial hypertrophy could be a compensatory response to the increased load imposed by hypertension as evident by the significant elevation in SBP and MAP in non-treated hyperthyroid (IR) rats compared to euthyroid control rats

This cardiac growth in response to excess thyroid hormones involves cardiac myocyte enlargement and proliferation of fibroblasts and vascular cells (Kenessey and Ojamaa, 2006). Moreover, contractile and calcium-handling proteins are up-regulated, resulting in a condition

frequently referred as physiological cardiac hypertrophy (Thomas *et al.*, 2005). The non-significant changes in initial baseline MFR and MFR/LV in non-treated hyperthyroid rats could further raise the assumption of compensated cardiac hypertrophy.

Unlike basal pre-ischemic coronary flow, ventricular performance was affected by excess thyroid hormones. Hearts from non-treated hyperthyroid rats displayed significant tachycardic response which was accompanied by enhanced ventricular contractility as evidenced by the significant increase in initial pre-ischemic baseline values of HR, PT, PT/LV and prolongation in TPT as well compared to their euthyroid controls. This observation was in agreement with previous reports (Venditti *et al.*, 2002) which also indicated the higher susceptibility of the heart from hyperthyroid rats to ischemia-reperfusion injury .

The increase in chronotropism in hyperthyroid rats is probably caused by unbalanced sympatho-vagal tone with a relative adrenergic overdrive (Biondi *et al.*, 2002). Moreover, (Thomas *et al.*, 2005) ascribed the enhanced contractility and thereby ejection fraction to the ability of thyroid hormones to potentiate calcium-induced calcium release from sarcoplasmic reticulum during systolic period by up-regulation of ryanodine receptor expression levels and sarcoplasmic calcium ATP ase, as well as down-regulation of phospholamban expression levels that help calcium uptake by sarcoplasmic reticulum.

Thus, our findings together with the previous studies reinforce the notion that

the hyper-dynamic cardiovascular state in hyperthyroidism is in fact an adaptive response to the increase in the peripheral metabolic needs produced by excess thyroid hormones.

Isolated hearts in this study were subjected to zero-flow global ischemia followed by reperfusion. Results of isolated perfused hearts of non-treated hyperthyroid group revealed increased bradycardic responses to ischemia-reperfusion (IR) as evidenced by significantly higher Δ HR and percentage change in HR compared to euthyroid control group. Additionally, non-treated hyperthyroid hearts appear to be more influenced by IR injury and showed significantly deteriorated post-ischemic recovery of inotropic parameters (PT, PT/LV and TPT) and significantly higher Δ and % changes of these parameters compared to euthyroid controls.

Such results were consistent with *Seara et al.* (2018) who provided evidence that the pathophysiological progression of myocardial IR injury can be distinctly affected by levels of thyroid hormones and demonstrated that post-ischemic recovery of LV end-diastolic pressure (LVEDP), LVDP and dP/dt was impaired in thyrotoxic rat hearts.

This myocardial contractile dysfunction observed during reperfusion in hyperthyroidism was imputed to an increase in myocardial oxidative stress, which has been reported in the present study as cardiac tissue MDA levels were significantly elevated in non-treated hyperthyroid rats compared to euthyroid rats. Hyperthyroid state is characterized by an increase of oxygen consumption associated with higher electron flux in

mitochondrial respiratory chain, a condition that may increase generation of ROS (*Fernandez and Videla, 1993*). Moreover, mitochondria are a possible site of ischemia-reperfusion damage. Actually, ischemia reduces in vitro mitochondrial respiration, an effect that is potentiated by reperfusion (*Borutaite et al., 1995*). Accordingly, *Venditti et al.* (2002) deduced that heart performance is strongly dependent on mitochondrial function and suggested that mitochondrial dysfunction during reoxygenation is due to an oxidative stress, which is more severe in hyperthyroid hearts.

Our results showed that, cardiac tissue TNF α significantly increased in non-treated hyperthyroid rats, a finding similar to *Al-Amran et al.* (2013) who provided evidence that both ROS and TNF- α have deleterious effect on the myocytes and endothelial cells thus causing myocardial injury. Also, post-ischemic LDH level in coronary efflux of those rats were found to be significantly higher compared to euthyroid controls. Similar to our results, *Shackebaei et al.* (2012) has reported increased LDH levels and CK-MB in first 10 minutes of reperfusion which indicates damage to the heart during the IR period. This finding could contribute to higher susceptibility of the heart of hyperthyroid rats to IR.

Myocardial ischemia induces degranulation of resident mast cells and cleavage of membrane-bound TNF α , both causes release of active TNF α in the ischemic myocardium to act in an autocrine, endocrine, and paracrine fashion (*Gilles et al., 2003*). Reperfusion of ischemic myocardium imposes an oxidant burden in which hydrogen

peroxide activates P38 MAP kinase and contributes to ischemia reperfusion-induced TNF production (Al-Amran et al., 2013). Thus, the overwhelming production of pro-inflammatory mediators namely TNF α could further explain contractile dysfunction reported in non-treated hyperthyroid rats of our study.

Accumulating evidence strongly support the promise of octreotide preconditioning against hepatic ischemia reperfusion injury (Yang et al. 2014). In addition, it could reduce the edema and MDA in ischemic rat brain (Chen et al., 2012). Earlier findings of Wang et al. (2005) indicated that octreotide can mimic ischemic preconditioning to provide cardioprotection against ischemia-reperfusion injury and to reduce infarct size in adult Wistar rats. But there is still no available data whether acute octreotide administration has a cardioprotective effect in ischemia-reperfusion model in conditions of diseased myocardium; in particular hyperthyroid states.

The results of the present study elucidated that hyperthyroid rats supplemented with octreotide, 20 minutes before scarification demonstrated significant improvement of the post-ischemic recovery of cardiac function compared to non-treated hyperthyroid rats. Interestingly, octreotide - treated hyperthyroid rats revealed significant decrease in the baseline tachycardia and the elevated developed peak tension and the prolonged TPT which was observed in non-treated hyperthyroid rats. Furthermore, it improved the chronotropic and some of inotropic parameters regarding their changes from baseline to post-ischemic values (Δ HR, Δ PT and Δ

PT/LV). The percent of decrease in HR following ischemia was significantly lower in octreotide treated rats (55%) compared to non-treated hyperthyroid rats (70%). Also, percent of TPT prolongation was significantly reduced in treated hyperthyroid rats (55.9%) compared to 84.7% in non-treated hyperthyroid rats. Such effects of octreotide were associated with reduction of myocardial injury, as denoted by the significantly low coronary flux LDH level in treated hyperthyroid group compared to non-treated hyperthyroid group.

The aforementioned protective effects of octreotide could be due its antioxidant effect and/or its anti-inflammatory effect (determined by significantly low cardiac tissue MDA and TNF α respectively).

Consistently, Chen et al. (2012) reported the anti-oxidant and effects of octreotide which prevented oxidative damage by enhancing the antioxidant defenses superoxide dismutase and glutathione reductase, thereby; it reduced MDA; a product of lipid peroxidation that is always used to evaluate the severity of oxidative damage.

In support of our findings, Wang et al. (2015) demonstrated that octreotide may exert anti-inflammatory and neuroprotective effects on retinal injury induced by ischemia through inhibition of the activation of NF-kappa; an important redox-sensitive transcription factor that regulates the genes of many inflammatory mediators thus plays an important role in ischemic tissue damage .

Studies have shown that somatostatin analogues may influence leukocytic activities e.g infiltration, adhesion, and chemotaxis and also inhibit the generation

of ROS from leukocytes (*Sener et al., 2005*) and decrease the production of TNF α and other inflammatory cytokines (IL1 β and IL6) (*Yang et al., 2013*), suggesting that this drug might exert anti-inflammatory properties.

Other mechanisms such as activation of protein kinase C, mitochondrial ATP-sensitive potassium channels (mitoKATP channels) and sarcolemmal KATP channels could be involved in octreotide induced cardioprotection. Opening of the mito KATP channel affects mitochondrial swelling and optimization of respiration, prevention of mitochondrial calcium overload and control of reactive oxygen radicals; all of which have possible linkages with cardioprotection during myocardial ischemia and infarction (*O'Rourke, 2000* and *Wang et al., 2005*).

CONCLUSION

Hyperthyroid hearts are less tolerant to ischemia/reperfusion injury with higher susceptibility to oxidative stress as well as overproduction of pro-inflammatory mediators. Thus, pharmacological targets that defend oxidative challenge and confer anti-inflammatory properties, namely octreotide (somatostatin analogue) are considered to have potential therapeutic effect and may protect against myocardial ischemic damage in hyperthyroidism. These effects inspire us to further explore how hyperthyroid patients with cardiovascular risks could benefit from long term octreotide administration.

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تأثير نظير السوماتوستاتين (اكتريوتيد) على إعادة التروية بعد الاقفار الدموي في الجرذان البيضاء البالغة المصابة بفرط نشاط الغدة الدرقية

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خلفية البحث : يرتبط اختلال وظيفة الغدة الدرقية بارتفاع معدل الامراض القلبية. ويزيد فرط نشاط الغدة الدرقية من قابلية القلب لإصابة نقص التروية و ضخه . ويمكن أن تزداد حدة نقص تروية عضلة القلب من خلال زيادة عبء العمل على عضلة القلب والأكسجين المطلوب الناتجين عن هرمونات الغدة الدرقية الزائدة.

الهدف من البحث: دراسة إضطرب وظيفة القلب الناجم عن نقص التروية في الجرذان المصابة بفرط نشاط الغدة الدرقية وإلقاء المزيد من الضوء على تأثير نظير السوماتوستاتين (اكتريوتيد) (OCT) علي الجرذان البيضاء البالغة المعرضة لفرط هرمونات الغدة الدرقية من إصابة نقص تروية الدم.

مواد و طرق البحث: تم إجراء هذه الدراسة على عدد ٢٤ من الجرذان الذكور البيضاء البالغة من سلالة محلية و تزن ١٤٠-٢٠٠ جم ، موزعة على ثلاث مجموعات متساوية. المجموعة الأولى (المجموعة الضابطة) معرضة لنقص تروية الدم ، تم إزالة قلوب هذه المجموعة و اعدادها في جهاز Langendorff. تعرضت القلوب المعزولة إلى ١٥ دقيقة من الاستقرار ، و ٢٠ دقيقة من نقص التروية الكامل صفر التدفق و ٣٠ دقيقة من ضخه. المجموعة الثانية: مجموعة فرط نشاط الغدة الدرقية و التي خضعت لنفس البروتوكول من نقص التروية. المجموعة الثالثة: تم فيها حقن جرذان مصابة بفرط نشاط الغدة الدرقية بنظير السوماتوستاتين (٣٥ ميكروجرام / كجم تحت الجلد) ، تليها دراسة لاستجابات القلب للإقفار و إعادة التروية. وقد تم جمع عينات الدم لقياس T3 و T4 و TSH. وقياس مستوي الشريان التاجي من الاكتات ديهيدروجينز، كما قدرت مستويات انسجة عضلة القلب من المالوندايلدهيد و معامل النخر ألفا.

النتائج: أظهرت جرذان فرط نشاط الغدة الدرقية ارتفاعاً ملحوظاً في مستوي T3 و T4 في البلازما مع انخفاض مصاحب في مستويات TSH. وقد لوحظ انخفاض كبير في وزن الجسم و نسبة الزيادة فيه مع ارتفاع الأوزان المطلقة من البطين الأيسر و مؤشر القلب مقارنة بالمجموعة الضابطة. وكان

الضغط الانقباضي ومتوسط ضغط الدم مرتفعين بشكل ملحوظ ، وأظهرت تلك الجرذان درجة أعلى بكثير من عدم انتظام دقات القلب والتغير في نسبة مئوية لهذا التغير، وكذلك زيادة كبيرة في مؤشرات التقلص العضلي بعد ضخه بالدم. وقد كانت هذه التغييرات مصحوبة بارتفاع ملحوظ في مستويات الشريان التاجي من الاكتات ديهيدروجينيز ونسيج القلب من المالوندايلدهيد ومعامل النخر ألفا.

بينما أظهر إعطاء الأوكثريوتيد إلى جرذان فرط نشاط الغدة الدرقية تحسناً في معايير ضربات القلب ومؤشرات التقلص العضلي . ويمكن إرجاع هذه النتائج جزئياً إلى الحد من الاستجابات المؤكسدة والالتهابية كما هو محدد بمستوي أنسجة القلب من المالوندايلدهيد ومعامل النخر ألفا التي انخفضت بشكل كبير.

الاستنتاج: قلوب الجرذان المصابة بفرط نشاط الغدة الدرقية هي أقل تحملاً لإصابة نقص التروية مع القابلية للإصابة بالأكسدة وكذلك الإفراط في إنتاج الوسطاء المؤيد للالتهابات. ولذلك ، يمكن ان يكون للأدوية التي تقاوم التحدي التأكسدي وتمنح خصائص مضادة للالتهابات ، مثل الاوكثريوتيد تأثيراً علاجياً محتملاً ، وربما حماية ضد تلف عضلة القلب الناتج عن نقص التروية في حالات فرط نشاط الغدة الدرقية.