Clinical and Experimental Health Sciences

Effects of Melatonin Administration on Vasomotor Activity and Histological Structure of Isolated Thoracic Aorta in Rats Treated with Thyroxine

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ABSTRACT

Objective: The goal of this study was to examine the effect of in vivo melatonin (MEL) administration on isolated thoracic aorta in rats with thyroxine treatment and its duty in aortic response to contractile agents, such as potassium chloride (KCl) and phenylephrine (PE). In addition, immunohistological alterations were also examined.

Methods: Experimental groups were as follows: control group (n= 5), thyroxine group (n= 5), melatonin group (n= 6), and thyroxine + melatonin group (n= 6). L-thyroxine was given by intraperitoneal (i.p) administration at 0.3 mg/kg/day for 14 days. MEL was administered i.p., at 3 mg/kg/day for 14 days. The thoracic aorta was isolated from rats euthanized by cervical dislocation. Then, vascular rings were prepared. Concentration-response curves for KCl and PE applications were recorded in an isolated organ bath. Tissue samples were fixed in 10% formalin for histopathological and immunohistological evaluation.

Results: KCl and PE-induced contractions were reduced significantly in the thoracic aortic rings of the thyroxine-treated rats. MEL administration partially attenuated the reduction in the contraction responses due to thyroxine treatment. Immunohistological findings showed that MEL inhibits the thickening of the vessel wall by probably suppressing collagen formation due to thyroxine treatment in the aortic tissue.

Conclusion: Our results suggest that MEL may attenuate the decrease in vascular resistance caused by thyroxine treatment.

Keywords: Aorta; histology; melatonin; thyroxine; phenylephrine; potassium chloride.

1. INTRODUCTION

Hyperthyroidism is an endocrine disease in which excess thyroid hormone (TH) is synthesized and secreted by the thyroid gland (1). Although TH is effective in almost all tissues and metabolic processes, it shows its effect significantly in the cardiovascular system. Thyroid hormones play a significant role in cardiac structure and function. Excessive thyroid hormone influences cardiovascular functions increasing cardiac output, which causes heart failure and, finally ends with dilated cardiomyopathy. In hyperthyroidism, an increase in cerebrovascular morbidity and mortality rates, especially in the cardiovascular system, as well as disorders in cardiac structures and functions are observed (2).

Melatonin is a hormone that is released at night with a circadian rhythm and is produced especially in the pineal gland (3). Since Lerner et al. described melatonin in 1958, this hormone has been shown to be involved in the regulation of many physiological functions including the cardiovascular system (4). Melatonin has effects on blood pressure and myocardial contractility, and it also increases cardiac antioxidant capacity (5). Melatonin receptors have been discovered in the heart and arteries (4). However, the mechanisms of melatonin actions on the cardiovascular system functions are still not fully understood.

It is obvious that melatonin has various beneficial effects in supportive treatments of cardiopathological conditions including high blood pressure, reperfusion injury, drug toxicity, and hypertrophy of the heart (6-12). These severe conditions and low toxicity of melatonin show that it may be correct to use this indole in clinical trials. In animal studies, findings suggest that melatonin may have much the same protective effects in the human heart, unless it is completely misleading (13). The relationship between thyroid and melatonin hormones has been recently reviewed (14,15).

Although thyroid hormones reduce systemic resistance in small and medium-sized arteries due to smooth muscle

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relaxation, there is limited literature on their effects on the histomorphological structure and mechanical properties of the aorta (16). Thus, the goal of this study was to investigate the in vivo effects of melatonin on the peristaltic activity of the aortic ring in an isolated organ bath and also histological effects in thyroxine-treated rats.

2. METHODS

2.1. Animals

Wistar Albino type male rats (300-350 g, n= 22) used in this study were provided by Ataturk University Experimental Research Center (ATADEM). Rats were housed where 12/12 hours light/dark light cycle, 22±0.5° C room temperature and humidity 50-60% in the environment were provided, and standard pellets and tap water were placed in cages. Experimental groups were as follows: control (n= 5) group, thyroxine (n= 5) group, melatonin group (n= 6), and thyroxine + melatonin group (n= 6) (Table 1). All procedures and protocols used in the study were approved by the Local Ethics Committee of Animal Experiments of the Atatürk University on January 27, 2017, with the document number 75296309-050.01.04-E.170.003.4126.

Table 1. *Experimental groups.*

Group	n	Treatment
I: Control group	5	0.9 % NaCl, i.p., for 14 days
II: Thyroxine	5	L-Thyroxine (0.3 mg/kg/day), i.p., for 14 days
III: Melatonin	6	Melatonin (3 mg/kg/day), i.p., at 21:00 o'clock, for 14 days
IV: Thyroxine + Melatonin	6	L-Thyroxine + melatonin

2.2. Isolated Organ Bath Experiments

2.2.1. Drugs

Phenylephrine (Sigma, CAS number: 61-76-7) was used to induce in vitro isolated rat thoracic aortic smooth muscle contractions at a concentration of 10^{-7} M. KCl (Sigma, CAS no: 7447-40-7) was used to induce isolated thoracic aortic contractions at a concentration of 40 mM. L-Thyroxine (Sigma, CAS number: 51-48-9) was given at 0.3 mg/kg/day intraperitoneally for 14 days as described by Shinohara et al. (17) . Melatonin (Santa Cruz, CAS number: 73-31-4) was also administered by i.p. way to each rat at 3 mg/kg/day at 21:00 o'clock in vivo. All drugs were dissolved in distilled water.

2.2.2. Preparation of isolated rat aorta

The isolated organ bath is a standard in vitro research method that needs basic pharmacological equipment. Experimental animals were sacrificed by cervical dislocation to prevent possible vascular complications due to anesthesia. The thoracal cavity was carefully opened from the median line. The thoracic aorta was reached by pushing the heart and lungs to the left. The descendent part of the thoracic aorta was removed and put in a Petri dish with Krebs solution in which the tissue was able to maintain its functions under optimum conditions. The surrounding connective tissues of the thoracic aorta were carefully cleaned and vascular rings of 3-4 mm size were prepared. Before the experiment, adjustment control was made by hanging a 2 gram (g) weight on the isometric transducer tip. The thoracic aortic ring was fixed on the retaining hook in a 10 milliliter isolated organ bath in a standard Kreps solution and the other end was attached to the hook connected to the transducer. Kreps solution, with pH 7.4, 37°C and 95% O_2-5% CO_2, was consisted of NaCl 119, KH_2PO_4 1.2, KCl 4.75, $MgSO_4$ 1.5, $CaCl_2$ 2.5, $NaHCO_3$ 25, and glucose 11 as nM. A contraction up to 1g weight tension level was created while the medium was renewed every 15 minutes and incubated for one hour to let the thoracic aortic rings adapt to the bath environment.

At the end of the incubation period, different concentrations of PE (10^{-4}-10^{-9} M) and KCl (20 mM-100 mM) are applied to the isolated organ bath via a micro-pipette where the thoracic aortic smooth muscle rings isolated from the rats were hanged, and the contraction responses to them were recorded. The determined concentrations were applied non-cumulatively to the bath environment, and after each concentration, washing was done three times with an interval of two minutes and the tissue was brought to baseline balance. Another upper concentration was applied to the bath when the tissue appeared to be in basal balance, and the process continued as specified in the test protocol until the last concentration. Concentration-response curves for KCl and PE applications were recorded by reading them on the computer screen. Contractions reported are the means of experiments repeated three times for each concentration.

2.3. Histopathological Examination

2.3.1. Histological examination

Tissue samples were fixed in 10% formalin solution for 2 days. Paraffin blocks were embedded due to tissue follow-up routine. Sections with 4 µm of thickness were captivated from each block. For histopathological examination, the slides were stained with hematoxylin-eosin (HE) and examined with a light microscope (Olympus, Japan). Sections were assessed as absent (-), mild (+), moderate (++), and severe (+++) according to their histopathological findings (18).

2.3.2. Immunohistochemical staining

All sections taken with adhesive (poly-L-Lysin) on slides were passed through the xylol and alcohol series for immunoperoxidase examination. The sections were washed with phosphate buffered saline (PBS), and endogenous peroxidase inactivation was achieved by keeping them in 3% hydrogen peroxide for 10 minutes. In order to determine the antigen in the tissues, they were exposed to antigen retrieval

solution for 2x5 minutes at 500 watts in a microwave oven and then left to cool. Tissues were exposed to primary antibody (COL 1A1, Pro-COL 3A1, Catalog no: sc-293182, sc-166316, Santa Cruz, USA) for 30 min at 37˚C and then followed according to the immunohistochemistry kit procedure (Ab-236466) in order to determine mature and young collagen deposits. The chromogen used was 3-3' Diaminobenzidine (DAB). Hematoxylin was used for floor staining (19).

2.4. Statistical Analyses

Statistical analyses of the experimental data were carried out using IBM SPSS Statistics 20 for Windows. One Way ANOVA with posthoc LSD test was used to find the statistical differences between groups. As the level of statistical significance, P <0.05 value was accepted. Data were presented as mean ± standard deviation (SD).

Five random areas were selected from each image to evaluate the intensity of positive staining using the Imaging Software Program of ZEISS Zen. Mann-Whitney U test was performed to compare positive immunopositive stained areas and immunoreactive cells with controls. As a result of the test, the data were presented as mean ± SD and a P value of <0.05 was considered statistically significant.

3. RESULTS

3.1. Contraction Responses of the Aorta in an Isolated Organ Bath

The contraction responses of rat isolated thoracic aortic endothelial smooth muscle rings of the control group (n= 5) to KCl and PE are shown in Figures 1 and 2. Contractions reported are the means of experiments repeated three times for each concentration.

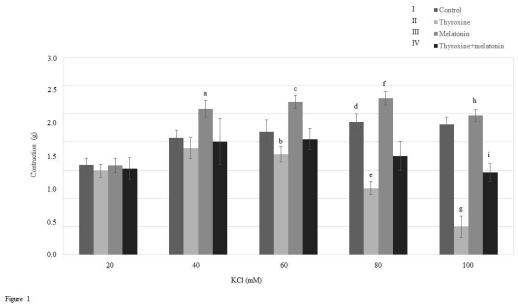


Figure 1. *Isolated thoracic aortic smooth muscle ring contraction responses (mean±SD) induced by potassium chloride (KCl) in control, melatonin, thyroxine treated and thyroxine plus melatonin groups. Contractions are the means of experiments repeated three times for each concentration. Statistical differences between groups are as follows: a: III vs I (p<0.05), II and IV (p<0.01); b: II vs IV (p<0.05); c: III vs I, II and IV (p<0.001); d: I vs II (p<0.001), III (p<0.01) and IV (p<0.001); e: II vs III and IV (p<0.001); f: II vs IV (p<0.001); g: II vs I (p<0.001); h: III vs II (p<0.001); i: IV vs I, II and III (p<0.001)*

As shown in Figure 1, although the contraction response of the isolated aorta to KCl was not affected below 60 mM, it decreased remarkably at 80 and 100 mM concentrations. Melatonin administration increased the aortic contraction compared with the control goup in all concentartions of KCl except 20 mM. At 80 and 100 mM KCl concentrations the decreases in aortic contraction due thyroxine treatment were prevented by melatonin administration.

As seen in Figure 2, contraction responses to PE were higher than all other groups in all concentrations tested. Thyroxine treatment decreased the contraction responses of aorta to PE remarkably in all concentrations of PE compared with control group. These decreases could only be prevented significantly by melatonin administration at 10^{-9} and 10^{-8} M concentrations. Although melatonin administration itself increased the contraction responses of aorta to PE in all concentrations of PE, the increases could have reached the statistical significance at 10^{-9}, 10^{-8} and 10^{-7} M concenrations of PE.

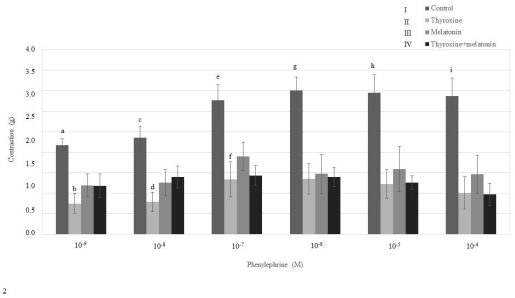


Figure 2. *Isolated thoracic aortic smooth muscle ring contraction responses (mean±SD) induced by phenylephrine (PE) in control, melatonin, thyroxine treated and thyroxine plus melatonin groups. Contractions are the means of experiments repeated three times for each concentration. Statistical differences between groups are as follows:a: I vs II, III and IV (p<0.001); b: II vs III and IV (p<0.05); c: I vs II, III and IV (p<0.001); d: II vs III and IV (p<0.05); e: I vs II, III and IV (p<0.001); f: II vs III (p<0.05); g: I vs II, III and IV (p<0.001); h: I vs II, III and IV (p<0.001); i: I vs II, III and IV (p<0.001).*

3.2. Histopathological Findings

3.2.1. Histopathology

When the aortic tissues were evaluated histopathologically, it was observed that they had a normal histological structure in the control group (Figure 3-A). On the other hand, severe degeneration of endothelial cells in the intima layer, the vessel wall thickening due to severe collagen increase in the media layer, and moderate edema in the adventitia layer were detected in the thyroxine treated group (Figure 3-B). However, the media and intima layers were in normal histological structure in the MEL group (Figure 3-C). Mild endothelial damage in the intima layer, thickening due to mild collagen increase, and mild edema in the adventitia layer were observed in MEL + thyroxine treated group (Figure 3-D).

A statistically significant decrease in aortic wall thickness was found when MEL + thyroxine treated group was compared with the only thyroxine treated group ($p<0.05$, Table 2). Histopathological findings are summarized in Table 3.

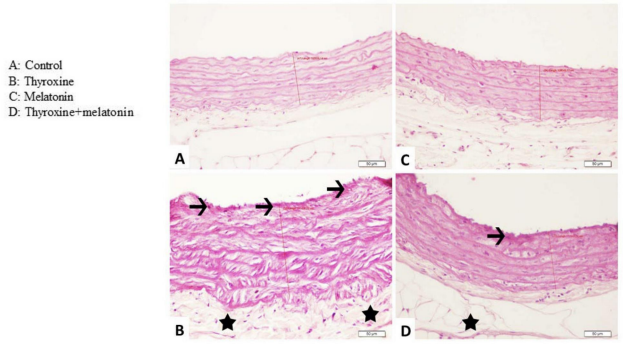


Figure 3. *Control and melatonin groups had normal histological appearance (A, C), thyroxine treated group had degeneration of endothelial cells (arrows), severe thickening of the vessel wall, and adventitial edema (stars) (B), melatonin + thyroxine group had mild thickening of the vessel wall and adventitial edema (star) (D). Aortic tissue, H&E, bar:50µm.*

Table 2. *The thickness of the aortic wall in experimental groups.*

Group	Thickness of the aortic wall (nm)
Control	103 288 ± 1241[a]
Thyroxine	195 425 ± 1062[b]
Melatonin	105 471 ± 1950[a]
Thyroxine + Melatonin	117 642 ± 1204[c]
Different letters (a, b, c) show statistically significant difference ($p<0.05$).	

Table 3. *Scoring of histopathological findings in aortic tissue.*

	Control	Thyroxine	Melatonin	Thyroxine + Melatonin
Endothelial damage	-	+++	-	+
Wall thickening	-	+++	-	+
Adventitial edema	-	+++	-	++

3.2.2. Immunohistochemical Findings

COL 1 and PRO-COL 3 expressions were evaluated as negative when aortic tissues were examined immunohistochemically in the control group (Figure 4-A and Figure 5-A). On the other hand, severe COL 1 and PRO-COL 3 expressions were detected in the medial layer in the thyroxine treated group (Figure 4-B and Figure 5-B).

COL 1 and PRO-COL 3 expressions were evaluated as negative when aortic tissues were examined immunohistochemically in the MEL group, also (Figure 4-C and Figure 5-C). However, mild COL 1 and PRO-COL 3 expressions were observed in the medial layer of the aorta in MEL+thyroxine treated group (Figure 4-D and Figure 5-D). Immunohistochemical findings are also summarized in Table 4.

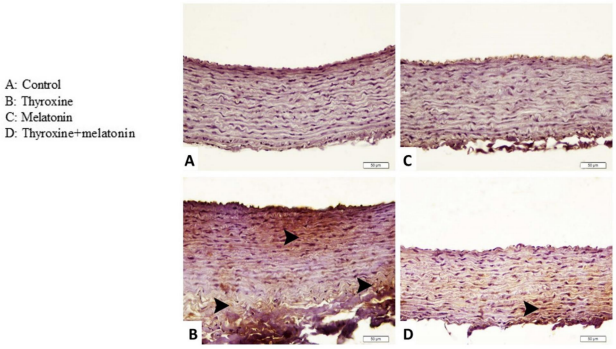


Figure 4. *Control and melatonin groups had negative COL 1 expression (A,C), the thyroxine treated group had severe COL 1 expression (arrow heads) (B), and the melatonin+thyroxine group had mild COL 1 expression (arrow heads) (D). Aortic tissue, immunohistochemistry paraffin protocol (IHC-P), bar: 50µm.*

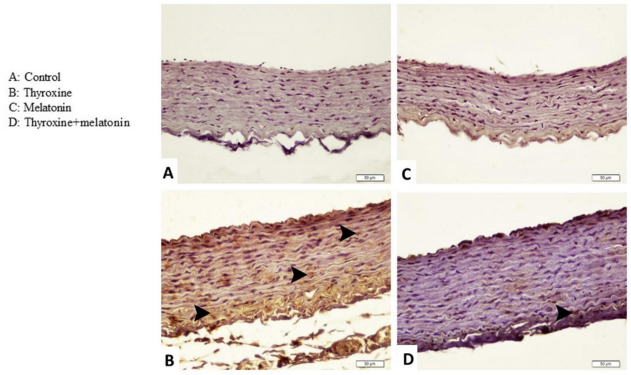


Figure 5. *Control and melatonin groups had negative PRO-COL 3 expression (A,C), the thyroxine treated group had severe PRO-COL 3 expression (arrow heads) (B), and the melatonin+thyroxine group had mild PRO-COL 3 expression (arrow head) (D). Aortic tissue, IHC-P, bar: 50µm.*

Table 4. *Scoring of immunohistochemical findings in aortic tissue.*

	Control	Thyroxine	Melatonin	Thyroxine + Melatonin
COL 1	21.3 ± 2.91[a]	83.1 ± 2.91[b]	20.9 ± 4.65[a]	41.4 ± 3.18[c]
PRO-COL 3	20.4 ± 4.25[a]	80.5 ± 7.16[b]	20.9 ± 5.14[a]	40.4 ± 3.18[c]

Different letters (a,b,c) in rows show statistically significant difference ($p < 0.05$).

4. DISCUSSION

Thyroid hormones have various effects on the cardiovascular system. Although the resistance of the systemic blood vessels and functions of the heart are mainly affected by the thyroid gland, hyperthyroidism decreases the total peripheral resistance and increases the cardiac output (20). However, the underlying mechanisms are still largely uncertain. In literature, although the changes in cardiac and vascular functions due to hyperthyroidism were commonly investigated in vivo (21-26), to the best of our knowledge the

changes in in vitro aortic reactivity in hyperthyroidism were studied in a few studies only (27,28).

In a study where McAllister et al. (28) have given triiodothyronine (Hyper, n=27; 300 µg/kg) to male rats for 6-12 weeks and investigated the effect of hyperthyroidism on vascular contraction and relaxation responses by comparing with the euthyroid control group (euthyroid n=27). They reported that the group with hyperthyroidism had developed hypertrophy in the left ventricle (euthyroid, 2.01±0.04 mg/g/kg; hyperthyroid, 2.70±0.06; $p<0.0005$), and also had higher oxidative enzyme activity in many skeletal muscle tissues ($p<0.0005$). When they evaluated the vascular responses of 2-3 mm vascular rings obtained from the abdominal aorta in vitro, and compared with the control group, they observed decreased contraction responses induced by the norepinephrine in the aortic rings with intact endothelium in the hyperthyroidism group ($p<0.05$), however, they did not find a significant difference when the endothelial layer is removed. They concluded that hyperthyroidism affects vascular contractile and relaxation responses of the blood vessels in male rats. Similar findings have also been reported by other researchers (29-31).

Although the excess amount of thyroid hormones is associated with several effects on the cardiovascular system, the adaptive changes in various arteries are not known well. In a study on acute hyperthyroidism induced vascular changes in isolated rat aorta reported by Honda et al. (27), acute hyperthyroidism was induced with a 3-day L-thyroxine (T_4) (0.5 mg/kg/day) subcutaneous injection to investigate whether muscarinic and adrenergic receptor-mediated responses of the blood vessels change. They stated that T_4 significantly increases tachycardia, cardiac hypertrophy, and weight loss. To see the effects of acute hyperthyroidism, the tension of aortic ring preparations was isometrically measured. As a result, they stated that the contractions induced by norepinephrine (NE) were significantly decreased in the aortic rings of T_4 treated rats compared to the control group, and vascular responses may change in the acute stage of hyperthyroidism and may result from an alteration in the endothelial NO system regardless of the amount of eNOS protein. In recent years, Gachkar et al. in the study of aortic effects of thyroid hormones in male mice, have shown that thyroid hormone affects aortic contractility via genomic and non-genomic mechanisms of action (32). In our study, we examined the effects of in vivo MEL application on contractile agents KCl and PE in isolated thoracic aorta smooth muscle rings in rats with thyroxine treatment, compared to the control group. KCl-induced relaxation responses of the aorta of rats treated with thyroxine at 80 mM and 100 mM concentrations were 49.79% and 78.35% of the control, respectively (Figure 1, $p<0.001$). When the PE responses were compared with the control group, the decrease in isolated thoracic aortic smooth muscle contractions in the thyroxine treated group was observed at all concentrations, and the highest relaxation response was found to be 70.03% of the control at PE concentration of 10^{-4} M (Figure 2, $p<0.001$). In rats with thyroxine treatment, the contractions created with KCl and PE showed a vasorelaxation response compared to the control group, and the reduction in vascular resistance in the literature was similar to our results.

Melatonin is involved in physiological processes including the cardiovascular system (33). It has been reported that melatonin causes vascular contraction (34) and contraction in isolated ring segments obtained from rat caudal artery is enhanced by adrenergic nerve stimulation (35). Mahle et al. first obtained pre-contraction induced by 30 mM KCl in the rings obtained from the 28 human coronary arteries, then they found increases in coronary artery rings induced by various concentrations of in vitro melatonin exposure (10^{-13}-10^{-5} M) (36). They also reported that melatonin induces concentration-dependent vasoconstriction in the rings of rat caudal and cerebral arteries.

In a study by Klemm et al., melatonin did not affect the relaxation caused by acetylcholine in the rat aorta; either this response did not possibly change the endothelial nitric oxide (NO) activity or vascular response would not be present to nitric oxide (37). In other studies, when vascular studies with the effect of melatonin in vitro and in vivo are evaluated, it can be seen that the endothelial layer is important in the effect of melatonin, and therefore melatonin may have a suppressive effect on NO (33, 38). Monroe et al. have found that agonist KCl (70 mM) induced contractions in rat aorta were inhibited by melatonin (10^{-5}-10^{-3} M) (33). Melatonin has been found to affect channels other than calcium channels, and in addition, it antagonizes calcium binding of calmodulin, the intracellular Ca^{+2} binding protein (39). In the literature, the fact that there are contradictory results in studies investigating the effects of melatonin on the vascular system suggests that responses to the melatonin used in the bath environment may vary due to different concentrations. In light of all this information, it can be said that the absence of suitable antagonists and agonists of the receptor subtypes of melatonin in the vascular endothelium makes it difficult to investigate the vasoconstrictive effects of melatonin (40).

In literature, there are many studies on the relations between the thyroid gland and pineal gland, which are important organs of the endocrine system. Although there are various results related to the hypothesis, material method and findings in the studies, it is concluded that the thyroid gland affects the pineal gland or vice versa. As a result of the literature review, it has been determined that there are differences between the application times of melatonin and thyroid gland functions. Two studies by Lewinski et al. (15) in rats and a study by Petterborg et al. (41) reveal that melatonin applied in the afternoon is more effective in thyroid inhibition. In the present study, 0.3 mg/kg/day L-thyroxine i.p was applied at 08:00 o'clock and 3 mg/kg/day melatonin i.p at 21:00 o'clock for fourteen days. Considering the results of the literature, it was aimed to increase the bioavailability of the hormone by applying melatonin in the evening.

Studies have shown that melatonin hormone, which has an endocrine effect on thyroid gland functions, has a general inhibitory effect. An increase in the secretion of

the T_4 hormone and hypertrophy in the thyroid gland have been reported in experimental animals with pinealectomy. Melatonin, on the other hand, suppresses T_4 released from the thyroid gland, causing a decrease in plasma T_4 levels. Melatonin also increases TSH (42). Vriend et al. have demonstrated that the administration of melatonin in hamsters with hypothyroidism decreases the high TSH hormone level suggesting that melatonin can play a role in regulating the release of TRH (43). In another study, it is reported that exogenous melatonin administration to blind hamsters reduces T_3 and T_4 levels (44). Studies have also shown high plasma melatonin levels in experimental animals with hyperthyroidism. This result demonstrates that hyperthyroidism can increase plasma levels of melatonin. It is widely agreed upon that there is an important relationship between the thyroid and the pineal gland and that disorders in thyroid function can alter the release of melatonin (45).

In recent studies, it has been shown by biochemical and immunohistochemical methods that MEL affects the cardiovascular system (11,42,46). In this study, severe degeneration of endothelial cells in the intima layer, thickening of the vessel wall due to severe collagen increase in the media layer, and moderate edema in the adventitia layer were observed in the thyroxine treated group. Additionally, mild COL 1 and PRO-COL 3 expressions were observed in the medial layer of the aortic wall in the thyroxine treated group. The aortic wall thickness in thyroxine treated group was significantly decreased by melatonin administration. Immunohistological alterations due to thyroxine treatment were attenuated by in vivo melatonin administration. The histopathological and immunohistological findings suggest that melatonin inhibits the thickening of the vessel wall by suppressing the formation of collagen 3, which is active young collagen, and its conversion to mature collagen, collagen 1, due to thyroxine treatment in the aortic tissue. In a study by Moulakaksis et al., it was stated that rats with thyrotoxicosis show significant changes in the mechanical and microstructural properties of the aortic wall (16). Similarly, there are studies showing that hyperthyroidism causes an increase in aortic stiffness (47). The increase in arterial wall stiffness carries a significant risk in the formation of atherosclerosis together with vascular aging. In SAMP8 mice, Rosei et al. have shown that chronic administration of melatonin reduces the contractile responses to norepinephrine in small arteries of the mesentery (48). In another study, it was demonstrated that in vitro administration of aortic rings of elderly rats with melatonin increases the relaxation response to the acetylcholine exposure (49). All of these studies show the beneficial effect of melatonin on arterial changes parallel to our results in this study.

Although many studies show the cardioprotective effects of melatonin in literature, it was not possible to make one-to-one comparison with our results since we could not find sufficient studies showing the effect of in vivo melatonin on vascular changes caused by thyroxine treatment. It seems that more studies are necessary to determine the mechanism/s of cellular action of melatonin on the vascular smooth muscle in thyroxine treated rats. With the techniques required to identify melatonin receptors, it will be possible to examine the physiological roles of the receptors and how they can act in various clinical situations, such as hyperthyroidism.

There are some limitations of this study. First, the experimental study period was relatively short (only 2 weeks) in our study evaluating the in vivo effect of melatonin on the thoracic aorta of rats treated with thyroxine. Second, studies using the nitric oxide synthesis inhibitor L-NAME for endothelium-independent relaxation could not be performed due to time and equipment limitations. Third, the effect of melatonin administration on the smaller peripheral arterioles of thyroxine-treated rats was beyond the scope of this study. Further studies are needed to investigate the possible role of melatonin in regulating compliance against resistance vessels (including venous capacitance vessels) in different regions and vascular beds of thyroxine-treated rats.

5. CONCLUSION

In summary, KCl and PE-induced contractions were reduced significantly in thoracic aortic rings isolated from thyroxine treated rats. MEL administration partially attenuated the reduction in the contraction responses due to thyroxine treatment. Immunohistological findings suggest that melatonin inhibits the thickening of the vessel wall by probably suppressing collagen formation due to thyroxine treatment in the aortic tissue. Our results suggested that MEL may attenuate the decrease in vascular resistance caused by thyroxine treatment.

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Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: HU, ES
Design of the study: HU, ES, MG
Acquisition of data for the study: HU, ES, SY
Analysis of data for the study: HU, ES, FC, MG
Interpretation of data for the study: HU, MG, FC, SY
Drafting the manuscript: HU, ES
Revising it critically for important intellectual content: HU, MG, FC, SY
Final approval of the version to be published: HU, ES, SY, FC, MG

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