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The effect of chronic hyperthyroidism and restored euthyroid state by methimazole therapy in rat small mesenteric arteries

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ABSTRACT

Not much has been reported about the effects of hyperthyroidism and its correction on resistance vessels, and just two inconsistent studies have investigated the impacts of restored euthyroidism on vascular reactivity. In this regard, we designed the current study to evaluate the vascular reactivity of the mesenteric arteries of hyperthyroid and restore euthyroid rats. Hyperthyroidism was induced by administration of triiodothyronine (T_3; 300 μg/kg, i.p., for 12 weeks in T_3 group). Euthyroidism was restored by administration of T_3 for 8 weeks and then T_3+Methimazole (0.003% in drinking water) for 4 weeks (T_3+MMI group). According to the McGregor method, vascular relaxation and contractility response were measured in response to acetylcholine or phenylephrine respectively. We found that maximal contractility response (E_{max}) to phenylephrine in the T_3 group was significantly decreased ($P<0.001$), and E_{max} to acetylcholine was significantly increased compared with the saline group ($P<0.05$). When N^G-nitro-L-arginine methyl ester (L-NAME, 3×10^{-4} M) was used, E_{max} to acetylcholine in the T_3 group was still higher than the saline group ($P<0.05$). However, decrease in maximal response of the T_3 group was significantly greater than the saline group ($P<0.01$). We also showed that when euthyroidism is restored by methimazole therapy, enhanced acetylcholine-induced vasorelaxation and impaired contractility response to phenylephrine were normalized, as there was no significant difference in E_{max} of the T_3+MMI group versus the saline group ($P>0.05$). In conclusion, synthesis of both nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) in mesenteric arteries significantly increased as a consequence of hyperthyroidism, and this abnormal vascular reactivity is corrected by methimazole therapy.

1. Introduction

A hyperthyroid state significantly alters cardiovascular functions to cause problems such as bradycardia, increased cardiac output, decreased in total peripheral resistance, arterial hypertension and enhanced endothelium-dependent vasodilatation. These vascular disorders usually play important roles in treatment strategies (Epstein et al., 2001; Vargas et al., 2006).

A considerable amount of studies have been conducted to address the possible mechanisms by which hyperthyroidism changes vascular reactivity. Although the exact mechanisms are still unknown, NO and EDHF pathways are known to be responsible for this (Büssemaker et al., 2003; Quesada et al., 2002; Rajfer et al., 1992; Sarac et al., 2006). It is assumed that enhanced endothelium dependent relaxation is a consequence of increased cardiac output, hypertension and fluid shear stress at the endothelial cell surface (McAllister et al., 1998b).

Available literature shows that, depending on the size and location, the artery endothelium is different (Clark and Fuchs, 1997). Moreover, the response of different vascular smooth muscles may vary due to inconsistency in types and densities of pharmacological receptors and ion transport mechanisms (d'Uscio et al., 1997; Mulvany and Aalkjaer, 1990). NO seems to play a major role in large conducting arteries, and EDHF appears to be of the primary importance in resistance vessels (Hwa et al., 1994).

There is ample of evidence indicating that mechanisms involved in enhanced endothelium-dependent vasorelaxation related to hyperthyroidism depend on different vascular beds and types of hyperthyroidism. Bussemaker et al. have shown that acute hyperthyroidism enhanced acetylcholine-induced relaxation is mediated by EDHF, whereas in chronic hyperthyroid rats NO plays the most important role (Büssemaker et al., 2003).

As resistance vessels are the major site of generation of vascular resistance (Mulvany and Aalkjaer, 1990), their dysfunction can play a

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pivotal role in cardiovascular disorders related to chronic hyperthyroidism. To our knowledge, the effect of hyperthyroidism on small resistance arteries has not been adequately defined yet. Therefore, the primary objective of this study was to investigate the impacts of hyperthyroidism on a mesenteric arterial bed, as a suitable model of small resistance vessels and potential mechanism by which hyperthyroidism affects vascular function.

Although medical therapy of hyperthyroidism may generally improve cardiovascular health, not much has been reported on the influence of hyperthyroidism treatment on vascular reactivity. Based on our knowledge, just two researches have been previously done related to this matter. Ortega et al. have reported that abnormal endothelium-dependent responses in thyroid arteries from methimazole treated patients are not corrected by medical therapy compared with untreated patients (Ortega et al., 2005). Conversely, Napoli et al. have shown that endothelial dysfunction (increased forearm blood flow and increased reactivity to acetylcholine in hyperthyroid patients) is normalized when euthyroidism is restored by methimazole therapy (Napoli et al., 2001). Because the results of previous studies are inconsistent and there is no data concerning endothelial function in resistance arteries from hyperthyroid rats treated with methimazole, this study also has been designed to see whether or not hyperthyroidism correction can normalize the endothelial dysfunctions in resistance arteries.

2. Materials and methods

2.1. Drugs

All drugs which were used in this study were purchased from Sigma Chemical (Sigma, St. Louis, MO, USA). All chemicals used to prepare the Krebs–Henseleit solution were analytical grade and purchased from Merck Chemicals, Germany. Krebs solution contained (in mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11, maintained at 37 °C and aerated with a 95% O_2 and 5% CO_2 gas mixture. All solutions were freshly prepared each day and concentrated stock solutions of vasoactive agents were made and then were serially diluted with distilled water.

2.2. Animals and treatment protocol

The animals were handled in accordance with protocols approved by the Ethics Committee of Tehran University of Medical Sciences and also adhered to guidelines of the US National Institute of Health (NIH publication no. 85.23, revised 1985) guides for the care of lab animals. Male Sprague–Dawley rats (initially weighing 220–250 g (8–10 weeks of age)) were housed in air filtered metabolic cages in a temperature and humidity controlled environment with 12 h light/dark cycle. Food consists of normal rat chow and water ad libitum. Animals were randomly divided into three study groups of age matched rats. The first group (T_3 group) received T_3 at the dose 300 μg/kg, i.p. (dissolved in Na_2Co_3 solution 1%, 1000 μg/ml) every other day for 12 weeks. The second group (T_3+MMI group) received T_3 at the same dosage as the T_3 group for 8 weeks (to induce hyperthyroidism) and then animals simultaneously received T_3 and methimazole (0.003% in drinking water) for four consecutive weeks. To obtain a stable euthyroidism, the dose of methimazole was chosen to obtain a normal serum level of thyroid-stimulating hormone (TSH), free thyroxine (FT_4) and free triiodothyronine (FT_3) (Virdis et al., 2009). Finally, the third group is the saline group, which received saline every other day for 12 weeks.

2.3. Assessment of treatment efficacy

The general condition and body weight changes were observed twice a week during the whole study. All animals were observed

Table 1
Effects of hyperthyroidism and restored hyperthyroidism on body weight gain, heart weight and heart rate. The T_3 group received T_3 (triiodothyronine) at the dose of every other day for 12 weeks. The T_3+MMI group received only T_3 for 8 weeks (hyperthyroid state) and then received co-administration of T_3 and methimazole for 4 weeks (restored hyperthyroidism). Base line of body weight and heart rate were measured before drug administrations. Data are presented as mean ± S.E.M of six rats.

	Baseline of bwt (g)	Baseline of hrt (bpm)	8-week treatment			12-week treatment		
			bwtg (g)	hwt (g)	hrt (bpm)	bwtg (g)	hwt (g)	hrt (bpm)
Saline group	233.4 ± 6.1	323 ± 8	110.2 ± 8.1	0.93 ± 0.03	325 ± 6	147.1 ± 4.8	1.03 ± 0.02	334 ± 9
T_3 group	239.1 ± 5.4	330 ± 10	69.7 ± 6.2[a]	1.09 ± 0.04 [a]	364 ± 11[b]	106.3 ± 5.6[b]	1.33 ± 0.05[b,d]	373 ± 11[c,e]
T_3+MMI group	230.9 ± 4.9	327 ± 9	68.7 ± 7.3[a]	1.07 ± 0.02[a]	361 ± 7[b]	129.1 ± 6.6	1.18 ± 0.07	325 ± 10

bwt: body weight, bwtg: body weight gain, hwt: heart weight and hrt: heart rate.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$ versus saline group.
[d] $P < 0.05$.
[e] $P < 0.001$ versus T_3+MMIgroup.

Table 2
Hormonal changes from saline group, T_3 group and T_3+MMI group after 8 and 12 weeks drug administrations. Data are presented as mean ± S.E.M of six rats.

	8-week treatment			12-week treatment		
	TSH (ng/ml)	FT_4 (pg/ml)	FT_3 (pg/ml)	TSH (ng/ml)	FT_4 (pg/ml)	FT_3 (pg/ml)
Saline group	4.7 ± 0.3	19.8 ± 1.2	4.5 ± 0.1	4.2 ± 0.4	18.5 ± 0.9	4.8 ± 0.2
T_3 group	2.8 ± 0.6[b]	26.6 ± 1.5[b]	5.3 ± 0.2[a]	2.8 ± 0.5[a]	29.1 ± 1.7[c,d]	6.5 ± 0.5[b,e]
T_3+MMI group	2.6 ± 0.5[a]	28.5 ± 2.1[b]	5.5 ± 0.7[a]	3.9 ± 0.6	20.2 ± 2.2	4.3 ± 0.6

TSH: thyroid-stimulating hormone, FT_4: free thyroxine and FT_3: free triiodothyronine.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$ versus saline group.
[d] $P < 0.05$ and.
[e] $P < 0.01$ versus T_3+MMI group.