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The influence of hyperthyroidism on pharmacologically induced contractions of isolated resistance arteries

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Abstract

We investigated the effect of hyperthyroidism on the responses of small mesenteric resistance arteries to several contractile and dilator agents. Hyperthyroidism was established by feeding rats for 28 days with 5 mg/kg L-thyroxine-containing rat chow. This treatment produced a stable hyperthyroid state, as indicated by the increased serum T4 levels (236 ± 7 vs. 60 ± 2; T4-treated vs. control). Preparations of small mesenteric arteries were mounted in an isometric wire myograph. Subsequently, concentration-effect curves were determined for K^+, Ca^{2+}, methoxamine, phenylephrine, 5-hydroxytryptamine (5-HT), 9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$ (U46619), methacholine and nitroprusside. Our results indicate that hyperthyroidism does not induce major changes in the sensitivity of isolated resistance vessels to K^+, Ca^+, the α-adrenoceptor agonists, methacholine and nitroprusside. Furthermore, neither the affinity of α-receptors for methoxamine, nor the α-receptor reserve was influenced by the hyperthyroid state of the animal. A clearly sensitizing influence of hyperthyroidism was found for the vasoconstrictor effects of both 5-HT (6.57 ± 0.04 vs. 6.29 ± 0.06; hyperthyroid vs. control) and the thromboxane A_2 receptor agonist U46619 (6.78 ± 0.13 vs. 6.30 ± 0.09; hyperthyroid vs. control). Sensitization to both 5-HT and U46619 was abolished in the presence of N^{ω}-nitro-L-arginine methylester HCl (L-NAME, 0.1 mM). 5-HT-induced contractions in vessels from hyperthyroid rats were diminished by prior incubation with indomethacin (10 μM). The present results indicate that during hyperthyroidism resistance vessels are sensitized to both 5-HT and U46619. This sensitization involves the nitric oxide/L-arginine pathway and probably also certain steps in the cyclooxygenase pathway.

Keywords: Resistance artery, small; Hyperthyroidism; Thromboxane A_2 receptor agonist; 5-HT (5-hydroxytryptamine, serotonin); Endothelial modulation

1. Introduction

Hyperthyroidism is known to be associated with several alterations of the cardiovascular system, such as an increased cardiac output, tachycardia and a decrease in peripheral vascular resistance (Klein, 1990; Levey and Klein, 1990). The spectrum of symptoms of hyperthyroidism suggests the existence of a hyperadrenergic state, although plasma levels of catecholamines appear to be normal or even somewhat reduced (Nagel-Hiemke et al., 1981; Coulombe et al., 1977). To explain this apparently paradoxical finding it is presumed that thyroid hormones may alter tissue sensitivity to catecholamines. In the heart this change has been attributed to an increased density of β-adrenoceptors (Williams and Lefkowitz, 1983; Bilezikian and Loeb, 1983; Ishac et al., 1983; Fox et al., 1985; Kunos et al., 1980).

In vascular smooth muscle, the effects of thyroid hormones on receptor numbers and pharmacological contractile responses appear to be controversial. Different smooth muscle preparations have been investigated, with contradictory results. Fox et al. (1985) reported that in the caudal artery of hyperthyroid rats the response to phenylephrine was markedly decreased. As reported by Field et al. (1973), the contractile potency of KCl was unchanged in isolated aorta preparations taken from hyperthyroid rats, whereas Gunasekera and Kuriyama (1990) reported that maximal contractions induced by high KCl concentrations appeared to be diminished by the hyperthyroid state of the donor

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animals. Grassby and McNeill (1988) observed a somewhat reduced response to the α_1-adrenoceptor agonist methoxamine in the isolated aorta from hyperthyroid rats. Studies reporting about the binding properties of α-adrenoceptors in tissues from hyperthyroid rats appear to be conflicting (Bilezikian and Loeb, 1983; Kunos et al., 1980; Ishac and Pennefather, 1983).

The contractile response to 5-hydroxytryptamine (5-HT) has been reported to be increased in isolated coronary and mesenteric artery preparations obtained from hyperthyroid rats (Miyazawa et al., 1989; Grassby and McNeill, 1988). The isolated superior mesenteric artery taken from hyperthyroid rats appeared to be more sensitive to methoxamine and 5-HT than control preparations (Grassby and McNeill, 1988), although no difference was found for the contractile responses induced by K^+ or Ca^{2+} (Ishikawa et al., 1989).

It should be emphasized that most of these studies were performed with isolated large arteries. Only few data concerning the influence of hyperthyroidism on small arteries are available, although these vessels are highly relevant for regulation of peripheral vascular resistance. For this reason we characterized the responses of small mesenteric arteries, taken from hyperthyroid rats, to various contractile stimuli. We also compared the affinity constants and relative receptor occupancies for the α-adrenoceptor agonist, methoxamine, in vessels from hyperthyroid and control rats.

Endocrine disorders may impair the activity of endothelial cells (Scivoletto et al., 1986). Therefore, we investigated whether changes in the endothelium were involved in the enhanced sensitivity of vessels from hyperthyroid rats to 5-HT and a thromboxane A_2 receptor agonist.

2. Materials and methods

2.1. Treatment of the rats

Male Wistar rats, weighing approximately 250 g at the beginning of the experiment, were rendered hyperthyroid by feeding with L-thyroxine (T4)-containing chow for 4 weeks. T4 was diluted with glucose (1 : 2000) and subsequently mixed with rat chow (10 g T4-glucose mixture/kg rat chow) by Hope Farms (Woerden, Netherlands) to provide a daily intake of 5 mg/kg T4. Age-matched control rats were fed with chow that contained the vehicle only (glucose, 10 g/kg). Food intake and body weight were recorded twice a week.

Rats were anaesthetized with pentobarbital (75 mg/kg, intraperitoneally) and subjected to artificial respiration via a tracheal cannula. Heparin (250 I.U.) was administered via a cannula (PE-50, Clay Adams, USA) inserted into a carotid artery. Subsequently the arterial pressure was measured by means of a Statham P23 pressure transducer and recorded by means of a Maclab data aquisition system (ADI, Australia).

Blood samples (1 ml) from hyperthyroid and control animals were collected at the end of T4 treatment via a cannula inserted into a carotid artery, to measure total serum T4 and thyroid-stimulating hormone (TSH) levels, using conventional radioimmunoassay methods (Wiersinga, 1974). For the rat TSH-RIA, the rat TSH reference preparation NIADDK-rTSH-RP-2 was used, provided by the UCLA Medical Centre, Torrance, USA.

2.2. Isolated small mesenteric arteries

Mesenteric arteries were removed and placed immediately in ice-cold Tyrode's solution (see below). Mesenteric vessels close to the gut with a length of about 3 mm were isolated from fat and surrounding tissues, and a 40-μm diameter stainless-steel wire was inserted into the lumen of the vessel. Subsequently, the vessel was dissected and transferred to the chamber of an isometric myograph (Mulvany and Halpern, 1976). The vessel was fixed to a micrometer screw and, after insertion of a second wire, to an isometric force transducer (Kistler Morse, DSG 6, Redmond, WA, USA).

The preparations were equilibrated in Tyrode's solution (composition (mM): NaCl 136; KCl 2.5; $CaCl_2$ 1.8; $MgCl_2$ 0.5; NaH_2PO_4 0.42; $NaHCO_3$ 11.9 and glucose 5.5) for 10 min at 37°C, gassed with carbogen (95% O_2-5% CO_2), at pH 7.4. The individual diameter was adjusted to a value that equals 90% of the diameter that a vessel would have at a transmural pressure of 100 mm Hg (13.3 kPa). Mechanical responses were expressed as active tension, ΔT, that is the developed active force divided by twice the vessel length.

The responsiveness and the viability of the vessels were tested as follows: at the beginning of each experiment, the preparations were contracted 3 times for 5 min with high-K^+ Tyrode's solution (117.5 mM NaCl was replaced by 120 mM KCl) and once with methoxamine (10 μM) for 5 min, at intervals of 15 min. After these precontractions and a 15-min equilibration period, cumulative concentration-effect curves were made for different agonists. Prior to each $CaCl_2$ concentration-effect curve, the preparations were exposed to Ca^{2+}-free Tyrode's solutions and repeatedly stimulated with high K^+ (120 mM), until the mechanical response had disappeared. One hour later, Ca^{2+} was added cumulatively to a Ca^{2+}-free high-K^+ Tyrode's solution.

To characterize the α-adrenoceptor dissociation constant, the vessels were pretreated for 20 min with phenoxybenzamine (30 nM). After pretreatment with this alkylating antagonist, the preparations were repeatedly washed over a period of 30 min before the concentration-effect curve for methoxamine. was made. N^{ω}-Nitro-L-arginine methylester HCl (L-NAME, 0.1 mM) and indomethacin (10 μM) were added to the bath 30 min before the concentration-effect curve was made. Concentration-effect curves for methacholine and nitroprusside were made after precontraction with the α_1-adrenoceptor agonist methoxamine (10 μM).

2.3. Calculations

Using a computer program (GraphPad, Institute for Scientific Information, USA), all curves were fitted to log concentration-effect data for 5–7 individual experiments. The underlying equation was $E = E_{max} \cdot A^P \cdot (A^P + EC_{50}^P)^{-1}$. In this equation, E is the response obtained with a given concentration A, E_{max} is the maximally attainable response, EC_{50} the concentration required for the half maximal effect, and the exponent P describes the slope of the relationship (Hill coefficient). Curves were fitted to averaged concentration-effect data.

The affinity constant (K_a) of methoxamine with respect to postjunctional α_1-adrenoceptors was determined as described by Furchgott and Bursztyn (1967). Two cumulative concentration-effect curves before and after partial receptor alkylation by phenoxybenzamine were determined. $[A]$ and $[A']$ are corresponding equieffective concentrations before and after receptor alkylation, respectively. From plots of the reciprocals of methoxamine concentrations that gave equivalent responses before ($1/[A]$) and after ($1/[A']$) phenoxybenzamine treatment, linear regression curves were generated and the slope and ordinate intercept were calculated. On the basis of this 'double reciprocal plot', the K_a and q (fraction of receptors not alkylated) were calculated using the following equation (Furchgott and Bursztyn, 1967):

$K_a = (\text{slope} - 1)/\text{intercept}$
$q = 1/\text{slope}$

2.4. Statistical evalution

Unless stated otherwise the values in the text are given as means ± standard error of the mean (S.E.M.). Differences between means were compared using a two-sided Student's t-test for unpaired data. The level of significance was set at $P < 0.05$.

2.5. Drugs used

The following drugs were used: methoxamine HCl, (acetyl-β)-methacholine chloride, L-phenylephrine HCl, 5-hydroxytryptamine oxalate, 9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$ (U46619), N^{ω}-nitro-L-arginine methylester HCl and L-thyroxine sodium salt, obtained from Sigma Chemical Co. (St. Louis, MO, USA); $CaCl_2$, KCl and sodium nitroprusside obtained from E. Merck (Darmstadt, Germany); phenoxybenzamine HCl obtained from Smith, Kline and French (Philadelphia, PA, USA); indomethacin obtained from Merck Sharpe and Dohme (Haarlem, Netherlands). All drugs were dissolved in distilled water, except for indomethacin, which was dissolved in 0.01 M Na_2CO_3.

3. Results

3.1. Effect of T4 treatment on the animals

Several parameters were monitored to establish the degree of hyperthyroidism resulting from T4 treatment for 28 days (Table 1). The growth rate of the T4-treated rats was decreased with respect to control animals, whereas the chow consumption of the T4-treated rats was significantly increased. T4 treatment of the animals resulted in significant increases in serum T4 levels and significant decreases in serum TSH levels.

The pulse pressure was significantly increased after T4 treatment of the animals. However, the most pronounced circulatory effect of T4 treatment was observed for heart rate, which increased by approximately 80 beats/min in T4-treated versus control animals (Table 1).

3.2. Responses of isolated small mesenteric arteries to contractile stimuli

Mesenteric vessels with a length of 1.65 ± 0.02 mm ($n = 142$) were adjusted to their individual optimal diameter: 302.4 ± 3.9 μm and 306.2 ± 3.9 μm, T4-treated versus control animals, respectively; n.s. According to Mulvany and Aalkjaer (1990), these vessels can be considered as resistance vessels.

3.2.1. Depolarizing K^+ solution and Ca^{2+}

K^+ (10–130 mM) produced concentration-dependent increases in tension of the mesenteric artery. Tissues from

Table 1
Biochemical, metabolic and haemodynamic parameters of hyperthyroid rats

	Growth rate (g/28 days)	n	Chow consumption (g/28 days)	n	Serum T4 (nmol · ml^{-1})	n	Serum TSH (ng · ml^{-1})	n	Mean arterial pressure (mm Hg)	n	Pulse pressure (mm Hg)	n	Heart rate (beats · min^{-1})	n
Controls	110 ± 4	42	593 ± 11	8	60 ± 2	39	0.69 ± 0.09	13	125 ± 3	40	47 ± 2	40	375 ± 5	40
T4-treated	81 ± 3 [a]	46	685 ± 12 [a]	8	236 ± 7 [a]	39	0.29 ± 0.03 [a]	23	127 ± 3	44	64 ± 2 [a]	44	453 ± 6 [a]	44

Effect of T4 treatment for 28 days on growth rate (body weight), chow consumption, serum T4 and TSH levels, blood pressure and heart rate of the donor animals. Values are given as means ± S.E.M. Differences between means were compared using a two-sided Student's t-test for unpaired data; [a] $P < 0.05$ compared to controls.

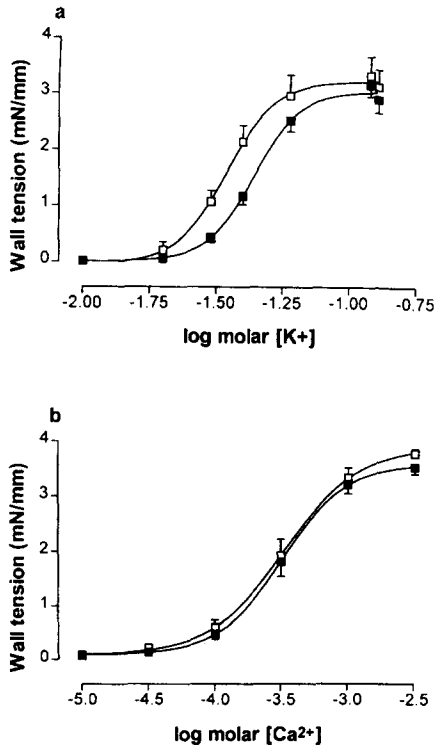

Fig. 1. Concentration-effect curves of K^+ (a) and Ca^{2+} (b) in small mesenteric arteries of control (□) and L-thyroxine (T4)-treated animals (■). Effects are expressed as absolute wall tension (mN/mm). Experimental points are expressed as means ± S.E.M. ($n = 6$).

T4-treated animals were significantly less sensitive to K^+, whereas the absolute maximal response was unaffected (Fig. 1a). The addition of Ca^{2+} also resulted in concentration-dependent increases in tension of the mesenteric artery, but neither the potency nor the absolute maximal response of the Ca^{2+}-induced contractions was affected by T4 treatment (Fig. 1b).

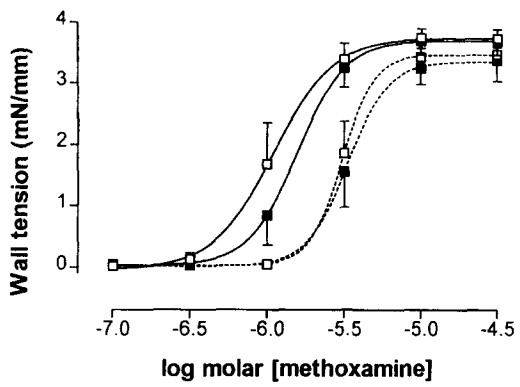

Fig. 2. Concentration-effect curves of methoxamine in small mesenteric arteries of control (□) and L-thyroxine (T4)-treated animals (■) without (solid lines) and in the presence of 30 nM phenoxybenzamine (dashed lines). Effects are expressed as absolute wall tension (mN/mm). Experimental points are expressed as means ± S.E.M. ($n = 6$–7).

3.2.2. *Contractile responses mediated by α_1-adrenoceptor stimulation*

The effect of α_1-adrenoceptor stimulation on the mesenteric artery of T4-treated animals was established by using the α_1-adrenoceptor agonists methoxamine and phenylephrine. Both compounds induced concentration-dependent contractions of the mesenteric vessels. T4 treatment of the donor animals influenced neither the methoxamine- nor the phenylephrine-induced contractions (Table 2). Phenoxybenzamine antagonized the methoxamine-induced contractions of resistance vessels of hyperthyroid and control rats, causing a rightward shift of both curves (Fig. 2). Affinity constants (pK_a values) and fractions of receptors not alkylated (q values) for methoxamine were calculated, by means of the receptor alkylation method as described in the Methods section (Furchgott and Bursztyn, 1967). For methoxamine pK_a values of 5.4 ± 0.2 and 5.3 ± 0.3 were calculated in vessels taken from hyper-

Table 2
pD_2 values and maximal responses in mesenteric arteries of hyperthyroid rats

Vasoconstrictor response	Control			T4-treated		
	pD_2 value	E_{max} (mN/mm)	n	pD_2 value	E_{max} (mN/mm)	n
KCl	1.47 ± 0.03	3.27 ± 0.32	6	1.36 ± 0.02 [a]	3.08 ± 0.23	6
$CaCl_2$	3.48 ± 0.08	3.93 ± 0.06	6	3.50 ± 0.06	3.60 ± 0.13	6
Methoxamine	5.92 ± 0.13	3.81 ± 0.15	7	5.75 ± 0.07	3.74 ± 0.23	7
Phenylephrine	5.92 ± 0.05	3.22 ± 0.16	7	5.96 ± 0.08	3.13 ± 0.19	6
5-HT	6.29 ± 0.06	2.58 ± 0.19	6	6.57 ± 0.04 [a]	3.28 ± 0.30	6
U46619	6.30 ± 0.09	2.96 ± 0.29	6	6.78 ± 0.13 [a]	3.24 ± 0.11 [a]	6
Vasodilator response	Control			T4-treated		
	pD_2 value	E_{max} (%)	n	pD_2 value	E_{max} (%)	n
Nitroprusside	7.19 ± 0.08	83.0 ± 1.7	6	7.30 ± 0.08	89.4 ± 2.2	6
Methacholine	6.65 ± 0.15	97.0 ± 1.0	7	6.45 ± 0.20	96.0 ± 1.4	7

Effect of T4 treatment of the rats for 28 days on the potency (expressed by negative log EC_{50} values) of several vasoconstrictors and vasodilators. For the vasoconstrictors the absolute maximal response (E_{max}, mN/mm) is presented whereas for the responses induced by the vasodilators the maximal relaxation is shown (E_{max}, %), expressed as percentage of the maximal response to 10 μM methoxamine. Values given as means ± S.E.M. Differences between means were compared using a two-sided Student's t-test for unpaired data; [a] $P < 0.05$ compared to controls.

thyroid and control rats, respectively (n.s.). The fraction of the receptors not alkylated with 30 nM phenoxybenzamine amounted to 0.52 ± 0.09 in vessels from hyperthyroid rats and to 0.48 ± 0.08 in control vessels (n.s.).

3.2.3. Contractile responses mediated by 5-HT and thromboxane receptors

The responses to 5-hydroxytryptamine and the thromboxane A_2 receptor agonist U46619 were examined in tissues from T4-treated and control animals. Both 5-HT (0.1–30 μM) and U46619 (0.3–30 μM) induced concentration-dependent contractions of the isolated vessels (Figs. 3 and 4). The concentration-effect curve of 5-HT was bell-shaped and the maximal contractions occurred at concentrations of 1 μM versus 3 μM, T4-treated versus control animals, respectively. The concentration-effect curves for both 5-HT- and U46619-mediated contractions of the small mesenteric arteries were shifted leftwards by T4 treatment of the animals.

Incubation with the nitric oxide synthase inhibitor, L-NAME (0.1 μM), did not influence the basal tone of the vessel. Fig. 3a shows that the contractions elicited by 5-HT were enhanced by L-NAME over the concentration range

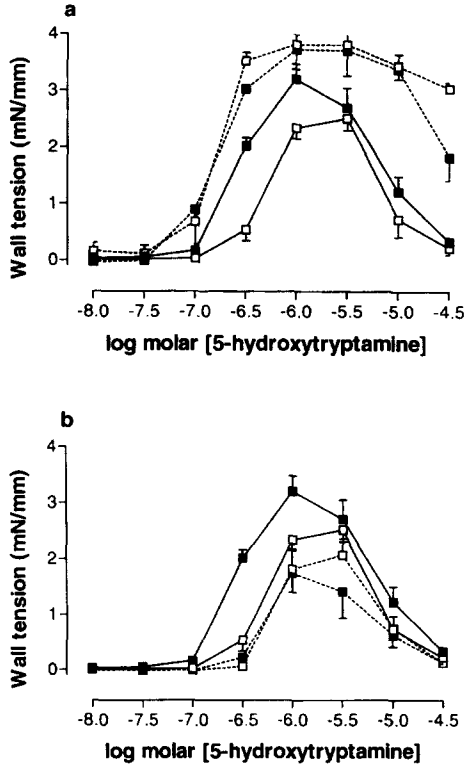

Fig. 3. (a) Concentration-effect curves of 5-hydroxytryptamine (5-HT) in small mesenteric arteries of control (□) and L-thyroxine (T4)-treated animals (■) without (solid lines) and in the presence of 0.1 mM L-NAME (dashed lines). (b) Concentration-effect curves of 5-HT in small mesenteric arteries of control (□) and L-thyroxine (T4)-treated animals (■) without (solid lines) and in the presence of 10 μM indomethacin (dashed lines). Effects are expressed as absolute wall tension (mN/mm). Experimental points are expressed as means ± S.E.M. ($n = 6$).

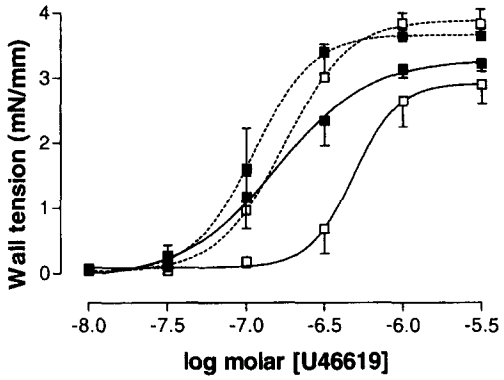

Fig. 4. Concentration-effect curves of the thromboxane A_2 receptor agonist U 46619 in small mesenteric arteries of control (□) and L-thyroxine (T4)-treated animals (■) without (solid lines) and in the presence of 0.1 mM L-NAME (dashed lines). Effects are expressed as absolute wall tension (mN/mm). Experimental points are expressed as means ± S.E.M. ($n = 6$).

of both the ascending and the descending limb of the bell-shaped curve. Final pD_2 values did not differ significantly in either type of preparation (6.75 ± 0.02 vs. 6.86 ± 0.09 in vessels taken from control and hyperthyroid rats, respectively). Incubation of the vessels with L-NAME (0.1 mM) also enhanced the U46619-mediated contractions and resulted in the same final pD_2 values (6.75 ± 0.12 vs. 6.98 ± 0.12 in vessels taken from control and hyperthyroid rats, respectively).

Indomethacin treatment did not influence the resting tone of the preparations. Preincubation of vessels taken from control animals with indomethacin affected neither the pD_2 value of the 5-HT-induced contractions (6.21 ± 0.08), nor the absolute maximal response (2.58 ± 0.19 mN/mm). However, in vessels taken from hyperthyroid animals, 5-HT-induced contractions were significantly attenuated by indomethacin. Both the pD_2 values (6.24 ± 0.06) and the maximal responses (1.91 ± 0.40 mN/mm) of the 5-HT-induced contractions were decreased.

3.2.4. Endothelium-dependent and -independent relaxation

Both methacholine (endothelium-dependent) and nitroprusside (endothelium-independent) evoked concentration-dependent relaxations of the mesenteric resistance arteries. T4 treatment of the animals did not affect the sensitivity of the vessels with respect to the methacholine- and nitroprusside-induced relaxations. Maximal relaxations were achieved in both preparations with methacholine, whereas nitroprusside induced near maximal dilator effects

4. Discussion

Several biochemical/metabolic and haemodynamic parameters indicate that T4 treatment for 28 days leads to a significant, stable and reproducible hyperthyroid state. The

elevated chow consumption together with the decreased growth rate are indicative for an increase in basal metabolic rate. Tachycardia and an increase in pulse pressure are known to be characteristic haemodynamic changes associated with hyperthyroidism. Finally, increased T4 and depressed TSH levels are characteristic biochemical changes associated with the hyperthyroid state.

In mesenteric resistance arteries obtained from T4-treated rats the vasoconstrictor potencies of the various agents investigated were influenced in a different manner. The responses of the isolated vessels to the α_1-adrenoceptor agonists methoxamine and phenylephrine were not influenced by the hyperthyroid state of the animal. The response to Ca^{2+} was unchanged, whereas that to a depolarizing K^+ solution was slightly depressed. This highlighted that the hyperthyroid state of the donor animals was associated with a marked increase in the contractile responses to both 5-HT and U46619. These data indicate that in mesenteric resistance vessels of hyperthyroid rats it is the different transduction mechanism of specific stimuli that is affected rather than the contractile system of the vascular smooth muscle cells.

α-Adrenoceptors were characterized by their affinity constant and the number of receptors not alkylated. The calculated α-adrenoceptor affinity for methoxamine and the number of receptors not alkylated were equal in vessels from hyperthyroid and control rats. These results indicate that hyperthyroidism does not induce major changes in sensitivity and α-adrenoceptor numbers in isolated resistance vessels.

5-HT and thromboxane A_2 are released from aggregating platelets as the major vasoreactive mediators (De Clerck and Van Nueten, 1983). In addition, thromboxane A_2 has been reported to be produced in blood vessels, including the rat mesenteric vascular bed (Soma et al., 1985). In blood vessels, both 5-HT_1-like and 5-HT_2 receptors are known to be present and to elicit opposing effects (Saxena and Villalon, 1990). The occupation of thromboxane A_2 receptors, which are present in vascular smooth muscle cells, produces vascular smooth muscle contraction (Dorn and Becker, 1993).

The bell-shaped concentration-effect curve for 5-HT in our preparations appears to be of interest. Endothelium-mediated relaxation and tachyphylaxis have been proposed earlier as an explanation for the smaller contractions seen at higher concentrations of 5-HT (Nyborg and Mikkelsen, 1985). The smaller contractions at higher concentrations of 5-HT have not been observed in larger arteries, such as the superior mesenteric and the coronary artery taken from T4-treated animals (Miyazawa et al., 1989; Ishikawa et al., 1989). A clear sensitizing influence of hyperthyroidism was found for the ascending part of the 5-HT-induced concentration-effect curve as well as for the vasoconstrictor effects of the thromboxane A_2 receptor agonist U46619.

By modulating endothelial cell function, we evaluated possible mechanisms underlying the supersensitivity of vessels obtained from hyperthyroid rats to both vasoconstrictors. To test the involvement of the nitric oxide (NO) pathway in the sensitization of resistance vessels from hyperthyroid rats to 5-HT and U46619, we studied the effect of prior incubation with L-NAME on 5-HT- and U46619-induced contractions. In the presence of the NO synthase inhibitor, L-NAME (0.1 mM), the contractions induced by 5-HT and U46619 were greater in both preparations. Basal tone was not affected by incubation with L-NAME, indicating that there is no basal release of endothelium-derived nitric oxide. It seems likely that both 5-HT and U46619 are able to stimulate the production of endothelium-derived NO, which may counteract the contractile effects of both agents. These findings are supported by the results of several studies, which have shown that the presence of an intact endothelial cell layer may profoundly depress the contractile responses of various isolated blood vessels to exogenous vasoconstrictor agents (Trezise et al., 1992; Whiting and Cambrigde, 1995; Frieden and Beny, 1995).

The sensitization to the constrictor effects of both 5-HT and U46619 in vessels taken from hyperthyroid animals was abolished in the presence of L-NAME. Since L-NAME inhibits the formation of NO from its precursor L-arginine in vascular endothelial cells (Moncada et al., 1991), it seems likely that the NO/L-arginine pathway is involved in the sensitization to 5-HT and U46619 of resistance vessels from hyperthyroid rats.

The hyperthyroid state of the rats did not affect the responsiveness of resistance vessels to either endothelium-dependent or endothelium-independent relaxations. We therefore assume that neither accelerated dilator responses, nor endothelial dysfunction is responsible for the sensitization to 5-HT and U46619.

To investigate the possible role of contractile factors, dependent on cyclooxygenase activity, in the sensitization to 5-HT of vessels from hyperthyroid animals, we studied the effect of prior incubation with the cyclooxygenase inhibitor indomethacin on the 5-HT-induced contractions of preparations from control and hyperthyroid animals. The lack of effect of indomethacin on the 5-HT-induced contractions in vessels of control rats substantiates the findings of Trezise et al. (1992), who showed that indomethacin did not influence 5-HT-induced contractions in the rabbit isolated basilar artery. The impairment of the 5-HT-induced contractions in the mesenteric arteries from hyperthyroid animals by prior incubation with indomethacin is of interest. This finding indicates that a contractile cyclo-oxygenase product may be involved in the sensitization to 5-HT of resistance vessels from hyperthyroid animals. Accordingly, in hyperthyroidism the balance of contractile cyclooxygenase products and products released via the NO/L-arginine pathway, released after 5-HT stimulation, appears to be changed in favour of cyclooxygenase products. These findings may explain the

overall increased sensitivity to 5-HT-induced contractions of vessels from hyperthyroid animals.

Our finding of a very clear sensitization to the constrictor effect of the thromboxane A_2-mimetic, U46619, is novel and may be of interest from a pathophysiological view, indicating a possible interrelationship between hyperthyroidism and enhanced effects of thromboxane A_2. It may be speculated that in hyperthyroidism thrombo-embolic events are made worse, as a result of a simultaneous contraction of blood vessels, which are more sensitive to the thromboxane released by aggregating platelets.

In conclusion, the present investigation in isolated resistance vessels indicates the occurrence of sensitization to 5-HT and a thromboxane A_2 receptor agonist associated with the hyperthyroid state. This sensitization may be explained, at least partially, by alterations in endothelial cell function.

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References

Bilezikian, J.P. and J.N. Loeb, 1983, The influence of hyperthyroidism and hypothyroidism on alpha-and beta-adrenergic receptor systems and adrenergic responsiveness. Endocr. Rev. 4, 378.

Coulombe, P., J.H. Dussault and P. Walker, 1977, Catecholamine metabolism in thyroid disease. II. Norepinephrine secretion rate in hyperthyroidism and hypothyroidism, J. Clin. Endocrinol. Metab. 44, 1185.

De Clerck, F. and J.M. Van Nueten, 1983, Platelet-mediated vascular contractions. Inhibition by flunarizine, a calcium-entry blocker, Biochem. Pharmacol. 32, 765.

Dorn, G.W. and M.W. Becker, 1993, Thromboxane A2 stimulated signal transduction in vascular smooth muscle, J. Pharmacol. Exp. Ther. 265, 447.

Field, F.P., R.A. Janis and D.J. Triggle, 1973, Relationship between aortic reactivity and blood pressure of renal hypertensive, hyperthyroid, and hypothyroid rats, Can. J. Physiol. Pharmacol. 51, 344.

Fox, A.W., E.N. Juberg, J.M. May, R.D. Johnson, P.W. Abel and K.P. Minneman, 1985, Thyroid status and adrenergic receptor subtypes in the rat: comparison of receptor density and responsiveness, J. Pharmacol. Exp. Ther. 235, 715.

Frieden, M. and J. Beny, 1995, Effect of 5-hydroxytryptamine on the membrane potential of endothelial and smooth muscle cells in the pig coronary artery, Br. J. Pharmacol. 115, 95.

Furchgott, R.F. and P. Bursztyn, 1967, Comparison of dissociation constants and of relative efficacies of selected agonists acting on parasympathetic receptors, Ann. NY Acad. Sci. 144, 882.

Grassby, P.F. and J.H. McNeill, 1988, Hyperthyroidism induces supersensitivity to biogenic amines in rat vascular tissue via a pre- and a postjunctional mechanism, J. Pharmacol. Exp. Ther. 244, 1027.

Gunasekera, R.D. and H. Kuriyama, 1990, The influence of thyroid states upon responses of the rat aorta to catecholamines, Br. J. Pharmacol. 99, 541.

Ishac, E.J. and J.N. Pennefather, 1983, The influence of the thyroid state upon responses to noradrenaline and phentolamine in perfused mesenteric arterioles from the rat, J. Pharm. Pharmacol. 35, 466.

Ishac, E.J., J.N. Pennefather and G.M. Handberg, 1983, Effect of changes in thyroid state on atrial alpha- and beta-adrenoceptors, adenylate cyclase activity, and catecholamine levels in the rat, J. Cardiovasc. Pharmacol. 5, 396.

Ishikawa, T., T. Chijiwa, M. Hagiwara, S. Mamiya and H. Hidaka, 1989, Thyroid hormones directly interact with vascular smooth muscle strips, Mol. Pharmacol. 35, 760.

Klein, I., 1990, Thryoid hormone and blood pressure regulation, in: Hypertension: Pathophysiology, Diagnosis, Management, eds. J.H. Laragh and B.M. Brenner (Raven Press, New York) p. 1661.

Kunos, G., L. Mucci and S. O'regan, 1980, The influence of hormonal and neuronal factors on rat heart adrenoceptors, Br. J. Pharmacol. 71, 371.

Levey, G.S. and I. Klein, 1990, Catecholamine-thyroid hormone interactions and the cardiovascular manifestations of hyperthyroidism, Am. J. Med. 88, 642.

Miyazawa, K., H. Hashimoto and M. Nakashima, 1989, Altered responsiveness of coronary arteries in hyperthyroid rats, Arch. Int. Pharmacodyn. Ther. 297, 120.

Moncada, S., R.M.J. Palmer and E.A. Higgs, 1991, Nitric oxide: physiology, pathophysiology and pharmacology, Pharmacol. Rev. 43, 109.

Mulvany, M.J. and C. Aalkjaer, 1990, Structure and function of small arteries, Physiol. Rev. 70, 921.

Mulvany, M.J. and W. Halpern, 1976, Mechanical properties of vascular smooth muscle cells in situ, Nature 260, 617.

Nagel-Hiemke, M., G. Gross, I. Lues and H.J. Schumann, 1981, Influence of hypo- and hyperthyroidism on plasma catecholamines in pithed rats, Naunyn-Schmied. Arch. Pharmacol. 317, 159.

Nyborg, N.C. and E.O. Mikkelsen, 1985, In vitro studies on responses to noradrenaline, serotonin, and potassium of intramyocardial and mesenteric resistance vessels from Wistar rats, J. Cardiovasc. Pharmacol. 7, 417.

Saxena, P.R. and C.M. Villalon, 1990, Cardiovascular effects of serotonin agonists and antagonists, J. Cardiovasc. Pharmacol. 15 (Suppl. 7), S17.

Scivoletto, R., Z.B. Fortes and J. Garcia-Leme, 1986, Thyroid hormones and vascular reactivity: role of the endothelial cell, Eur. J. Pharmacol. 129, 271.

Soma, M., M.S. Manku, D.K. Jenkins and D.F. Horrobin, 1985, Prostaglandins and thromboxane outflow from the perfused mesenteric vascular bed in spontaneously hypertensive rats, Prostaglandins 29, 323.

Trezise, D.J., G.M. Drew and A.H. Weston, 1992, Analysis of the depressant effect of the endothelium on contractions of rabbit isolated basilar artery to 5-hydroxytryptamine, Br. J. Pharmacol. 106, 587.

Whiting, M.V. and D. Cambrigde, 1995, Canine renovascular responses to sumatriptan and 5-carbox-amidotryptamine: modulation through endothelial 5-HT1-like receptors by endogenous nitric oxide, Br. J. Pharmacol. 114, 969.

Wiersinga, W.M., 1974, The Peripheral Conversion of Thyroxine (T4) into Triiodothyronine (T3) and Reverse Triiodothyronine (rT3) (PhD Thesis, University of Amsterdam, Amsterdam).

Williams, R.S. and R.J. Lefkowitz, 1983, The effects of thyroid hormone on adrenergic receptors, in: Molecular Basis of Thyroid Hormone Action (Academic Press, New York) p. 325.