The influence of thyroid states upon responses of the rat aorta to catecholamines

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1 Mechanical responses to various stimulants and the distribution of adrenoceptor subtypes were investigated in smooth muscle cells of the rat aorta in eu- (control), hyper- and hypo-thyroid states.

2 Concentration-response relationships for K showed that the KCl EC₅₀ value was slightly higher (28.4 mM K) in hypothyroid than in euthyroid and hyperthyroid states (22.5 mM and 22.8 mM K, respectively). The order of maximum amplitudes of KCl contraction was control > hypothyroid > hyperthyroid.

3 The EC₅₀ values for the noradrenaline (NA)-induced contraction for eu-, hypo- and hyper-thyroid states were 7.4, 12.5 and 5.5 nM, respectively. The density of α_1 -adrenoceptors was increased to 1.4 times the control in the hypothyroid state and reduced to 0.26 times the control in the hyperthyroid state.

4 The EC₅₀ values for the phenylephrine-induced contraction in eu-, hypo- and hyper-thyroid states were 43.4, 247 and 41.5 nM, respectively, and those for clonidine 104, 1360 and 56.5 nM, respectively. The IC₅₀ values for prazosin against 0.1 μ M NA-induced contractions in eu-, hypo- and hyper-thyroid states were 5.8, 3.5 and 0.7 nM, respectively, and those for yohimbine 8.9, 5.4 and 4.1 μ M, respectively.

5 The IC₅₀ values for isoprenaline against phenylephrine-induced contractions were 63 nM and 2.4 μ M in hyper- and eu-thyroid states, respectively, and the corresponding IC₂₅ values for eu- and hypo-thyroid states were 72 and 423 nM, respectively. The density of β -adrenoceptors was increased to 3.91 times the control in the hyperthyroid and reduced to 0.68 times the control in the hypothyroid states. The amount of adenosine 3':5'-cyclic monophosphate (cyclic AMP) synthesized by isoprenaline increased in parallel with the density of β -adrenoceptors in the three thyroid states.

6 NA consistently increased the amount of inositol 1,4,5-trisphosphate (IP₃) within 30 s in the three thyroid states. In the hypothyroid state, the amount of IP₃ was slightly increased and in the hyperthyroid condition, it occurred to a lesser extent than in the euthyroid state.

7 The results indicate that in hypothyroidism, the density of α_1 -adrenoceptors is increased and that of β -adrenoceptors reduced in vascular beds. The latter but not the former is accompanied by corresponding mechanical responses. In hyperthyroidism, the density of α_1 -adrenoceptors is decreased and β -adrenoceptors increased with parallel changes in mechanical responses.

Introduction

In the hyperthyroid state, there is general agreement that the mechanical and electrical responses of cardiac muscle tissues induced by β -adrenoceptor stimulation are enhanced and those induced by α -adrenoceptor stimulation are markedly reduced. In contrast, responses to α -adrenoceptor stimulation are markedly enhanced in the hypothyroid state (Nakashima *et al.*, 1971; Rosenqvist & Boreus, 1972; Young & McNeill, 1974; Kunos, 1977; Bilezikian & Loeb, 1983; Threatte *et al.*, 1983; Ishac & Pennefather, 1983; Gross & Lues, 1985; Fox *et al.*, 1985).

However, no consensus has been reached concerning the density of α -adrenoceptors in either hyperthyroidism (unchanged: Ciaraldi & Marinetti, 1978; decreased: Ciaraldi & Marinetti, 1977; Sharma & Banerjee, 1978; Williams & Lefkowitz, 1979; McConnaughey *et al.*, 1979) or hypothyroidism (increased: Sharma & Banerjee, 1978; Kunos *et al.*, 1980; Chang & Kunos, 1981; unchanged: Ciaraldi & Marinetti, 1978; decreased: Ciaraldi & Marinetti, 1977; 1978; McConnaughey *et al.*, 1979; Noguchi & Whitsett, 1983). Several studies have also revealed that the number of β -adrenoceptors is altered in different thyroid states. In hyperthyroidism, the number of β -adrenoceptors and the responses to β -agonists are increased (Williams *et al.*, 1977; Tse *et al.*, 1980; Scarpace & Abrass, 1981; Stiles & Lefkowitz, 1981; Krawietz *et al.*, 1982; Bilezikian & Loeb, 1983; O'Donnell & Wanstall, 1986; O'Donnell *et al.*, 1987) or unchanged (Banerjee & Kung, 1977). In hypothyroid animals, the β -adrenoceptor population

in cardiac muscles decreases (Banerjee & Kung, 1977; Chang & Kunos, 1981; Ciaraldi & Marinetti, 1977; McConnaughey *et al.*, 1979; Kunos *et al.*, 1980; Tse *et al.*, 1980; Stiles & Lefkowitz, 1981; Krawietz *et al.*, 1982; Noguchi & Whitsett, 1983).

In vascular smooth muscle tissues, the effects of thyroid hormones on adrenoceptors are also controversial. In rat vascular beds, it was shown that the density of smooth muscle α_1 -adrenoceptors is not altered by any thyroid state (Fox *et al.*, 1985). However, others have found that in the rat pulmonary artery and aorta, responses to β_1 - but not to β_2 -adrenoceptor stimulation are enhanced in the hyperthyroid state (O'Donnell & Wanstall, 1986; O'Donnell *et al.*, 1987). In the hypothyroid rat aorta, Rahmani *et al.* (1987) demonstrated that the α_1 -adrenoceptor-mediated response was significantly reduced and shifted to the right, and that α_2 -adrenoceptor responses were blocked. In contrast, responses to the α_1 - and α_2 -adrenoceptors were not altered in the hyperthyroid state. These results confirmed previous observations (MacMillan & Rand, 1962; Rosenqvist & Boreus, 1972), but were in contrast to the enhanced α -adrenoceptor-mediated responses to NA obtained in rat aorta in the hypothyroid but not in the hyperthyroid state (Field *et al.*, 1973).

In vascular smooth muscle tissues, no systematic investigation has been carried out on the density of adrenoceptor distribution and its relationship to receptor stimulation in different thyroid states. The present experiments were thus designed to clarify responses of the smooth muscle of rat aorta in relation to the distribution of the catecholamine receptors

in eu-, hyper- and hypo-thyroid states. The possible actions of thyroid hormones on smooth muscle tissues are also discussed.

Methods

Materials

Male Wistar King A (WKA) rats weighing 225–350 g (8–12 weeks old) were used. The animals were caged separately and given rat purina chow and drinking water *ad libitum*. Hypothyroidism was induced by total thyroidectomy and to prevent the occurrence of hypoparathyroidism, parathyroid glands were autotransplanted. One or two parathyroid glands were identified under the binocular microscope, carefully dissected out from the thyroid lobes and re-implanted into the hyoid muscle. The hypothyroid animals were killed not less than 3 weeks after the thyroidectomy.

Hyperthyroidism was induced by subcutaneous injections of 0.75 mg kg^{-1} L-thyroxine sodium in alkaline saline solution (0.001 N NaOH in $0.9\% \text{ NaCl}$) daily for 7 days (Threatte *et al.*, 1983). The animals were killed at least 20 h after the last injection. The control rats (eu-thyroid status) were either sham-operated or injected with alkaline saline for the same period of time. Since the thyroxine-treated animals were sensitive to overdoses and vulnerable to spontaneous death during the treatment period, especially when the room temperature exceeded 25°C , the room temperature was maintained at 25°C .

Assessment of thyroid states

Body weight was measured at the commencement of treatment and the average daily change in body weight was compared with controls. Colonic temperature was measured under anesthesia (sodium pentobarbitone $6.5 \text{ mg } 100 \text{ g}^{-1}$ i.p.) at an ambient temperature of $25 \pm 2^\circ\text{C}$ by a thermistor probe (Nihon Kohden model NGA 111, type 219), which was inserted 6 cm beyond the anus for 1 min. Subsequently animals were exsanguinated and then blood and tissues were collected. Heart weights were measured in all animals as was the weight of the thyroid in eu- and hyper-thyroid rats. Heart and thyroid weights were expressed relative to body weight ($\text{mg } 100 \text{ g}^{-1}$ body weight). A blood sample taken from each rat on the day of experiment was centrifuged at 3000 r.p.m. for 10 min, and the serum was separated immediately. Serum Ca estimation was carried out for the hypothyroid rats by use of a commercially available calcium C-test (Wako kits). The rest of the serum samples were stored at -20°C . Total serum thyroxine (T_4) and tri-iodothyronine (T_3) levels of each rat were determined by radioimmunoassay with a commercially available conventional double antibody technique (provided by Eiken Chem. Co. Ltd.) and newly developed solid phase antibody-coated tubes (provided by Mallinkrodt Diagnostica, Daiichi Radioisotopes, Japan).

Measurements of isometric contraction

The isolated aorta was cleaned of surrounding connective tissue and a 2 mm wide helical strip was cut with scissors from a 6 mm length of upper thoracic aorta. The endothelium was gently removed with a cotton ball. The tissue was suspended in an organ bath and perfused with warmed Krebs solution ($35\text{--}37^\circ\text{C}$), by a thermistor-controlled perfusion pump. The tissue was suspended for 1 h without stretch, and then 50–60 mg tension was applied for a further 1 h. Isometric tension was recorded by a force transducer (Nihon Kohden FD pick up, TB-621), and the measurements were started when the amplitude of contraction showed a constant value in response to repetitive application of a solution containing 39 mM KCl .

To obtain concentration-response relationships, agonists and antagonists were applied cumulatively.

Krebs solution of the following composition was used (mm): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 15.5, H_2PO_4^- 1.2, Cl 134, glucose 11.5. The solution was bubbled with 97% O_2 and 3% CO_2 , and the pH of the solution was maintained at about 7.3.

Cyclic AMP and inositol 1,4,5-trisphosphate assays

Rat aorta devoid of connective tissue and endothelium was cut into 5 segments and incubated at 37°C in Krebs solution for 1 h. Tissues were stimulated with $1 \mu\text{M}$ isoprenaline for 0–5 min and the reaction was halted by quickly immersing the muscle strip in liquid N_2 . The samples were homogenized in cold trichloroacetic acid (0.3 mol l^{-1}) at 4°C and the concentration of adenosine 3':5'-cyclic monophosphate (cyclic AMP) and protein content were measured by a radioimmunoassay kit (Yamasa Shoyu, Tokyo, Japan) and the method of Lowry *et al.* (1951), respectively.

In the case of inositol 1,4,5-trisphosphate (IP_3) measurements, tissues were stimulated with $50 \mu\text{M}$ NA for 30 s to 3 min, and homogenized in cold trichloroacetic acid (0.75 mol l^{-1}). The amount of IP_3 was measured by a radioimmunoassay kit (Amersham, Japan, plc., code TRK, 1000).

Radioligand binding assay

β -Adrenoceptor radioligand binding assay Aortae from 6–7 rats devoid of connective tissue and endothelium were minced with scissors in 50 mM tris-maleate buffer (pH 7.2) at 4°C , and homogenized with a polytron homogenizer (Type NS 500; Nihon Seimitsu Kogyo, Japan) for 20 s at half-maximal speed. The above procedure was repeated twice and the homogenate was then centrifuged at $10,000 g$ for 10 min and the supernatant was centrifuged again at $100,000 g$ for 1 h at 4°C . The pellet was re-suspended in buffer and protein determination was performed as described by Lowry *et al.* (1951). [^{125}I]-iodocyanopindolol ([^{125}I]-CYP; $2200 \text{ Ci mmol}^{-1}$) was incubated with $20 \mu\text{g}$ of membrane protein in triplicate in a final volume of 0.5 ml at 25°C for 1 h. The suspension was filtered through a GF/F glass fibre filter (Whatman 25) under reduced pressure. The filters were washed with 10 ml of 15 mM Tris-maleate buffer at pH 7.2, dried and counted in a gamma counter. Non-specific binding of [^{125}I]-CYP was determined as binding remaining following treatment with $50 \mu\text{M}$ isoprenaline.

α_1 -Adrenoceptor radioligand binding assay α_1 -Adrenoceptor binding experiments were carried out with [^{125}I]-iodo-4-hydroxy phenyl-ethyl-aminomethyl-tetralone ([^{125}I]-HEAT; $2200 \text{ Ci mmol}^{-1}$), as described by Jones *et al.* (1987), using a crude membrane fraction of 3–4 aortae in 25 mM glycylglycine buffer (pH 7.6). Briefly, the procedure was the same as for the [^{125}I]-CYP binding assay; $20 \mu\text{g}$ of crude membrane was incubated for 40 min, filtered through glass fibre filters and radioactivity counted. The determination of non-specific binding of [^{125}I]-HEAT was made by treatment with $100 \mu\text{M}$ NA.

Drugs

The following drugs were used: L-thyroxine, (–)-noradrenaline (NA), and isoprenaline hydrochloride were obtained from Sigma Chem. Co.; phenylephrine, prazosin were obtained from Taito-Pfizer Pharmac. Co., and yohimbine and clonidine from Tokyo Kasei Pharmac. Co. [^{125}I]-iodocyanopindolol ([^{125}I]-CYP) and β -[^{125}I]-iodo-4-hydroxy phenyl-ethyl-aminomethyl-tetralone ([^{125}I]-HEAT) were obtained from NEN Research Products. Triiodo-thyronine (T_3) solid phase antibody coated kits (T_3 -SPAC) were kindly donated by Daiichi Radioisotopes, Japan.

Statistics

The experimental values are expressed as the mean $\pm$ s.e. Statistical significance was determined by use of Student's *t* test.

Results

Table 1 summarizes the measurements of various parameters (see Methods) following altered thyroid states in rats. In the hypothyroid animals, significant reductions in weight gain, colonic temperature, heart/body weight ratio and serum T_4 and T_3 levels were observed. However, there was no significant change in the serum Ca level to suggest hypoparathyroidism. In contrast, significant increases in colonic temperature, heart/body weight ratio, serum T_4 and T_3 levels were observed in the hyperthyroid rat. In addition, there were significant reductions in the weight of the thyroid gland and in the amount of weight gain. In the hyperthyroid rats, the serum Ca level remained the same as that observed in the controls.

K- and NA-induced contractions observed in three different thyroid states

Figure 1 shows the concentration-response (contraction) relationships observed on application of various concentrations of KCl and NA to aortae from eu-, hyper- and hypo-thyroid rats. In the hypothyroid state, the KCl concentration-response relationship was shifted to the right and the maximum amplitude of contraction evoked by 118 mM KCl was inhibited compared with that observed in euthyroid rats. In hyperthyroid aortae, the maximum amplitude of the KCl-induced contraction (118 mM) was markedly inhibited, but the 11.7 mM KCl-induced contraction was not modified in comparison to the control (Table 2).

Figure 1 Concentration-response relationships for (a) KCl- and (b) noradrenaline (NA)-induced contractions of rat aorta in eu-(control; $\circ$), hypo-($\bullet$) and hyper-thyroid states ($\square$). Vertical lines denote s.e.mean; in (a) $n = 6-10$ and (b) $n = 15-17$. Asterisks indicate responses significantly different from the control (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Similar experiments were carried out with NA as the agonist. As shown in Figure 1b, the minimum concentration of NA required to produce a contraction was approximately the same in the three states (> 0.1 nM). However, the contractions evoked at any given concentration of NA in aortae in the euthyroid rats were consistently larger than those observed in the hyper- and hypo-thyroid states, and the maximum amplitude of the NA ($10 \mu\text{M}$)-induced contraction evoked in tissues from the hyperthyroid rat was much smaller than that observed from eu- and hypothyroid rats. The EC_{50} values for NA in eu-, hypo- and hyper-thyroid conditions are listed in Table 2.

Table 1 Various somatic parameters measured following altered thyroid states in rats

Variable	Euthyroid (control)	Treatment group Hypothyroid	Hyperthyroid
% decrease of wt. gain over control animals	a	$40.3 \pm 1.7^*$ (79)	$87.8 \pm 7.2^*$ (90)
Colonic temp. in $^{\circ}\text{C}$	38.2 ± 0.1 (45)	$36.7 \pm 0.1^*$ (47)	$39.7 \pm 0.1^*$ (63)
Heart/body weight ($\text{mg } 100 \text{ g}^{-1}$ body wt.)	256 ± 5.6 (48)	$204 \pm 2.1^*$ (74)	$403 \pm 5.1^*$ (90)
Thyroid/body weight ($\text{mg } 100 \text{ g}^{-1}$ body wt.)	4.81 ± 0.11 (54)	0	$3.35 \pm 0.07^*$ (44)
Serum thyroxine (T_4) ($\mu\text{g dl}^{-1}$)	3.16 ± 0.29 (58)	$0.44 \pm 0.04^*$ (79)	$23.7 \pm 1.62^*$ (90)
Serum triiodothyronine (T_3) (ng dl^{-1})	71.8 ± 2.95 (58)	$35.2 \pm 2.46^*$ (79)	$403 \pm 25.7^*$ (90)
Serum Ca^{2+} (mg dl^{-1})	8.65 ± 0.11 (32)	$8.57 \pm 0.09^{\text{NS}}$ (39)	$8.68 \pm 0.24^{\text{NS}}$ (26)

Results are expressed as mean $\pm$ s.e. (number of observations).

* Significantly different from euthyroid group by Student's *t* test, $P < 0.001$.

NS – not significant.

a, weight gains of alkaline saline injected and sham-operated rats during the treatment period were 28.7 ± 2.5 and $18.3 \pm 1.5 \text{ g week}^{-1}$, respectively.

Table 2 The EC_{50} values measured for high K-, noradrenaline-, phenylephrine- and clonidine-induced contractions and the IC_{50} values for prazosin- and yohimbine-induced inhibition of the noradrenaline ($0.1 \mu\text{M}$)-induced contraction measured from smooth muscle tissues of the rat aorta in eu-, hypo- and hyper-thyroid states

	Control	Hypothyroid	Hyperthyroid
High-K	$22.5 \pm 0.8 \text{ mM}$ ($n = 8$)	$28.4 \pm 0.7 \text{ mM}^{**}$ ($n = 10$)	$22.8 \pm 1.5 \text{ mM}$ ($n = 9$)
Noradrenaline	$7.4 \pm 1.2 \text{ nM}$ ($n = 15$)	$12.5 \pm 1.7 \text{ nM}^*$ ($n = 17$)	$5.5 \pm 1.0 \text{ nM}$ ($n = 17$)
Phenylephrine	$43.4 \pm 3.1 \text{ nM}$ ($n = 15$)	$247 \pm 58 \text{ nM}^{**}$ ($n = 12$)	$41.5 \pm 3.7 \text{ nM}$ ($n = 11$)
Clonidine	$104 \pm 12.3 \text{ nM}$ ($n = 14$)	$1360 \pm 251 \text{ nM}^{***}$ ($n = 14$)	$56.5 \pm 18.6 \text{ nM}^{**}$ ($n = 9$)
Prazosin	$5.8 \pm 0.7 \text{ nM}$ ($n = 25$)	$3.45 \pm 0.45 \text{ nM}^{**}$ ($n = 9$)	$0.66 \pm 0.2 \text{ nM}^{***}$ ($n = 8$)
Yohimbine	$8.9 \pm 0.2 \mu\text{M}$ ($n = 12$)	$5.44 \pm 0.83 \mu\text{M}^{***}$ ($n = 8$)	$4.07 \pm 0.34 \mu\text{M}^{***}$ ($n = 8$)

The EC_{50} and IC_{50} values were obtained from concentration-response curves in each tissue. Data are mean $\pm$ s.e. (n = number of observations).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Effects of α -adrenoceptor agonists and antagonists

To clarify further the response of smooth muscle tissues to activation of α -adrenoceptors, the effects of phenylephrine and clonidine were studied (Figure 2a and b). The minimum concentration of phenylephrine required to produce a contraction was 1 nM, and the maximum amplitude of contraction evoked by phenylephrine was larger in the control than the hypo- and hyper-thyroid rats. The minimum concentration of clonidine required to evoke a contraction was about 10 nM and the maximum amplitudes of contraction evoked by 10 μ M clonidine were much smaller than those evoked by 10 μ M phenylephrine or NA. The EC_{50} values for phenylephrine and clonidine in the eu-, hypo- and hyper-thyroid states are given in Table 2.

To investigate further the action of thyroid hormone on responses to α -adrenoceptor subtypes, the effects of prazosin and yohimbine on NA-induced contractions were investigated (Figure 3a and b). Prazosin and yohimbine each inhibited the NA (0.1 μ M)-induced contraction in the three different thyroid states. Yohimbine was less potent than prazosin (Table 2). The inhibition of the standard NA-induced contraction by yohimbine occurred at concentrations above 1 μ M and even at a concentration of 10 μ M, yohimbine never completely blocked the

Figure 2 Concentration-response relationships for (a) phenylephrine- and (b) clonidine-induced contractions in rat aorta in eu- (○; $n = 15$ in (a) and (14) in (b)), hypo- (●; $n = 12$ in (a) and 14 in (b)) and hyper- (□; $n = 11$ in (a) and 9 in (b))-thyroid states. Vertical lines show s.e.mean * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, responses significantly different from control.

Figure 3 Concentration-dependent inhibition by prazosin (a) and yohimbine (b) of the noradrenaline (NA, 0.1 μ M)-induced contraction in rat aorta in eu- (○; $n = 25$ in (a) and 12 in (b)), hypo- (●; $n = 9$ in (a) and 8 in (b)) and hyper- (□; $n = 8$ in (a) and (b)) thyroid states. Vertical lines show s.e.mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, responses significantly different from control.

contraction in any of the three thyroid states (Figure 3b and Table 2).

The effects of the β -adrenoceptor agonist, isoprenaline, on the phenylephrine-induced contraction

To evaluate the role of β -adrenoceptors in the three different thyroid states, the effects of isoprenaline were studied in tissues precontracted by phenylephrine (1 μ M) (Figure 4). In the euthyroid state, 1 nM isoprenaline did not relax the phenylephrine (1 μ M)-induced contraction and more than 1 μ M isoprenaline was required to produce 50% relaxation of the contracted tissue. A potent relaxation was induced by isoprenaline in tissues excised from the hyperthyroid rats (1 μ M isoprenaline induced 0.09 ± 0.02 times the maximum amplitude of phenylephrine-induced contractions), whereas in the hypothyroid state, 1 μ M isoprenaline relaxed the tissue to 0.66 ± 0.02 times the maximum amplitude of contraction observed. The IC_{50} values for isoprenaline against the phenylephrine-induced contraction were $2.4 \pm 0.4 \mu$ M and 63.2 ± 5.9 nM ($n = 17$) in the eu- and hyper-thyroid states, respectively, and the IC_{25} values in eu- and hypo-thyroid states were 72.4 ± 19.5 and 423 ± 94.4 nM ($n = 16-17$), respectively.

The effects of thyroid states on [125 I]-CYP and [125 I]-HEAT binding

To investigate β -adrenoceptor distribution on smooth muscle cells of the rat aorta in eu-, hyper- and hypo-thyroid states, [125 I]-CYP was used. β -Adrenoceptor density was estimated from a Scatchard analysis of the saturation isotherm specific for [125 I]-CYP binding in the three thyroid states (Figure 5a). The total binding of [125 I]-CYP to the fragmented membrane increased in a time-dependent manner and reached a steady level at 45 min. Therefore the maximum binding (B_{max}) was measured after 1 h exposure to [125 I]-CYP. The specific binding of [125 I]-CYP was obtained by subtraction of non-specific binding from total binding (Methods). The B_{max} values in the eu-, hypo- and hyper-thyroid states are given in Table 3. The equilibrium dissociation constants, K_D , for the specific

Figure 4 The concentration-response relationship of the isoprenaline-induced relaxation in rat aorta precontracted by phenylephrine (1 μ M) in eu- (○; $n = 17$), hypo- (●; $n = 16$) and hyper- (□; $n = 17$) thyroid states. The contractions evoked by phenylephrine in each of the three different thyroid states were normalized as 1 and complete relaxation of precontracted tissues was normalized as 100%, respectively. Vertical lines show s.e.mean. ** $P < 0.01$, *** $P < 0.001$, responses significantly different from control.

Figure 5 Scatchard analysis of saturation isotherms of β -adrenoceptor specific [125 I]-iodocyanopindolol ([125 I]-CYP) binding (a) and α_1 -adrenoceptor specific [125 I]-iodo-4-hydroxyphenyl-ethyl-amonimethyl-tetralone ([125 I]-HEAT) binding (b) to membrane fractions of smooth muscle cells of the rat aorta in eu- (○), hypo- (●) and hyper-thyroid states (□). Numbers of observations are 5–6 (34–45 animals) in (a) and 7–10 (15–20 animals) in (b).

binding of [125 I]-CYP in the above three different thyroid states were similar (Figure 5a and Table 3). Therefore, hyperthyroidism caused a 3.91 times increase and hypothyroidism caused a 0.68 times reduction in the density of specific [125 I]-CYP binding sites, without any change in the binding affinity in the rat aorta.

The effects of thyroid states on the specific binding of [125 I]-HEAT to the α_1 -adrenoceptor were determined by a Scatchard analysis, using the same procedure as for the [125 I]-CYP binding and the results are summarised in Figure 5b and Table 3. In hyperthyroidism, the density of β -adrenoceptors on smooth muscle cell membranes was increased but that of α -adrenoceptors decreased, while the reverse sequence in receptor density was observed in hypothyroidism. However, the K_D values derived from application of radioligands were not modified in the three different thyroid states.

Effect of thyroid state on the synthesis of cyclic AMP induced by isoprenaline

Figure 6a shows the effects of $1 \mu\text{M}$ isoprenaline on the synthesis of cyclic AMP in smooth muscle cells in the three different thyroid states. The basal cyclic AMP level of smooth muscle tissues obtained from the hyperthyroid rat was increased ($0.86 \pm 0.07 \text{ pmol mg}^{-1} \text{ protein}$, $n = 8$) in comparison to that from euthyroid rats ($0.56 \pm 0.08 \text{ pmol mg}^{-1} \text{ protein}$, $n = 8$), while that obtained from the hypothyroid rat was decreased ($0.33 \pm 0.02 \text{ pmol mg}^{-1} \text{ protein}$, $n = 7$). Following application of $1 \mu\text{M}$ isoprenaline, the amount of cyclic AMP was markedly increased within 1 min to 1.56, 1.60 and 1.84 times in the eu-, hypo- and hyper-thyroid rats, respectively and then gradually reduced with time. In the hypothyroid rat, the amount of

Figure 6 Time-dependent synthesis of cyclic AMP (a) and inositol 1,4,5-trisphosphate (IP_3) (b), following application of $1 \mu\text{M}$ isoprenaline or $50 \mu\text{M}$ noradrenaline, respectively in eu- (○), hypo- (●) and hyper- (□)-thyroid states. Individual points indicate the mean ($n = 7$ –9); vertical lines show s.e. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significantly different from control.

cyclic AMP measured 5 min after application of isoprenaline was reduced to almost the same level as that observed before the application, while in the control (euthyroid) rat, the amount of cyclic AMP returned to a level just above the basal level and in the hyperthyroid rat, the amount of cyclic AMP remained at a higher level than the control (Figure 6a).

Effect of thyroid state on the synthesis of IP_3

Figure 6b shows the effects of $50 \mu\text{M}$ NA on the synthesis of IP_3 in smooth muscle cells of the eu-, hyper- and hypothyroid rats. The basal level of IP_3 in the tissue was increased in the hypothyroid rats ($4.14 \pm 0.54 \text{ pmol mg}^{-1} \text{ protein}$, $n = 7$) and decreased in the hyperthyroid rats ($2.22 \pm 0.39 \text{ pmol mg}^{-1} \text{ protein}$, $n = 7$) when compared with the euthyroid rats ($3.33 \pm 0.40 \text{ pmol mg}^{-1} \text{ protein}$, $n = 7$). In all three thyroid conditions, the amount of IP_3 was markedly increased within 30 s following NA stimulation in a similar manner and gradually decreased with time over a period of 3 min. When the maximum amount of synthesized IP_3 , measured at 30 s after application of NA, was compared to the basal levels, the increases were 2.54, 2.31, and 2.24 times basal ($n = 7$ each) in eu-, hyper- and hypo-thyroid animals, respectively. Thus, the hyperthyroid state was associated with a relatively decreased level of IP_3 , whereas in the hypothyroid state, the amount of IP_3 measured following agonist stimulation was not significantly different from the controls, although the basal value was high.

Discussion

In cardiac and vascular smooth muscle tissues, figures for the density of adrenoceptor distribution in different thyroid states are controversial as described in the Introduction. Recently, Limas & Limas (1987) showed that the density of β -adrenoceptors in rat cardiac muscle increased progressively

Table 3 [125 I]-iodocyanopindolol ([125 I]-CYP) and [125 I]-iodo-4-hydroxyphenyl-ethyl-amonimethyl-tetralone ([125 I]-HEAT) binding to smooth muscle membranes of the rat aorta in eu-, hypo- and hyper-thyroid states

	[125 I]-CYP	Control	Hypothyroid	Hyperthyroid
B_{max} (fmol mg^{-1} protein)		28.3 ± 3.1 ($n = 5$)	$19.3 \pm 1.5^*$ ($n = 6$)	$110.6 \pm 9.2^{***}$ ($n = 6$)
K_D (pM)		45.3 ± 3.3 ($n = 5$)	33.9 ± 2.9 ($n = 6$)	47.3 ± 3 ($n = 6$)
[125 I]-HEAT				
B_{max} (fmol mg^{-1} protein)		72.9 ± 1.7 ($n = 7$)	$101.6 \pm 3.3^{***}$ ($n = 8$)	$18.6 \pm 1.9^{***}$ ($n = 10$)
K_D (pM)		15.1 ± 0.66 ($n = 7$)	16.5 ± 0.38 ($n = 8$)	13.4 ± 0.8 ($n = 10$)

The maximum binding (B_{max}) and dissociation constant (K_D) are presented.

Results are expressed as mean $\pm$ s.e. (n = number of observations). Asterisks denote significantly different from control animals determined by Student's t test (* $P < 0.05$, *** $P < 0.001$).

during daily injections of L-thyroxine. In contrast the number of α_1 -adrenoceptors declined rapidly and following cessation of thyroxine administration, the receptor number returned rapidly to normal values within 2 days. These results may indicate that the biological activity of thyroxine in the rat is a critical factor in determining the density and responsiveness of the cardiac adrenoceptor.

Rahmani *et al.* (1987) found that in aortae from hypothyroid rats, the α_1 -adrenoceptor-mediated response was significantly decreased, the maximum mechanical response was reduced and the concentration-response relationship shifted to the right, compared with the euthyroid aortae, whereas the α_2 -adrenoceptor-mediated response was completely inhibited. In contrast, hyperthyroidism did not alter the α_1 - and α_2 -adrenoceptor responses. These observations did not confirm the previous findings of Rosenqvist & Boreus (1972) and Field *et al.* (1973).

In the present experiments, the somatic condition of rats was significantly modified following thyroidectomy or injections of thyroxine (Table 1). In the hypothyroid state, responses of the α_1 -adrenoceptor to NA or phenylephrine were markedly inhibited and those of the α_2 -adrenoceptor to NA or clonidine almost ceased. The density of α_1 -adrenoceptors in the hypothyroid rat was increased, as estimated from the B_{max} value of [125 I]-HEAT binding experiments, without any significant change in the K_D value. Furthermore, the basal level of IP₃ synthesis was enhanced. However, in the hypothyroid state, the increased number of the α_1 -adrenoceptors did not directly correlate with the α_1 -adrenoceptor-induced mechanical responses of the smooth muscle.

In the rat pulmonary artery, O'Donnell *et al.* (1987) observed a selective decrease in the responses mediated by the β_1 -adrenoceptor in hypothyroidism. In the present study, thyroxine increased the density of β -adrenoceptors and the associated mechanical responses. The reverse was observed in the hypothyroid aorta, as estimated from the maximum relaxation of the tissue, the IC₅₀, the B_{max} and synthesis of cyclic AMP. However, the K_D values remained the same in all three thyroid states. These results are thus consistent with those previously observed on the β -adrenoceptor in cardiac and other smooth muscle tissues.

In the hypothyroid rat, an increased number of α_1 -adrenoceptors was accompanied by an increase in the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PI-P₂) and the resulting synthesis of IP₃ under resting conditions. However, following agonist stimulation, time-dependent IP₃ synthesis was not significantly different, when compared with the control animals, whilst mechanical responses mediated by α_1 -adrenoceptor agonists were reduced. These discrepancies in the synthesis of IP₃ and the associated mechanical response may be explained by alterations in the functions of the sarco-

plasmic reticulum and myosin ATPase. Suko (1973) showed that Ca uptake and the hydrolysis of Ca-dependent myosin ATPase in cardiac muscles were markedly reduced in the hypothyroid state, presumably due to a deficiency of protein synthesis in this condition (Rovetto *et al.*, 1972; Yazaki & Raben, 1975).

In the hyperthyroid rat, a decreased α -adrenoceptor density together with a decreased basal level of IP₃, but a normal pattern of stimulated IP₃ synthesis, was observed. This was accompanied by an increased density of β -adrenoceptors, resulting in an enhanced production of cyclic AMP. These factors may combine to reduce the overall mechanical response. Cyclic AMP is known to accelerate Ca uptake into the sarcoplasmic reticulum and partly to inhibit the Ca-calmodulin-myosin light chain kinase (MLCK) complex through phosphorylation of MLCK by cyclic AMP-dependent protein kinase (Conti & Adelstein, 1981; Kuriyama *et al.*, 1982). In smooth muscle tissues, thyroid hormones have also been shown to have an inhibitory action on Ca-calmodulin binding, and this may also induce a reduction in myosin phosphorylation through inhibition of the Ca-calmodulin-MLCK complex (Hagiwara *et al.*, 1989). These results suggest that in the rat aorta, thyroid hormones negatively regulate the synthesis of the α -adrenoceptor but positively regulate the synthesis of the β -adrenoceptor.

The thyroid gland has multiple actions on cardiac and smooth muscle tissues, e.g. in cardiac ventricular but not atrial muscles, thyroid hormones regulate the actin-activated ATPase and isoenzyme patterns (Banerjee, 1983), and they also accelerate the accumulation of Ca into the sarcoplasmic reticulum through activation of Ca-ATPase (Suko, 1973). These actions may be related to an increase in cyclic AMP concentration (Suematsu *et al.*, 1985) by increasing the number of β -adrenoceptors. Furthermore, thyroid hormones also decrease the density of Ca channels (Hawthorn *et al.*, 1988). Moreover, additional multiple actions of thyroid hormones on cellular processes and components, including protein synthesis through nuclear transcription, mitochondrial activation and plasma membrane effects are well documented (Sterling, 1979; Oppenheimer, 1979; 1985).

In conclusion, thyroid hormones regulate cell functions including the distribution of α - and β -adrenoceptors in rat aorta. Thyroid hormones accelerate the production of the β -adrenoceptor with concomitant synthesis of cyclic AMP, while these hormones suppress the production of the α -adrenoceptor and thereby the synthesis of IP₃, without affecting the cellular process involved in their synthesis. A deficiency of thyroid hormones reverses the above sequence, again without affecting the enzymatic reactions that synthesize cyclic AMP or IP₃. An investigation of the molecular mechanisms involved in the action of thyroid hormones on the coding system in the nucleus is urgently needed.

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