Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 493 (2004) 191–199

www.elsevier.com/locate/ejphar

Acute hyperthyroidism alters adrenoceptor- and muscarinic receptor-mediated responses in isolated rat renal and femoral arteries

Takeshi Iwata[a], Hideo Honda[a,b,*]

[a] *Second Department of Physiology, School of Medicine, Showa University, Hatanodai 1-5-8, Shinagawa, Tokyo, 142-8555, Japan*
[b] *Department of Pharmacology, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1, Horinouchi, Hachioji, Tokyo 193-0392, Japan*

Received 27 November 2003; received in revised form 15 April 2004; accepted 21 April 2004

Abstract

The effects of acute hyperthyroidism on the vasorelaxing responses to isoprenaline and acetylcholine were investigated in isolated rat renal and femoral arteries. In the renal artery, isoprenaline- and acetylcholine-induced relaxations were significantly greater in hyperthyroid rats than in control rats. In the femoral artery, only the acetylcholine-induced relaxation was significantly greater in hyperthyroid rats than in control rats. In the renal artery, N^G-nitro-L-arginine (L-NOARG), an inhibitor of nitric oxide (NO) synthase, reduced isoprenaline- and acetylcholine-induced relaxations in both hyperthyroid and control rats and the isoprenaline-induced relaxation was still greater in hyperthyroid rats than in control rats, but no difference in the acetylcholine-induced relaxation was seen between the two groups of rats since L-NOARG almost abolished the acetylcholine-induced relaxation. In the femoral artery, L-NOAGR reduced the isoprenaline-induced relaxation in control rats but not in hyperthyroid rats, while it almost abolished the acetylcholine-induced relaxation in both groups of rats. 17-Octadecynoic acid (17-ODYA), a cytochrome P-450 monooxygenase inhibitor, reduced the isoprenaline-induced relaxation in renal and femoral arteries from hyperthyroid and control rats, but it did not change the acetylcholine-induced relaxation in both arteries. These results indicate that acute hyperthyroidism significantly enhances β-adrenoceptor-mediated relaxation of the renal artery and muscarinic receptor-mediated relaxation of both renal and femoral arteries, suggesting that these effects may be due to an alteration in the NO and cytochrome P-450 systems of the artery.

Keywords: Hyperthyroidism, acute; Adrenoceptor; Muscarinic receptor; Renal artery; Femoral artery

1. Introduction

Hyperthyroidism is associated with alterations in the cardiovascular system, such as an elevation in cardiac output and a decrease in vascular resistance (Klein, 1990; Levey and Klein, 1990; Polikar et al., 1993). It has been demonstrated that some of these changes are accompanied by physiological alterations. The heart is a major target organ for thyroid hormone action, and several investigators have shown that thyroid hormones can modulate the number of cardiac β-adrenoceptors (Williams et al., 1977; Bilezikian and Loeb, 1983; Tsujimoto and Hashimoto, 1986; Hawthorn et al., 1988). In vascular smooth muscle, the effects of β-adrenoceptors are heterogeneous, which may be explained by the different tissues and models used. In isolated aortae obtained from hyperthyroid rats, the relaxation induced by isoprenaline (Gunasekera and Kuriyama, 1990) or noradrenaline (O'Donnell et al., 1987) was reported to be enhanced compared with that in control aortae. Similar findings were observed in pulmonary artery preparations from L-thyroxine (T_4)-treated rats (O'Donnell and Wanstall, 1986; Zwaveling et al., 1996), although the responses to procaterol, a β_2-adrenoceptor agonist, were reduced. In coronary arteries from hyperthyroid rats, the vasodilator response to isoprenaline was reported to be enhanced (Miyazawa et al., 1989). In addition, the role of the endothelium in the modulation of vascular reactivity in the hyperthyroid state remains unclear,

* Corresponding author. Department of Pharmacology, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1, Horinouchi, Hachioji, Tokyo 193-0392, Japan. Tel./fax: +81-426-76-4529.
E-mail address: hhonda@ps.toyaku.ac.jp (H. Honda).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.04.033

but there are a few reports that endothelium-dependent relaxation responses are enhanced in hyperthyroid rats (Vargas et al., 1995; McAllister et al., 1998; Bussemaker et al., 2003).

We have found that isoprenaline- and acetylcholine-induced blood pressure responses are enhanced (Honda et al., 2002) by acute hyperthyroidism (T_4 treatment for 3 days) in isolated rat aorta (Honda et al., 2000). However, the synthesis of endothelium nitric oxide synthase (eNOS) in rat aorta is inhibited by T_4 administration (Grieve et al., 1999). It is also suggested that thyroid hormones alter several functions in the kidney (Bradley et al., 1974). T_4 treatment enhanced reactivity to phenylephrine, vasopressin and barium chloride in a perfused rat kidney preparation (Sabio et al., 1994). Thyroid hormone actions on hemodynamics have therapeutically desirable effects in the treatment of various cardiovascular diseases (Klemperer et al., 1996; Bettendorf et al., 2000). Therefore, understanding the mechanisms of action of thyroid hormone on the vasculature could be of clinical importance. To our knowledge, no one has considered the relaxing effects of β-adrenoceptor and muscarinic receptor agonists on the vasculature of the acute hyperthyroid rat. So, the purpose of this study was to determine and compare the vasorelaxant actions of isoprenaline and acetylcholine on vascular reactivity in renal and femoral arteries isolated from euthyroid and acute hyperthyroid rats.

2. Materials and methods

2.1. Animals and induction of acute hyperthyroidism

This investigation conformed with the Guide for the Care and Use of Laboratory Animals, published by the U.S. National Institute of Health (NIH Publication No. 85-23, revised 1996). Seven-week-old male rats of Wistar–Imamichi strain, which were supplied by Imamichi Institute for Animal Reproduction (Ibaraki, Japan), were used. The rats were maintained in temperature (23 ± 1 °C)-, humidity (55 ± 5%)- and light (12-h light/day)-controlled quarters, and were given rat chow and drinking water ad libitum. Acute hyperthyroidism was induced by s.c. injection of 0.5 mg/kg T_4 sodium in alkaline saline solution (0.001 N NaOH in 0.9% NaCl) at 9:00 a.m. daily for 3 days. Experiments were performed 24 h after the last injection.

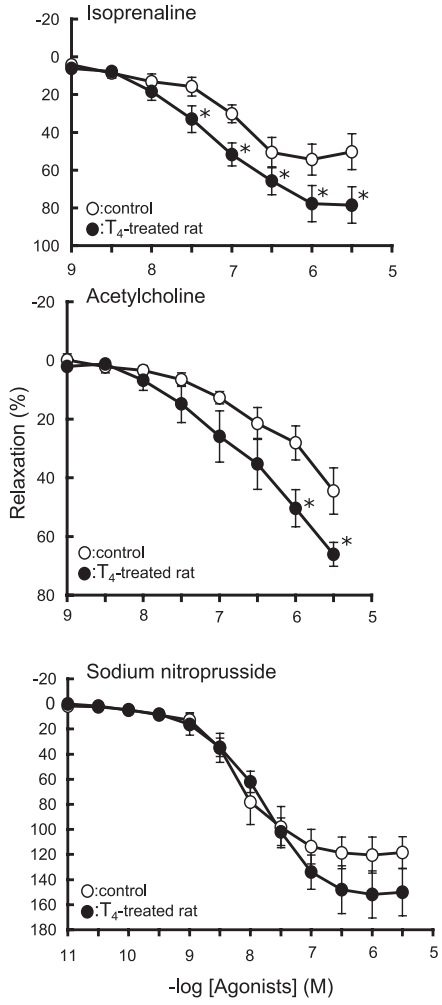

Fig. 1. Isoprenaline-, acetylcholine- and sodium nitroprusside-induced relaxation of renal artery from saline (○: control)- or thyroxine (●: T_4)-treated rats. Saline or thyroxine (500 μg/kg/ml) was injected s.c. for 3 days. Each value is the mean ± S.E.M. from seven to eight experiments. *$P < 0.05$ from control.

Control rats were injected with alkaline saline solution for the same periods of time.

2.2. Assessment of hyperthyroidism

A blood sample taken from the abdominal artery was centrifuged at 3000 rpm for 10 min and the serum sample was stored at −20 °C until assay. Serum T_4

Table 1
Morphological parameters in control and hyperthyroid rats

Group	GBW (g)	TW (mg/100 g)	HW (mg/100 g)	HR (beats/min)	Serum T_4 (ng/ml)
Control	18.8 ± 1.3	7.59 ± 0.28	316.2 ± 7.0	294.5 ± 8.8	26.0 ± 2.9
T_4-treated rat	6.5 ± 2.2[a]	5.36 ± 0.22[b]	381.1 ± 9.8[b]	351.2 ± 9.8[b]	55.3 ± 10.3[b]

GBW = gain in body weight, TW = thyroid weight, HW = heart weight.
Each value is the mean ± S.E.M. from six to eight rats.
[a] $p < 0.05$ from control.
[b] $p < 0.01$ from control.

Table 2
Values of − log (ED_{50}) and maximal relaxation in renal artery or femoral artery

	− Log (ED_{50})	Maximum (%)
Renal artery		
Isoprenaline		
Control	6.95 ± 0.06	58.4 ± 8.1
T_4-treated rat	7.30 ± 0.14[a]	83.5 ± 8.0[a]
Acetylcholine		
Control	6.36 ± 0.15	44.5 ± 7.9
T_4-treated rat	6.90 ± 0.20[a]	66.3 ± 3.9[a]
Sodium nitroprusside		
Control	8.13 ± 0.10	120.1 ± 14.3
T_4-treated rat	7.95 ± 0.24	150.1 ± 18.8
Femoral artery		
Isoprenaline		
Control	6.88 ± 0.11	88.5 ± 9.6
T_4-treated rat	7.03 ± 0.24	90.4 ± 7.0
Acetylcholine		
Control	6.27 ± 0.11	37.7 ± 6.8
T_4-treated rat	6.92 ± 0.29[a]	63.4 ± 11.7[a]
Sodium nitroprusside		
Control	8.40 ± 0.13	118.2 ± 5.1
T_4-treated rat	8.51 ± 0.11	141.5 ± 27.6

Each value is the mean ± S.E.M. from six to eight rats.
[a] $p < 0.05$ from control.

levels were determined by radioimmunoassay with a commercially available conventional double-antibody technique. Heart and thyroid weights are expressed relative to body weight (mg/100 mg body weight) (Honda et al., 2000).

2.3. Measurement of isometric tension

The renal and femoral arteries were isolated and placed in modified Krebs–Henseleit solution (pH 7.4) with the following composition (mM): NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; glucose, 11 at 37 °C the solution was gassed with 95% O_2 and 5% CO_2. The tissue was cleaned by removing connective tissue. The renal and femoral arteries were cut into rings about 3 mm long. Contraction and relaxation were measured by suspending the rings between two stainless-steel hooks, one of which was attached to the end of a bathing tube and the other was connected to a force transducer (45196A NEC San-ei, Japan). Isometric tension changes were recorded on a polygraph (LECT-HORIZ-8K NEC San-ei) as previously described (Tamura et al., 1997).

2.4. Relaxation of renal and femoral arteries precontracted with noradrenaline

Each preparation was equilibrated in 10 ml of bathing solution for 90–120 min before the experiment. The resting tension was 0.7 g. This was found to be the optimal preload for force development in these blood vessels in preliminary studies (Honda et al., 1999; Unemoto et al., 2003). After equilibration, the rings were exposed to KCl (50 mM). When the contractile responses plateaued, the rings were rinsed with the solution and allowed to equilibrate for an additional 60 min before the application of noradrenaline (300 nM). For the relaxation studies, submaximal tone (approximately 80% of the maximum tone) was induced with noradrenaline (300 nM) and then isoprenaline, acetylcholine or sodium nitroprusside was added in a cumulative fashion, and the relaxing effects were compared between rings isolated from control rats and rats treated with T_4. Endothelial integrity was confirmed with acetylcholine. The responses are expressed as the percent relaxation of noradrenaline-induced tone, and the relaxation in the absence of the

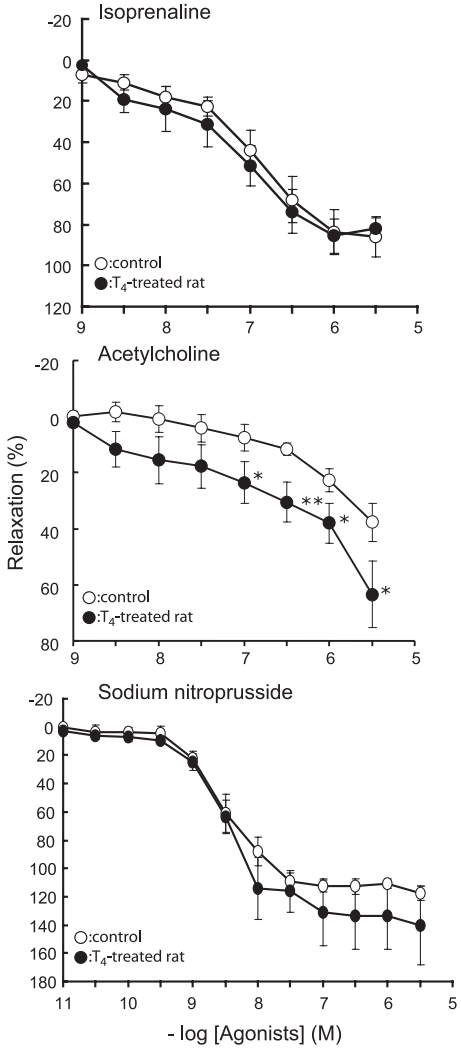

Fig. 2. Isoprenaline-, acetylcholine- and sodium nitroprusside-induced relaxation of femoral artery from saline (○: control) or thyroxine (●: T_4)-treated rats. Saline or thyroxine (500 μg/kg/ml) was injected s.c. for 3 days. Each value is the mean ± S.E.M. from six to eight experiments. *$P < 0.05$, **$P < 0.01$ from control.

drugs was taken as 0% (Ampong et al., 2002; Unemoto et al., 2003).

2.5. Effects of cyclo-oxygenase inhibitor, NO inhibitor or cytochrome P-450 mono-oxygenase inhibitor on isoprenaline- and acetylcholine-induced relaxation

To observe the effects of isoprenaline- or acetylcholine-induced relaxation, indomethacin, an inhibitor of cyclo-oxygenase, N^G-nitro-L-arginine (L-NOARG), an inhibitor of nitric oxide (NO) synthase, or 17-octadecynoic acid (17-ODYA), a cytochrome P-450 mono-oxygenase inhibitor, was added to the solution 10 min before treatment with noradrenaline.

2.6. Drugs and chemicals

T_4 sodium (Sigma, St. Louis, MO, USA) was dissolved in alkaline saline solution (0.001 N NaOH in 0.9% NaCl). Noradrenaline hydrochloride, isoprenaline, acetylcholine chloride, sodium nitroprusside, L-NOARG and 17-ODYA (Sigma) were dissolved in distilled water. Indomethacin (Sigma) was dissolved in 4% (weight/volume) $NaHCO_3$. Other chemicals were of analytical grade and obtained from Wako (Osaka, Japan).

2.7. Statistical analysis

Values are expressed or plotted as mean ± S.E.M. and statistical analysis was done with Student's *t*-test. Differences were considered significant at $P<0.05$.

3. Results

3.1. Effects of T_4 treatment on biological parameters of rats

Table 1 summarizes the effect of T_4 treatment on the various parameters monitored. The growth rate (body weight gain) was significantly inhibited with respect to that of control animals. Heart rate, heart weight and serum T_4 levels were significantly increased but thyroid weight was significantly decreased by T_4 treatment.

3.2. Relaxation induced by isoprenaline, acetylcholine and sodium nitroprusside

Isoprenaline (10^{-9}–3×10^{-6} M), acetylcholine (10^{-9}–3×10^{-6} M) and sodium nitroprusside (10^{-11}–3×10^{-6} M) induced dose-dependent vascular relaxation in renal and femoral arteries precontracted with noradrenaline in

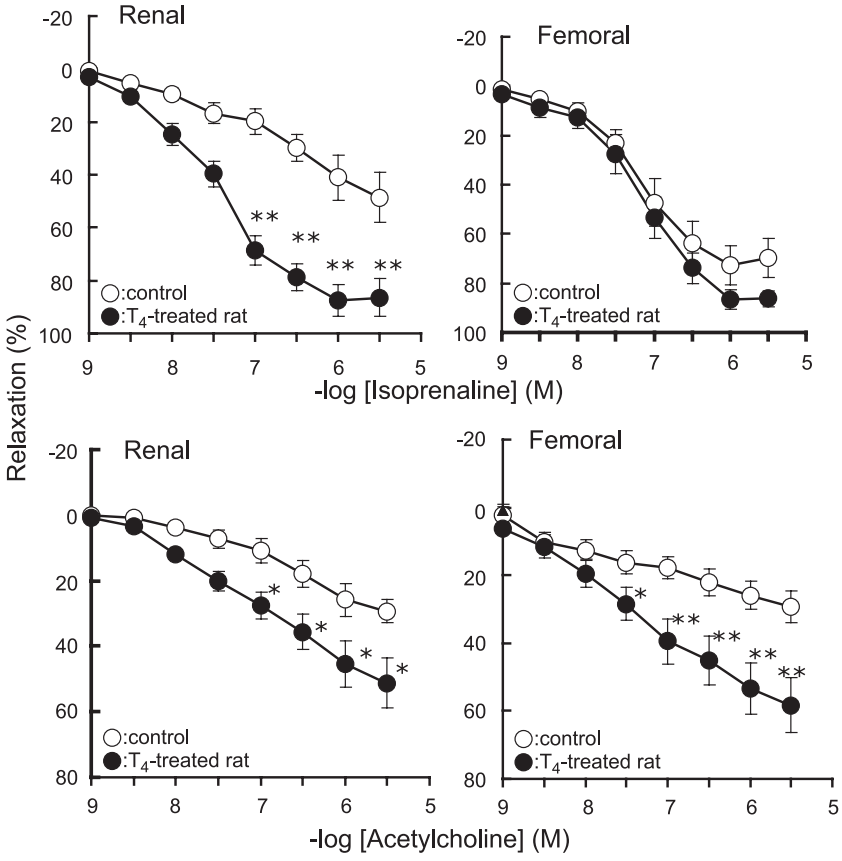

Fig. 3. Effect of indomethacin on isoprenaline- and acetylcholine-induced relaxation of renal artery or femoral artery from saline (○: control) or thyroxine (●: T_4)-treated rats. Saline or thyroxine (500 μg/kg/ml) was injected s.c. for 3 days. Indomethacin (10^{-4} M) was added 10 min before noradrenaline. Each value is the mean ± S.E.M. from seven to eight experiments. *$P<0.05$, **$P<0.01$ from control.

both T_4-treated and control rats (Figs. 1 and 2). Isoprenaline-induced relaxation of the renal artery was significantly greater in T_4-treated rats than in control rats (Fig. 1). The $-\log(ED_{50})$ values and the maximal relaxation are shown in Table 2. The $-\log(ED_{50})$ value of isoprenaline was significantly higher and the maximal relaxation was significantly greater in T_4-treated rats than in control rats, respectively. There were no significant differences in isoprenaline-induced relaxation of the femoral artery between T_4-treated and control rats (Fig. 2, Table 2).

Acetylcholine-induced relaxation of the renal and femoral arteries was significant greater in T_4-treated rats than in the control rats (Figs. 1 and 2). The $-\log(ED_{50})$ values of acetylcholine in the renal and the femoral arteries were significantly higher in T_4-treated rats than in control rats. The maximal relaxation was significantly greater in the renal and femoral arteries of T_4-treated rats than of control rats.

There were no significant differences in sodium nitroprusside-induced relaxation of renal and femoral arteries between T_4-treated and control rats (Figs. 1 and 2, Table 2).

3.3. Effects of cyclo-oxygenase inhibitor on isoprenaline- and acetylcholine-induced relaxation

Pretreatment with indomethacin (10^{-4} M) did not affect isoprenaline- and acetylcholine-induced relaxation of the renal and femoral arteries (Fig. 3). The $-\log(ED_{50})$ values and the maximal relaxation are shown in Table 3. The increase induced by T_4 treatment in $-\log(ED_{50})$ values and the maximum relaxation caused by isoprenaline in the renal artery and by acetylcholine in both arteries remained in the presence of indomethacin (Table 3).

3.4. Effects of NO inhibitor on isoprenaline- and acetylcholine-induced relaxation

Pretreatment with L-NOARG (10^{-4} M) reduced isoprenaline-induced relaxation of the renal artery from both T_4-treated and control rats, and the relaxation induced by isoprenaline was still greater in T_4-treated rats than in control rats (Fig. 4). The $-\log(ED_{50})$ value of isoprenaline was significantly higher and the maximal relaxation was significantly greater in T_4-treated rats than in control rats (Table 3). L-NOARG reduced the isoprenaline-induced relaxation of the femoral artery from T_4-treated or control rats and the reduction was greater in control rats than in T_4-treated rats. So, an enhancement by T_4 treatment of isoprenaline-induced relaxation was observed in the femoral artery (Fig. 4). In both arteries, the $-\log(ED_{50})$ value of isoprenaline was significantly higher and the maximal relaxation was significantly greater in arteries from T_4-treated rats than in arteries from control rats (Table 3).

L-NOARG almost abolished the acetylcholine-induced relaxation of the renal and femoral arteries from both T_4-treated rats and control rats (Fig. 4). There were no significant differences in the $-\log(ED_{50})$ values and maximal relaxation induced by acetylcholine in the renal

Table 3
Values of $-\log(ED_{50})$ and maximal relaxation in renal artery or femoral artery

Renal artery	$-$ Log (ED_{50})	Maximum (%)	Femoral artery	$-$ Log (ED_{50})	Maximum (%)
Isoprenaline			*Isoprenaline*		
+ Indomethacin			+ Indomethacin		
Control	6.70 ± 0.21	49.9 ± 8.6	Control	7.08 ± 0.09	74.2 ± 7.8
T_4-treated rat	7.45 ± 0.08[a]	88.2 ± 6.3[a]	T_4-treated rat	7.15 ± 0.14	88.1 ± 3.1
+ L-NOARG			+ L-NOARG		
Control	6.83 ± 0.17	41.6 ± 2.7	Control	6.62 ± 0.10	61.2 ± 7.5
T_4-treated rat	7.23 ± 0.13[b]	52.0 ± 3.1[a]	T_4-treated rat	6.91 ± 0.11[a]	84.7 ± 9.4[a]
+ 17-ODAY			+ 17-ODAY		
Control	6.72 ± 0.09	44.2 ± 5.9	Control	6.65 ± 0.12	51.0 ± 5.9
T_4-treated rat	6.65 ± 0.14	47.2 ± 7.3	T_4-treated rat	6.78 ± 0.14	53.1 ± 4.2
Acetylcholine			*Acetylcholine*		
+ Indomethacin			+ Indomethacin		
Control	6.60 ± 0.18	31.2 ± 3.6	Control	7.11 ± 0.12	30.0 ± 4.5
T_4-treated rat	7.05 ± 0.14[a]	52.0 ± 7.7[a]	T_4-treated rat	7.43 ± 0.13[a]	58.3 ± 9.0[b]
+ L-NOARG			+ L-NOARG		
Control	6.29 ± 0.10	17.0 ± 2.5	Control	6.51 ± 0.24	24.5 ± 2.4
T_4-treated rat	6.48 ± 0.13	17.5 ± 1.8	T_4-treated rat	6.52 ± 0.27	29.4 ± 6.5
+ 17-ODAY			+ 17-ODAY		
Control	6.21 ± 0.09	41.0 ± 8.9	Control	6.30 ± 0.15	27.4 ± 5.1
T_4-treated rat	6.57 ± 0.17[a]	66.1 ± 7.0[a]	T_4-treated rat	6.57 ± 0.16	61.4 ± 7.4[b]

Each value is the mean ± S.E.M. from six to eight experiments.
[a] $P < 0.01$ from control.
[b] $P < 0.05$ from control.

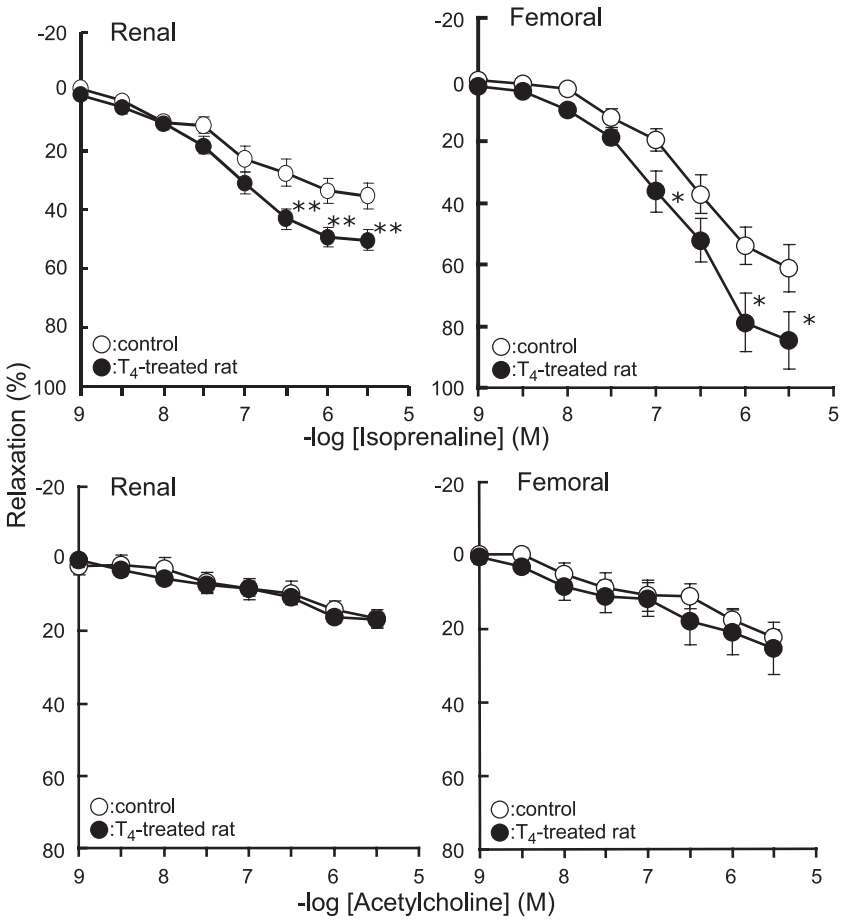

Fig. 4. Effect of L-NOARG on isoprenaline- and acetylcholine-induced relaxation of renal artery or femoral artery from saline (○: control) or thyroxine (●: T_4)-treated rats. Saline or thyroxine (500 μg/kg/ml) was injected s.c. for 3 days. L-NOARG (10^{-4} M) was added 10 min before noradrenaline. Each value is the mean ± S.E.M. from seven to eight experiments. *$P<0.05$, **$P<0.01$ from control.

and femoral arteries between T_4-treated and control rats (Table 3).

3.5. Effects of P-450 mono-oxygenase inhibitor on isoprenaline- and acetylcholine-induced relaxation

In the renal and femoral arteries, pretreatment with 17-ODYA (10^{-4} M) reduced the isoprenaline-induced relaxation in both T_4-treated and control rats (Fig. 5). The reduction was greater in T_4-treated rats than in control rats. So, the enhancement by T_4 treatment of the isoprenaline-induced relaxation of the renal artery was abolished in the presence of 17-ODYA. There were no significant differences in the isoprenaline-induced relaxation of the renal and femoral arteries between T_4-treated and control rats. The $-\log (ED_{50})$ values and the maximal relaxation induced by isoprenaline were not altered by T_4 treatment (Table 3). In contrast with isoprenaline, 17-ODYA did not change the acetylcholine-induced relaxation in the renal and femoral arteries, and in the presence of 17-ODYA, the enhanced relaxation induced by T_4 treatment remained in both arteries (Fig. 5). The maximal relaxation is given in Table 3.

4. Discussion

We have previously reported that T_4 treatment (0.5 mg/kg/days) for 3 days is sufficient to induce a significant degree of thyroid weight loss, tachycardia, cardiac hypertrophy and elevation of serum T_4 levels (Honda et al., 2000, 2001). In this study, we also confirmed that T_4 treatment for 3 days could produce acute hyperthyroidism in rats. The present results demonstrated that T_4 treatment for 3 days significantly enhanced the isoprenaline-induced relaxation of the renal artery, while it did not affect the isoprenaline-induced relaxation of the femoral artery. The effects of thyroid hormones on β-adrenoceptor-mediated responses in various tissues are frequently opposite. It was therefore of interest to compare the effect of T_4 treatment on rat tissues with different β-adrenoceptor populations. Rat aorta and pulmonary artery both contain β-adrenoceptor subtypes, with the β_2-subtype predominating (O'Donnell and Wanstall, 1981, 1986). Furthermore, the rat vas deferens has a homogeneous population of β_2-adrenoceptors (Krstew et al., 1982; May et al., 1985), while the rat trachea contains both β-adrenoceptor subtypes, with the β_1-subtype predominating (Henry et al., 1981). In addition, no change in the

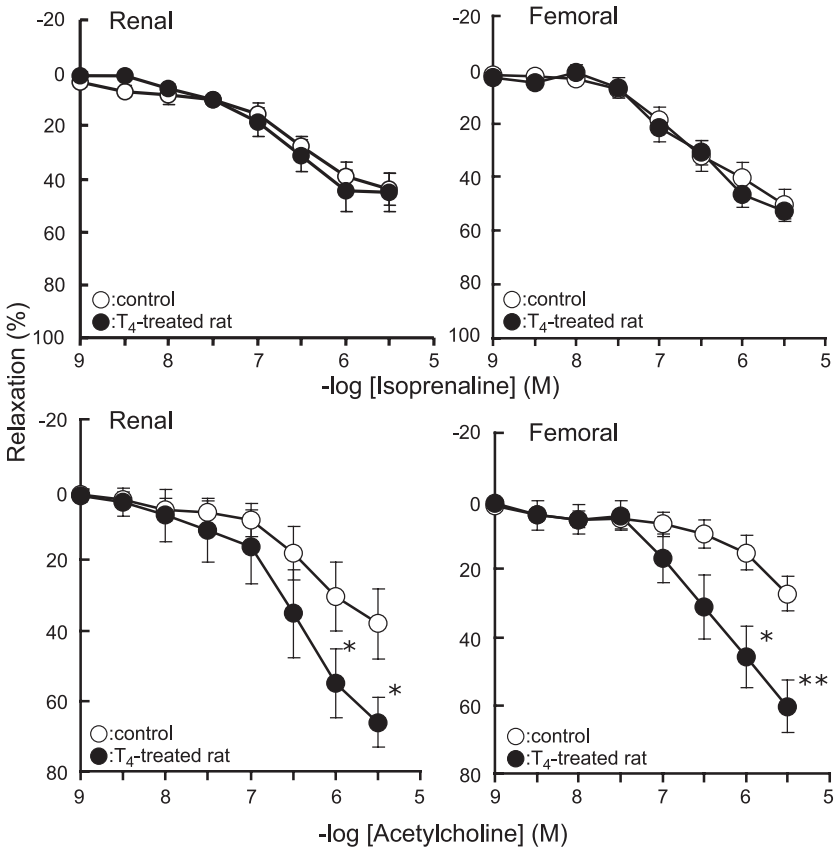

Fig. 5. Effect of 17-ODYA on isoprenaline- and acetylcholine-induced relaxation of renal artery or femoral artery from saline (○: control) or thyroxine (●: T_4)-treated rats. Saline or thyroxine (500 μg/kg/ml) was injected s.c. for 3 days. 17-ODAY (10^{-4} M) was added 10 min before noradrenaline. Each value is the mean ± S.E.M. from seven to nine experiments. *$P<0.05$, **$P<0.01$ from control.

number of β-adrenoceptors was found in mesenteric arteries from hyperthyroid rats (Tsujimoto and Hashimoto, 1986; Tsujimoto et al., 1987). It appears that the effect of thyroid hormones on β-adrenoceptor-mediated responses also differs quantitatively among tissues.

The acetylcholine-induced relaxation of the renal and femoral arteries was significantly greater in T_4-treated rats than in control rats, while no significant difference in sodium nitroprusside-induced relaxation was found between T_4-treated rats and control rats. We have previously reported that acetylcholine-induced vasorelaxation of the thoracic aorta is enhanced by T_4 treatment; however, the amount of endothelial NOS (eNOS) protein in the thoracic aorta was not influenced by T_4 treatment for 3 days (Honda et al., 2000). Considering these results, the enhancement of the activity of NO synthase (NOS) may be in part due to shear stress through an increase in blood flow. Indeed, it is generally accepted that blood flow in the skeletal muscle (Frery, 1967; Martin et al., 1992; McAllister et al., 1995) and the kidney (Govind et al., 1994) is elevated in hyperthyroid humans and rats. It is suggested that muscarinic mediated vasorelaxation responses are significantly increased in an early stage of hyperthyroidism and may be involved in the change in blood flow.

The cyclo-oxygenase inhibitor indomethacin did not alter the isoprenaline- or acetylcholine-induced relaxation of the renal and femoral arteries. These results suggest that the isoprenaline- or acetylcholine-induced relaxation of rat renal and femoral arteries dose not involve relaxant prostanoids, including prostacyclin I_2. The eNOS inhibitor L-NOARG inhibited the isoprenaline-induced relaxation of the renal artery from both T_4-treated and control rats, and the relaxation induced by isoprenaline was greater in T_4-treated rats than in control rats. These findings suggest that isoprenaline stimulates two different kinds of relaxing factors: the L-NOARG-sensitive factors NO and the L-NOARG-insensitive factor endothelium-derived hyperpolarizing factor (EDHF). EHDF is involved in the vasorelaxation induced by acetylcholine and its action is resistant to L-NOARG and indomethacin (Cohen and Vanhoutte, 1995; Garland et al., 1995). Although the nature of EDHFs has not yet been elucidated, recent studies indicate that one of EDHF may be a cytochrome P-450 mono-oxygenase-arachidonic acids metabolite (Hecker et al., 1994; Fulton et al., 1995; Garland et al., 1995; Lischke et al., 1995; Campbell et al., 1996; Honda et al., 2001). 17-ODYA, which is an irreversible inhibitor of the long-chain fatty acid metabolizing cytochrome P-450 enzyme (Harder et al., 1995), markedly inhibited the

N^G-nitro-L-arginine methyl ester, an inhibitor of NO synthase/indomethacin-insensitive response to acetylcholine in the rabbit carotid artery (Oyekan et al., 1994). In the present study, we showed that 17-ODYA inhibited the isoprenaline-induced relaxation of the renal artery, and that there was no significant difference in the response between arteries from T_4-treated and control rats. These results suggest that the isoprenaline-induced relaxation of the renal artery is significantly increased in an early stage of hyperthyroidism through the cytochrome P-450 system in the artery. In the femoral artery, the isoprenaline-induced relaxation was not influenced by T_4 treatment; however, in the presence of L-NOARG, the isoprenaline-induced relaxation was greater in T_4-treated rats than in control rats. 17-ODYA inhibited the isoprenaline-induced relaxation of the femoral artery and no significant difference was seen in the isoprenaline-induced relaxation of arteries from T_4-treated and control rats. These results suggest that the isoprenaline-induced relaxation of the femoral artery is mediated by the release of both NO and cytochrome P-450 metabolites, and that NO-dependent relaxation of the femoral artery is increased in an early stage of hyperthyroidism. L-NOARG abolished the acetylcholine-induced relaxation of the renal and femoral arteries from both hyperthyroid rats and control rats. These results suggest that acetylcholine-induced relaxation is mainly mediated by the release of NO, and that NO-dependent relaxation is increased in an early stage of hyperthyroidism in both the renal and femoral arteries. Recently, Bussemaker et al. (2003) indicated that hyperthyroidism enhanced endothelium-dependent relaxing responses induced by acetylcholine in the rat renal artery, most probably by increasing the vascular cyclic AMP content. They carried out mainly biochemical/immunochemical experiments in addition to a functional assay, while the present study focused on vascular function. In contrast with our results, Bussemaker et al. indicated that an enhanced acetylcholine-induced relaxation still remained in the presence of L-NOARG, a NOS inhibitor. The reason for this difference is not immediately obvious. However, it may be due to a difference in disease stage, since their acute hyperthyroidism model was induced by a single injection of triiodothyronine (T_3) and it had no influence on heart weight and body weight gain, in contrast with our present model. It may be also due to strain differences since they used Wistar–Kyoto rats.

In conclusion, we have demonstrated that isoprenaline-induced relaxation of the renal artery is greater in T_4-treated rats than in control rats, and suggest that this may be due to an alteration in cytochrome P-450 systems in T_4-treated rats. The isoprenaline-induced relaxation of the femoral artery is dependent on NO in an early stage of hyperthyroidism. The acetylcholine-induced relaxation of the renal and femoral arteries is greater in T_4-treated rats than in control rats, and may be mediated by increased NO in the arteries.

Acknowledgements

We wish to thank Dr. J. Taniguchi (Department of Internal Medicine, Tokyo Medical University) for great help.

References

Ampong, B., Honda, H., Kogo, H., 2002. Beta-adrenoceptor-mediated relaxation in the rat thoracic aorta. Vasc. Pharmacol. 38, 149–155.

Bettendorf, M., Schmidt, K.G., Grulich-Henn, J., Ulmer, H.E., Heinrich, U.E., 2000. Tri-iodothyronine treatment in children after cardiac surgery: a double-blind randomised, placebo-controlled study. Lancet 356, 529–534.

Bilezikian, J.P., Loeb, J.N., 1983. The influence of hyperthyroidism and hypothyroidism on α- and β-adrenergic receptor systems and adrenergic responsiveness. Endocr. Rev. 4, 378–388.

Bradley, S.E., Stephan, F., Coelho, J.B., Reville, P., 1974. The thyroid and the kidney. Kidney Int. 6, 346–365.

Bussemaker, E., Popp, R., Fisslthaler, B., Larson, C.M., Fleming, I., Busse, R., Brandes, R.P., 2003. Hyperthyroidism enhances endothelium-dependent relaxation in the rat renal artery. Cardiovasc. Res. 1, 181–188.

Campbell, W.B., Gebremedhin, D., Pratt, P.F., Harder, D.R., 1996. Identification of epoxyeicosatrienoic acid as endothelium-derived hyperpolarizing factors. Circ. Res. 78, 415–423.

Cohen, R.A., Vanhoutte, P.M., 1995. Endothelium-dependent hyperpolarization. Beyond nitric oxide and cyclic GMP. Circulation 92, 3337–3349.

Frery, H.M., 1967. Peripheral circulatory and metabolic consequences of thyroxicosis: 1. Blood flow and oxygen consumption of resting and working skeletal muscle in experimental thyrotoxicosis in the dog. Scand. J. Clin. Lab. Invest. 19, 4–14.

Fulton, D., Mahboubi, K., McGiff, J.C., Quilley, J., 1995. Cytochrome P450-dependent effects of bradykinin in the rat heart. Br. J. Pharmacol. 114, 99–102.

Garland, C.J., Plane, F., Kemp, B.K., Cocks, T.M., 1995. Endothelium-dependent hyper polarization: a role in the control of vascular tone. Trends Pharmacol. Sci. 16, 23–30.

Govind, S., Avadhesh, C.S., Emmanuel, B.T., Anil, G., 1994. Renal endothelium mechanism in altered thyroid sate. Life Sci. 54, 1901–1908.

Grieve, D.J., Fletcer, S., Pitsillidis, A.A., Botham, K.M., Elliott, J., 1999. Effect of oral propylthiouracil treatment on nitric oxide production in rat aorta. Br. J. Pharmacol. 127, 1–8.

Gunasekera, R.D., Kuriyama, H., 1990. The influence of thyroid states upon responses of the rat aorta to catecholamines. Br. J. Pharmacol. 99, 541–547.

Harder, D.R., Campbell, W.B., Roman, R.J., 1995. Role of cytochrome P-450 enzymes and metabolites of arachidonic acid in the control of vascular tone. J. Vasc. Res. 32, 79–92.

Hawthorn, M.H., Gengo, P., Wei, X.Y., Rutledge, A., Moran, J.F., Gallant, S., Triggle, D.J., 1988. Effects of thyroid status on beta-adrenoceptors and calcium channels in rat cardiac and vascular tissue. Naunyn-Schmiedeberg's Arch. Pharmacol. 337, 539–544.

Hecker, M., Bara, A.T., Bauersachs, J., Busse, R., 1994. Characterization of endothelium-derived hyperpolarizing factor as a cytochrome P450-derived arachidonic acid metabolites in mammals. J. Physiol. 481, 407–414.

Henry, P.J., Lulich, K.M., Paterson, J.W., 1981. Classification of beta-adrenoceptors in isolated rat trachea. Clin. Exp. Pharmacol. Physiol. 8, 622.

Honda, H., Unemoto, T., Kogo, H., 1999. Different mechanisms for testosterone-induced relaxation of aorta between normotensive and spontaously hypertensive rats. Hypertension 341, 1232–1236.

Honda, H., Iwata, T., Mochizuki, T., Kogo, H., 2000. Changes in vascular reactivity induced by acute hyperthyroidism in isolated rat aorta. Gen. Phamacol. 34, 429–434.

Honda, H., Moroe, H., Fujii, H., Arai, K., Notoya, Y., Kogo, H., 2001. Short term hypercholesterolemia artery N^G-nitro-L-arginin- and indomethacin-resistant endothelium-dependent relaxation by acetylcholine in rabbit renal artery. Jpn. J. Pharmacol. 85, 203–206.

Honda, H., Iwata, T., Mochizuki, T., Kogo, H., 2002. A fluctuation in adrenoceptor- and muscarinic receptor-mediated blood pressure responses in acute hyperthyroid rats. Vasc. Pharmacol. 40, 1–6.

Klein, I., 1990. Thyroid hormone and blood pressure regulation. In: des Laragh, J.H., Brenner, B.M. (Eds.), Hypertension: Pathophysiology, Diagnosis, Management. Raven Press, New York, pp. 1661–1672.

Klemperer, J., Ojamaa, K., Klein, I., 1996. Thyroid hormone therapy in cardiovascular disease. Prog. Cardiovasc. Dis. 38, 329–336.

Krstew, E., Malta, E., Raper, C., 1982. Comparison of guinae pig uterine and rat vas deferens preparations for assessment of β_2-adrenoceptor-mediated activity. J. Pharmacol. Methods 8, 279–289.

Levey, G.S., Klein, I., 1990. Catecholamine-thyroid hormone interactions and the cardiovascular manifestations of hyperthyroidism. Am. J. Med. 88, 664–670.

Lischke, V., Busse, R., Hecker, M., 1995. Selective inhibition by barbitutates of the synthesis of endothelium-derived hyperpolarizing factor in the rabbit carotid artery. Br. J. Pharmacol. 115, 969–974.

Martin, W.H., Korte, E., Tolley, T.K., Saffitz, J.E., 1992. Skeletal muscle β-adrenoceptor distribution and responses to isoproterenol in hyperthyrodism. Am. J. Physiol. 262, E504–E510.

May, J.M., Abel, P.W., Minneman, K.P., 1985. Binding of agonists and antagonists to β-adrenoceptors in rat vas deferns: relationship to functional response. Naunyn-Schmiedeberg's Arch. Pharmacol. 331, 324–333.

McAllister, R.M., Sansone Jr., J.C., Laughlin, M.H., 1995. Effects of hyperhtyrodism on muscle blood flow during exercise in rats. Am. J. Physiol. 268, H330–H335.

McAllister, R.M., Grossenburg, V., Delp, M.D., Laughlin, M.H., 1998. Effects of hyperthyroidism on vascular contractile and relaxation res ponse. Am. J. Physiol., E946–E953.

Miyazawa, K., Hashimoto, H., Nakashima, M., 1989. Altered responses of coronary arteries in hyperthyroid rats. Arch. Int. Prarmacodyn. Ther. 297, 120–132.

O'Donnell, S.R., Wanstall, J.C., 1981. Demonstration of both β_1- and β_2-adrenoceptors mediating orelaxation of isolated ring preparations of rat-pulmonary artery. Br. J. Pharmacol. 74, 547–552.

O'Donnell, S.R., Wanstall, J.C., 1986. Thyroxine treatment of aged or young rats demonstrates that vascular responses mediated by β-adrenoceptor subtypes can be differentially regulated. Br. J. Pharmacol. 88, 41–49.

O'Donnell, S.R., Wanstall, J.C., Mustafa, M.B., 1987. Influence of thyroid states on responses of rat isolated pulmonary artery, vas deferens and trachea to smooth muscle relaxant drugs. Br. J. Pharmacol. 92, 221–229.

Oyekan, A.O., McGift, J.C., Rosencrantz, W.P., Quilley, J., 1994. Relaxant responses of rabbit aorta: influence of cytochrome P-450 inhibitors. J. Pharmacol. Exp. Ther. 268, 262–269.

Polikar, R., Burger, A.G., Scherrer, U., Nicod, P., 1993. The thyroid and the heart. Circulation 87, 1435–1441.

Sabio, J.M., Luna, M.R., Cipriano, G.R., Vargas, F., 1994. Vascular reactivity to vasoconstrictors in aorta and renal vasculature of hyperthyroid and hypothyroid rats. Pharmacology 49, 257–264.

Tamura, K., Honda, H., Mimaki, Y., Sashida, Y., Kogo, H., 1997. Inhibitory effect of a new steroidal saponin, OSW-1, on ovarian functions in rats. Br. J. Pharmacol. 121, 1796–1802.

Tsujimoto, G., Hashimoto, K., 1986. A unique pressor response to isoprenaline in the pithed rat during triiodo-L-thyronine (T_3)-induced hyperthyrodism. Naunyn-Schmiedeberg's Arch. Pharmacol. 334, 138–144.

Tsujimoto, G., Hashimoto, K., Hoffman, B.B., 1987. Effects of thyroid hormone on beta-adrenergic responsiveness of aging cardiovascular system. Am. J. Physiol. 252, H516–H520.

Unemoto, T., Honda, H., Kogo, H., 2003. Differences in the mechanisms for relaxation of aorta induced by 17β-estradiol or progesterone between normotensive and hypertensive rats. Eur. J. Pharmacol. 472, 119–126.

Vargas, F., Fernadez-Rivas, A., Estan, J.G., Rio, C.G., 1995. Endothelium-dependent and endothelium-independent vasodilatation in hyperthyroid rats and hypothyroid rats. Pharmacology 51, 308–314.

Williams, L.T., Lefkowitz, R.J., Watanabe, A.M., Hathaway, D.R., Besch Jr., H.R., 1977. Thyroid hormone regulation of beta-adrenergic receptor number. J. Biol. Chem. 252, 2787–2789.

Zwaveling, J., Winkler, J., Prins, E.A., Pfaffendorf, M., Zwieten, P.A., 1996. The influence of hyperthyrodism on β-adrenocepter-mediated relaxation of isolated small mesenteric arteries. Naunyn-Scamiedebarg's Arch. Pharmacol. 353, 438–444.