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Vascular Responses to Vasoactive Drugs in Propylthiouracil-Treated Rat Aorta

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ABSTRACT

The vascular responses to the vasoactive drugs were evaluated using aortic ring preparations obtained from propylthiouracil (PTU)-treated rats.

The body weights and the levels of serum thyroxine (T_4) and triiodothyronine (T_3) were significantly decreased in propylthiouracil-treated rats as compared with those in age-matched control rats.

The contractile responses to norepinephrine and potassium and calcium ions were significantly attenuated in aortic rings of PTU-treated rats 4 weeks after when compared with those from age-matched control animals. By the PTU treatment, however, the sensitivity to norepinephrine but not to calcium was decreased while the maximal responses to norepinephrine and calcium were reduced together. The attenuated contractile responses to the vasoconstrictors in PTU-treated rats are ascribed to the decreased ability of the muscle cells to contract.

On the other hand, the relaxation responses induced by acetylcholine and histamine (endothelium-dependent relaxants) and isoproterenol and sodium nitroprusside (endothelium-independent relaxants) had tendencies to be augmented in aortic rings of PTU-treated rats when compared with those of age-matched control animals. However, the sensitivities to the endothelium-independent relaxants were different between PTU-treated and control rats whereas those to the endothelium-dependent relaxants were not.

These results suggest that the altered vascular responsiveness in the PTU-treated rats seems to be due to the alteration of smooth muslce cells rather than the influence of endothelium, and that this change is slowly progressive after hypothyroidism is evident.

Key Words: Vasoactive Drugs, Propylthiouracil-treated, Endothelium, Rat aorta
Abbreviations: PTU, Propylthiouracil

INTRODUCTION

Clinically, hypothyroidism brings about a reduction in cardiac output, stroke volume, heart rate, blood pressure, and pulse pressure (Colucci and Braunwald, 1987). Frequent manifestation in hypothyroidism is hypercholesterolemia which accelerates the development of atherosclerosis (Brown and Goldstein, 1985).

Experimentally, cholesterol feeding impairs endothelium-dependent relaxation of rabbit aorta and damage to endothelial cells is a consistent feature (Jayakody *et al.*, 1985). These findings led to the question whether hypothyroidism might produce an impairment of endothelium-dependent relaxation or not.

There have been few studies on the effects of thyroid status on the vascular responses to the vasoactive drugs in connection with endothelium. With these considerations, this study was designed to evaluate the effects of thyroid status on the

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vascular responses to endothelium-dependent and -independent vasoactive drugs in PTU-treated and control rat aorta.

MATERIALS AND METHODS

PTU treatment

Female 6-week-old Sprague-Dawley rats weighing 120-150g were used in this study. Rats were rendered hypothyroid by maintenance on a drinking water containing 0.05% propylthiouracil for 4 and 12 weeks. Control rats were litter mates or rats of the same weight as the experimental animals and were maintained on laboratory chow and tap water ad libitum.

Serum triiodothyronine and thyroxine assays

Under anesthesia with sodium pentobarbital (35 mg/kg i.p.), the blood was obtained from abdominal aorta. After centrifugation (3000 rpm for 10 min), the serum was frozen below $-20°C$ for the measurement of serum triiodothyronine and thyroxine levels by radioimmunoassay. Serum triiodothyronine and thyroxine were determined by the GAMMA-COAT™ [^{125}I] T_3 and Total T_4 Radioimmunoassay kits, respectively (Baxter Travenol Diagnostics, Inc., U.S.A.).

Organ bath study

The thoracic aorta was excised immediately and cleaned of fat and connective tissue on the moistened filter paper. The preparations were cut into four ring segments (4mm in length). Each aortic ring was suspended in a water-jacketed organ bath containing 15ml of modified Krebs-bicarbonate solution of the following composition (millimolar concentrations): NaCl, 115.0; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; and dextrose, 10.0. In addition, calcium-depleted, potassium-depolarizing solution was prepared in a way that $CaCl_2$ was omitted and 80mM NaCl was replaced by an equimolar concentration of KCl. The bathing solutions were maintained at $37 \pm 0.5°C$ and aerated with a gas mixture of 95% O_2 and 5% CO_2.

Two stainless steel triangles were inserted through each vessel ring. Care was taken to avoid rubbing the endothelial surface of the vessel. One was anchored to a stationary support and the other was connected to an isometric force transducer (Myograph F-60, Narco Bio-Systems, U.S.A.) Aortic rings were stretched passively by imposing the optimal resting tension, 2.0g, which was maintained throughout the experiment.

The segments were equilibrated for 120 min during which time the solution were replaced every 30 min. Thereafter, the contractile response to 50mM KCl was elicited first to ensure stabilization of the muscles. The isometric contractions were recorded on an ink-writing physiograph (Physiograph MK-IV-P, Narco Bio-Systems, U.S.A.) After a stable plateau had developed, the rings were washed with several changes of fresh buffer. A period of 60 min was allowed for reequilibration, after which norepinephrine and calcium were added to the physiological salt solution (PSS) and calcium-depleted, potassium (80mM)-depolarizing solution, respectively, in a cumulative fashion to obtain complete concentration-response relationships. Complete dose-response curves for relaxation were performed on the precontracted aortic ring segments of contol (0.1μM norepinephrine) and PTU-treated (1μM norepinephrine) rats. Preliminary experiments showed that these concentrations of norepinephrine induced approximately 70% of maximum contractile responses. The contractile responses were presented by mg tension per mg tissue weight. The relaxation responses were expressed as a percentage of maximum relaxation induced by several washouts of the bath.

Drugs

Drugs used in the present study include; norepinephrine bitartrate, acetylcholine chloride, histamine dihydrochloride, isoproterenol hydrochloride (Sigma Chemical Co., U.S.A.), and sodium nitroprusside (Katayama Chemical Co., Japan).

Statistical analysis

The EC_{50} and IC_{50} values were calculated by computerized Parafit program. The data were expressed as mean ± S.E.M. and were analyzed by Mann-Whitney test except the data of body weights by Student's *t*-test. P values less than 0.05 were regarded as statistically significant.

RESULTS

At sacrifice, PTU-treated and control rats weighed (g): 155 ± 1.5 and 203 ± 3.4 after 4 weeks; 153 ± 5.0 and 248 ± 9.4 after 12 weeks, respectively (Fig. 1).

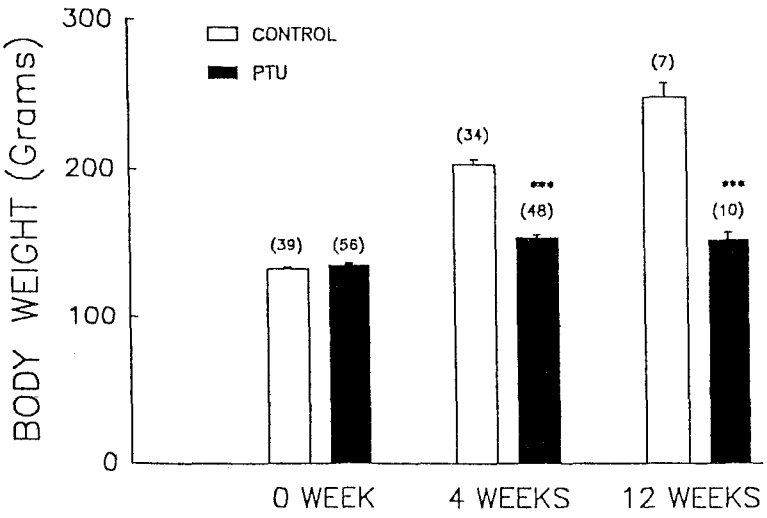

Fig. 1. Body weights in control and propylthiouracil (PTU)-treated rats for 4 and 12 weeks. Vertical bars represent S.E.M. The number of preparation is given in parentheses. *** $p<0.001$ compared with control.

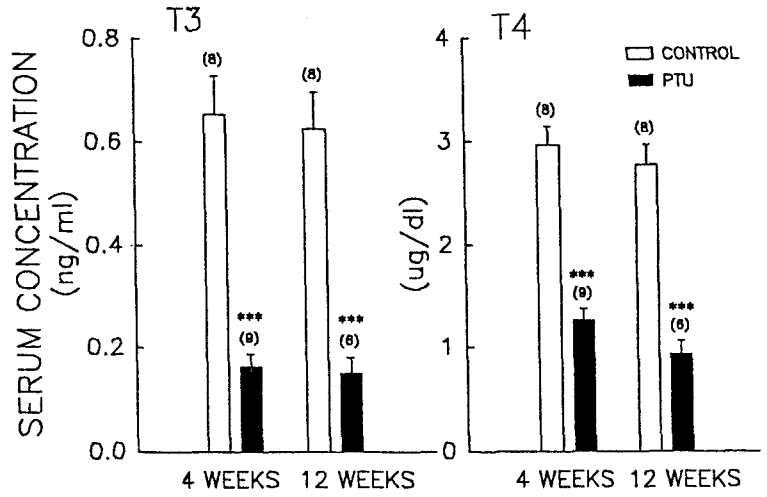

Fig. 2. Serum concentrations of triiodothyronine (T_3, left) and thyroxine (T_4, right) in control and propylthiouracil (PTU)-treated rats for 4 and 12 weeks. Vertical bars represent S.E.M. The number of preparation is given in parentheses. *** $p<0.001$ compared with control.

As shown in Figure 2, Serum triiodo thyronine concentrations (ng/ml) of the PTU-treated and control rats were: 0.16 ± 0.02 and 0.65 ± 0.08 after 4 weeks; 0.15 ± 0.03 and 0.63 ± 0.07 after 12 weeks, respectively. Serum thyroxine concentrations (μg/dl) of the PTU-treated and control rats were: 1.27 ± 0.11 and 2.97 ± 0.18 after 4 weeks; 0.93 ± 0.31 and 2.78 ± 0.20 after 12 weeks, respectively. These data showed that propylthiouracil treatment produced a significant hypothyroidism.

Contractile response to norepinephrine

The concentration-response relationship for norepinephrine was shown in Figure 3. The maximum contraction in the aortic ring of the control rats were significantly greater than that of PTU-treated rats (Table 1). However, EC_{50} values for the contraction to the norepinephrine was greater in the aortic ring of the PTU-treated rats than in that of control rats (Table 1).

Contractile resonse to calcium ions

The concentration-response relationship for calcium was illustrated in Figure 4. The maximum contraction in the aortic rings of the control rats were significantly greater than that of PTU-treated rats. However, EC_{50} values for the contraction to the calcium ions in the aortic ring of the control rats were

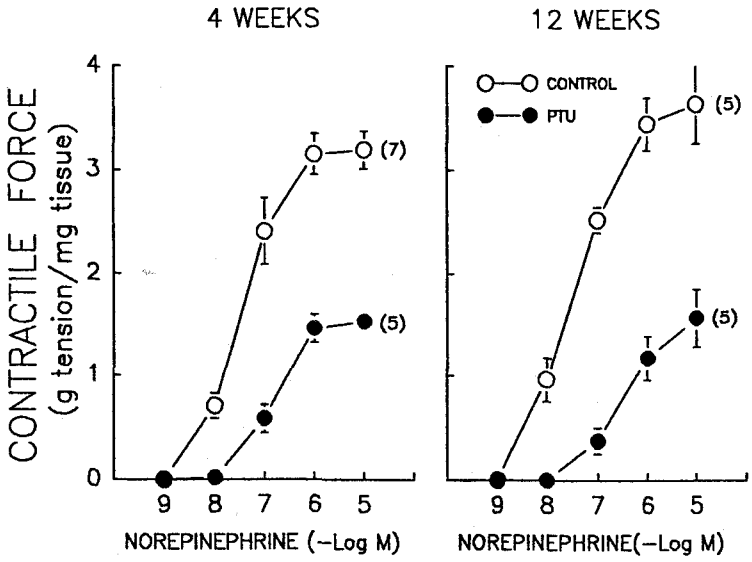

Fig. 3. Cumulative concentration-resonse curves for the contractile effects of norephinephrine in the aortic rings of control and propylthiouracil (PTU)-treated rats for 4 (left) and 12 (right) weeks. Vertical bars represent S.E.M. The number of preparation is given in parentheses.

Table 1. Maximum contractile effects and EC_{50} values of norepinephrine and calcium ion in the aortic rings of control and Propylthiouracil (PTU)-treated rats for 4 and 12 weeks

	Maximum Contraction (mg tension/mg tissue)		EC_{50} (−log M)	
	Control	PTU	Control	PTU
Norepinephrine				
4 wks	3232±187 (7)	1545±106 (5)**	7.44±0.13	6.82±0.10*
12 wks	3660±379 (5)	1724±380 (5)*	7.48±0.16	6.40±0.19*
Calcium ion				
4 wks	4852±327 (5)	2059±260 (6)**	2.94±0.18	3.03±0.10
12 wks	4462±324 (4)	2484±285 (4)*	3.13±0.09	3.13±0.09

Numbers in parentheses indicate the number of preparations used.
Data are expressed as mean±S.E.M.
*, **: Significantly different when compared with control $p<0.05$ and $p<0.01$, respectively.

not different from that of PTU-treated rats (Table 1).

Contractile response to KCl

The contractile response to 50mM KCl was determined in the aortic rings of the control rats and PTU-treated rats (Fig. 5). The contractile force in the aortic rings of the control rats were significantly greater than that of PTU-treated rats.

Relaxation response to acetylcholine

In the presence of active muslce tone induced by 0.1μM (control rats) and 1μM (PTU-treated rats) norepinephrine, the relaxation response to acetylcholine in a cumulative fashion was shown in Figure 6. Such concentrations of norepinephrine induced approximately 70% of maximum contractile responses. For the 12 week-treated groups, the maximum relaxation in the aortic ring of the PTU-treated rats were significantly greater than that of control rats (Fig. 6). However, IC_{50} values of the relaxation to the acetylcholine in the aortic rings of the PTU-treated rats were not different from that of control rats (Table 2).

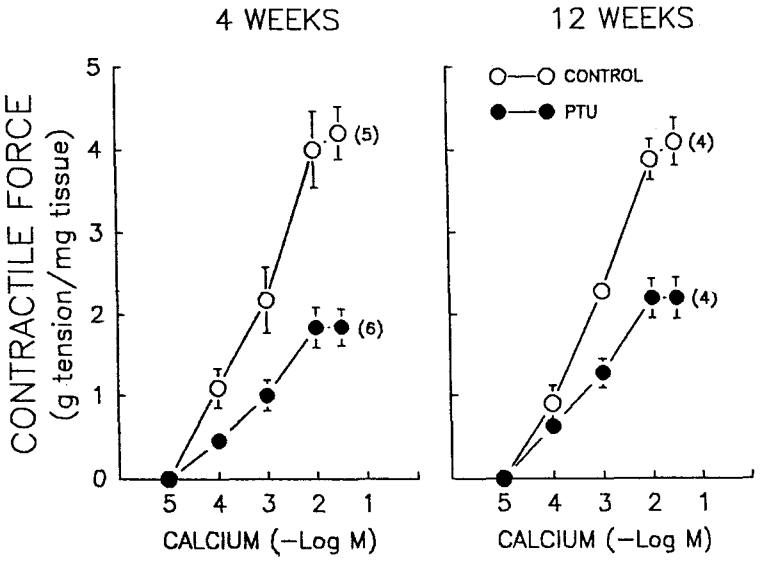

Fig. 4. Cumulative concentration-response curves for the contractile effects of calcium ions in the aortic rings of control and propylthiouracil (PTU)-treated rats for 4 (left) and 12 (right) weeks. Vertical bars represent S.E.M. The number of preparation is given in parentheses.

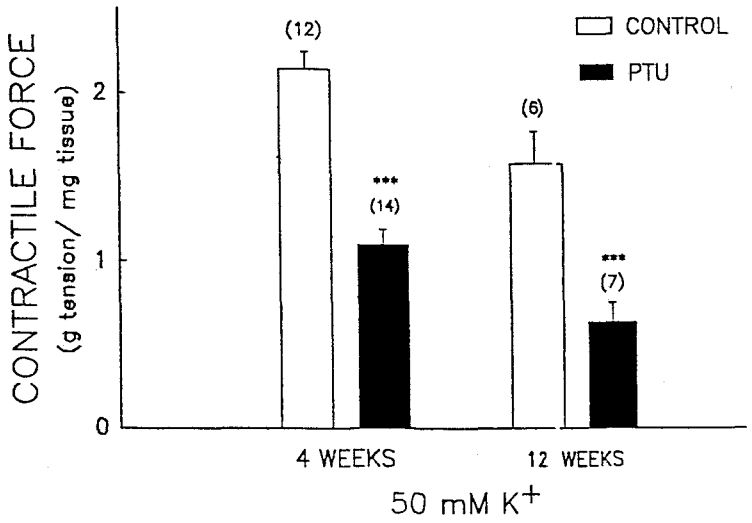

Fig. 5. Contractile effects of potassium ions (K^+) in the aortic rings of control and propylthiouracil (PTU)-treated rats for 4 and 12 weeks. Vertical bars represent S.E.M. The number of preparation is given in parentheses. *** $p<0.001$ compared with control.

Relaxation response to histamine

In the presence of active muscle tone, the relaxation response to histamine was shown in Figure 7. The maximum relaxations and IC_{50} values of the aortic rings of the PTU-treated rats were not significantly different from those of control rats (Table 2).

Relaxation response to isoproterenol

In the presence of active muslce tone, the relaxation response to isoproterenol were shown in Figure 8. For the 12 week-treated groups, the IC_{50} value of the aortic rings of the PTU-treated rats was significantly lower than that of control rats. However, the maximum relaxations to the isoproterenol from the aortic ring of the PTU-treated rats were not different from those of control rats (Table 2).

Relaxation response to nitroprusside

In the presence of active muscle tone, the relaxation response to nitroprusside were shown in Figure

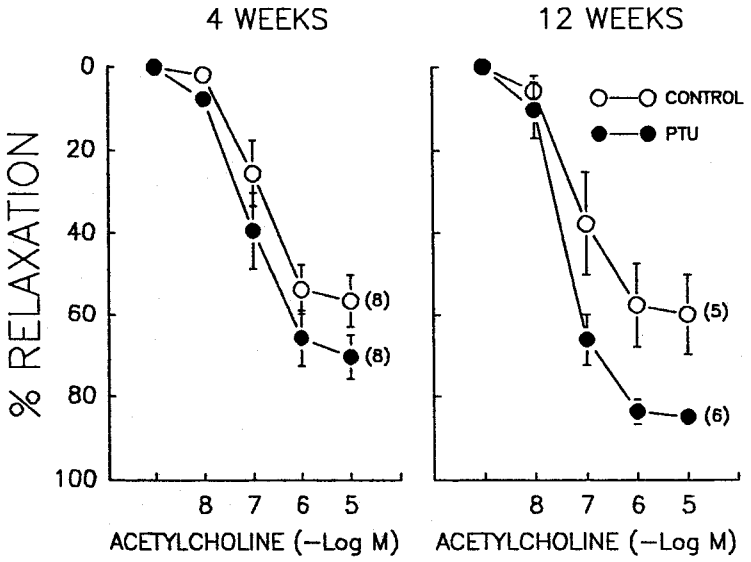

Fig. 6. Cumulative concentration-response curves for the relaxant effects of acetylcholine in the aortic rings of control and propylthiouracil (PTU)-treated rats for 4 (left) and 12 (right) weeks. Vertical bars represent S.E.M. The number of preparation is given in parentheses.

Table 2. Maximum relaxant effects and IC_{50} values of acetylcholine, histamine, isoproterenol, and nitroprusside in the precontracted aortic rings with norepinephrine in the control and propylthiouracil (PTU)-treated rats for 4 and 12 weeks

	Maximum Relaxation (%) change)		IC_{50} (−log M)	
	Control	PTU	Control	PTU
Acetylcholine				
4 wks	56± 6.2 (8)	71±5.3 (8)	6.91±0.13	7.01±0.18
12 wks	60± 9.7 (5)	85±2.9 (6)*	7.10±0.17	7.35±0.13
Histamine				
4 wks	59± 7.5 (6)	72±4.0 (7)	5.62±0.21	5.44±0.10
12 wks	58±14.2 (4)	68±3.5 (4)	5.68±0.34	6.10±0.23
Isoproterenol				
4 wks	83± 4.1 (9)	74±4.8 (9)	7.04±0.14	7.40±0.16
12 wks	72± 6.0 (5)	74±5.1 (4)	6.78±0.14	7.48±0.16*
Nitroprusside				
4 wks	100 (8)	100 (9)	7.56±0.19	8.04±0.14
12 wks	100 (5)	100 (4)	7.62±0.09	8.18±0.31*

Numbers in parentheses indicate the number of preparations used.
Data are expressed as mean±S.E.M.
*, $p<0.05$, compared with control.

9. For the 12 week-treated groups, the IC_{50} value in the aortic rings of the PTU-treated rats was significantly lower than that of control rats. However, the maximum relaxations to the nitroprusside from the aortic rings of the PTU-treated rats were not differnet from those of control rats (Table 2).

DISCUSSION

The contractile responses to norepinephrine (Fig. 3) and calcium (Fig. 4) and potassium ions (Fig. 5) were significantly attenuated in aortic rings from PTU-treated rats 4 weeks after when compared with

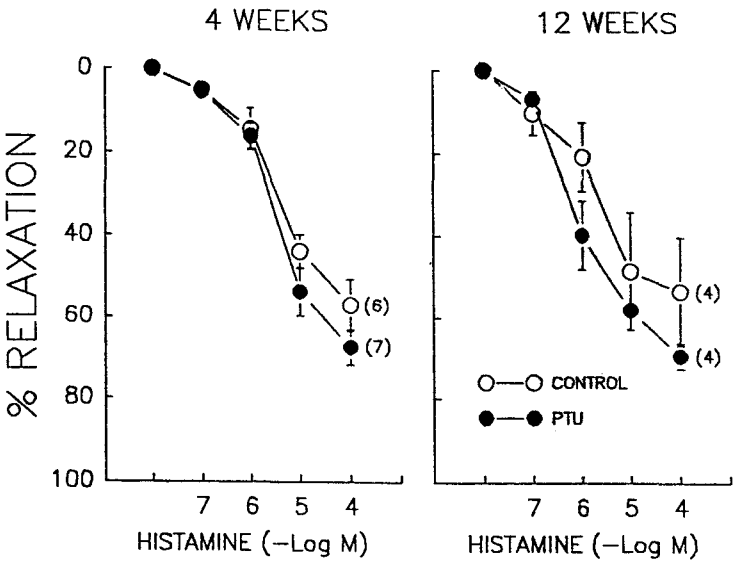

Fig. 7. Cumulative concentration-response curves for the relaxant effects of histamine in the aortic rings of control and propylthiouracil (PTU)-treated rats for 4 (left) and 12 (right) weeks. Vertical bars represent S.E.M. The number of preparation is given in parentheses.

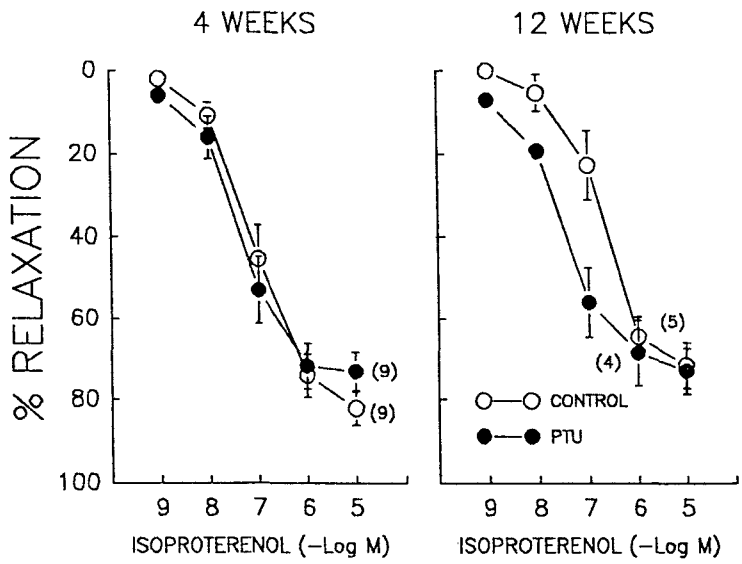

Fig. 8. Cumulative concentration-response curves for the relaxant effects of isoproterenol in the aortic rings of control and propylthiouracil (PTU)-treated rats for 4 (left) and 12 (right) weeks. Vertical bars represent S.E.M. The number of preparation is given in parentheses.

those from age-matched control animals. It is considered that the attenuated contractile responses to the vasoconstrictors in PTU-treated rats resulted from the decreased ability of the muscle cells to contract. The possibility may be put forward by the finding that the inotropic effect of thyroid hormone in the heart is induced by a change in the isozyme pattern of myosin (Hoh *et al.*, 1978). Defective synthesis of one of the heavy chains of the protein (Flink *et al.*, 1979) affects the Ca^{2+}-ATPase activity of the myosin, a reaction that may be responsible for generation of force (Morkin *et al.*, 1983).

By the PTU treatment, the sensitivity to norepinephrine but not to calcium was decreased whereas the maximal responses to norepinephrine and calcium were reduced together (Table 1). The different responses to norepinephrine and calcium might exclusively be related with the presence of endothelium. In the aortic rings of control rats, the contractile responses of endothelized aorta to the norepinephrine showed a significant increase in the EC_{50} value with no significant alteration of maximum

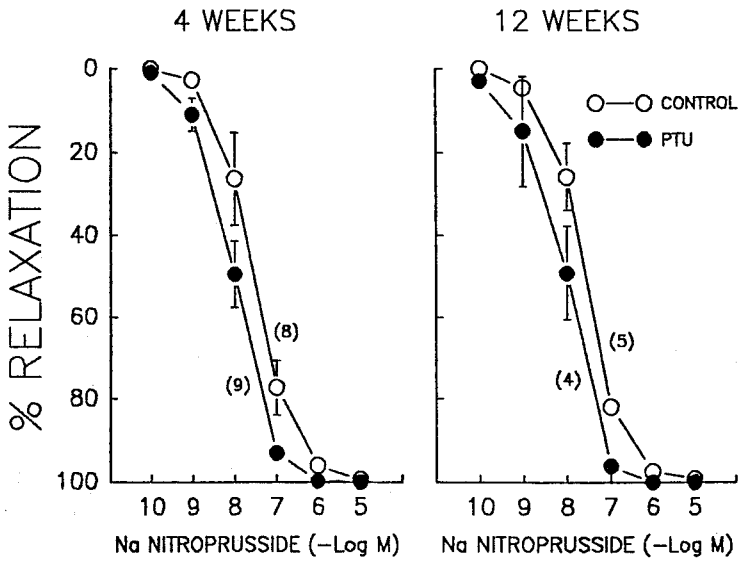

Fig. 9. Cumulative concentration-response curves for the relaxant effects of nitroprusside in the aortic rings of control and propylthiouracil (PTU)-treated rats for 4 (left) and 12 (right) weeks. Vertical bars represent S.E.M. The number of preparation is given in parentheses.

contractile force (Carrier and White, 1985) while the contractile responses to calcium in a high potassium-depolarizing medium were not influenced by the intactness of endothelium (Shirasaki *et al.*, 1988). The removal of endotehlium enhanced the contractile response of isolated rat aorta to potassium, BAY K 8644 and GCP 28392 (Mikkelsen *et al.*, 1988) as well as norepinephrine (Carrier and White, 1985). The contractile effect of calcium not only in K-depolarized but also in BAY K 8644-pretreated vessels was not influenced by endothelium (Mikkelsen *et al.*, 1988). Though it may be related to an inhibitory action of EDRF on the release of activator calcium (Mikkelsen *et al.*, 1988), the exact mechanism of the potentiating effect in the deendothelized vessels remains to be elucidated.

There is a tendency that the relaxation responses are generally augmented in aortic rings from PTU-treated rats when compared with those from age-matched control animals. However, the IC_{50} values to the endothelium-independent relaxants, such as isoproterenol and sodium nitroprusside (Katzung and Chatterjee, 1989), in the aortic rings of PTU-treated rats for 12 weeks were different from control rats whereas those to the endothelium-dependent relaxants, such as acetylcholine and histamine (Oyama *et al.*, 1986) were not. This result gives also support to the previous suggestions.

In addition, isoproterenol is known to stimulate adenylate cyclase by activation of β-adrenergic receptors and increase the cyclic AMP formation. Activated cAMP-dependent protein kinase phosphorylates myosin light chain kinase (MLCK) and reduces its affinity to Ca-calmodulin complex, eventually suppressing the contractile machinery (Adelstein *et al.*, 1978; Bülbring and Tomita, 1987). Nitroprusside and other nitrovasodilators, such as nitrites and organic nitrates, lead to the formation of the reactive free radical, nitric oxide (NO), which interacts with and activates guanylate cyclase and increases the synthesis of cyclic GMP in smooth muscle cells (Rapaport and Murad, 1983). A cyclic GMP-dependent protein kinase is thus stimulated, with resultant alteration of the phosphorylation of various proteins in smooth muscle. This eventually leads to the dephosphorylation of the light chain of myosin (Rapaport *et al.*, 1983), resulting in relaxation of smooth muslce.

Altered muscular sensitivities to isoproterenol and nitroprusside in the PTU-treated rats for 12 weeks but not for 4 weeks indicate that PTU-induced hypothyroidism is not severe or the cellular change is slowly progressive or both.

The release of nitric oxide (NO) by endothelial cells accounts for the biological activity of endothelium-derived relaxing factor (Palmer *et al.*, 1987). If the sensitivity to nitroprusside, which activates guanylate cyclase, was enhanced, the relaxation responses to endothelium-dependent relaxants, which also activate guanylate cyclase indirectly, should be hypersensitive, at least, in PTU-treated rats for 12 weeks. In support of this, it was observed that the maximal relaxation to acetylcholine was significantly augmented in the PTU-treated rats for 12 weeks

as compared with age-matched control rats (Table 2). Nevertheless, IC_{50} values of the relaxation to the endothelium-dependent relaxants in the aortic rings of PTU-treated rats were not different from that of control rats. This disparity could be explained at least partially by the facts that secondary hypercholesterolemia in the hypothyroidism might impair endothelium-dependent relaxation.

The altered vascular responsiveness in the PTU-treated rats may reflect the alteration of smooth muscle cells rather than the influence of endothelium. This change have proceeded slowly and continuously after hypothyroidism is evident biochemically. Furthermore, the effects of PTU-treatment on the vascular responses to vasoactive drugs are likely to be consistent with clinical hypothyroidism with respect to a reduction of blood pressure.

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= 국문초록 =

PTU-처치가 흰쥐대동맥의 수축 및 이완 반응에 미치는 영향

경북대학교 의과대학 약리학교실

심 일 청 · 김 인 겸 · 김 중 영

Propylthiouracil(PTU)을 4주 및 12주간 투여한 흰쥐의 대동맥을 적출하여 혈관수축제와 이완제에 대한 반응을 관찰하여 다음과 같은 결과를 얻었다.

PTU를 처치한 실험군에서는 대조군에 비해 현저한 체중저하와 혈중 갑상선호르몬의 감소를 보였다.

PTU를 처치한 흰쥐의 대동맥에 대한 norepinephrine(NE)과, calcium 및 potassium 이온에 의한 최대수축 반응은 대조군에 비하여 유의하게 감소되었다. 그러나 NE에 대한 중간유효량은 증가되었으나, calcium 이온에 대한 중간 유효량은 유의한 차이가 없었다.

그리고 acetylcholine, histamine, isoproterenol 및 nitroprusside에 의한 대동맥의 이완작용은 대조군에 비해 증가된 경향을 보였다. PTU를 12주간 처치한 군에 있어서 acetylcholine에 의한 최대 이완반응은 대조군에 비해 유의하게 증가되었지만 다른 이완제에 의해서는 유의한 차이가 없었다. PTU를 4주간 처치한 군에 있어서는 대조군에 비하여 혈관이완제에 대한 중간억제량은 유의한 차이가 없었지만, 12주 처치군에 있어서는 isoproterenol및 nitroprusside에 대한 중간억제량은 감소 되었으나 acetylcholine 및 histamine에 대한 중간억제량은 유의한 차이가 없었다.

이상의 결과로 미루어 보아 PTU-처치에 의한 혈관 반응성의 변화는 혈관 내피세포보다는 혈관 평활근세포자체의 변화에 기인되며, 이러한 세포내의 변화는 갑상선 기능이 저하된 후에도 계속되고 있음을 알 수 있었다.