Effects of Triiodo-L-Thyronine (T\textsubscript{3})-induced Hyperthyroidism on the Cardiovascular Alpha-Adrenoceptor System in Young and Older Rats

Gozoh TSUJIMOTO and Keitaro HASHIMOTO
Department of Pharmacology, Yamanashi Medical College, Tamaho-cho, Nakakoma-gun, Yamanashi 409-38, Japan
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Abstract—We have compared the vasopressor response of alpha-adrenoceptor agonists in young (2 month-old) and older (12 month-old) pithed rats both in the presence and absence of triiodo-L-thyronine (T\textsubscript{3})-induced hyperthyroidism. There was no age-related changes in the pressor response to either the alpha\textsubscript{1}-selective agonist phenylephrine or the alpha\textsubscript{2}-selective agonists clonidine and UK-14,304. However, T\textsubscript{3}-induced hyperthyroidism caused a selective reduction in postjunctional alpha\textsubscript{2}-adrenoceptor-mediated pressor responses but not alpha\textsubscript{1}-adrenoceptor-mediated ones in both age groups. In contrast to vascular alpha-adrenoceptors, there was an age-related decrease in the number of myocardial alpha\textsubscript{1}-adrenoceptor sites measured by specific binding of [\textsuperscript{3}H]prazosin. With thyroid hormone treatment, the density of ventricular alpha\textsubscript{1} receptors was found to be further reduced even in the older rats. These results suggest that thyroid hormone and aging have different influences on the cardiovascular alpha-adrenoceptor system and that these two physiological variables are independently interrelated.

Cardiovascular impairment is one of the most significant functional manifestations of the aging process. Consequently, elucidation of the mechanism responsible for this alteration is of major importance. It is now well documented that cardiovascular responsiveness to beta-adrenoceptor stimulation declines with age in several species (for review, see Ref. 1–3). Thus, the positive inotropic and chronotropic responses to sympathetic stimulation or infusion of catecholamines have been reported to be significantly reduced in aged heart (4–6). Also, similar findings of a loss in responsiveness of blood vessels to beta-adrenoceptor agonists have been reported by several investigators (7–9). As one of the important factors that could underlie age-related alterations in beta-adrenoceptor responsiveness of the cardiovascular system, much attention has been given to thyroid hormone (10, 11). We have recently observed that exogenous administration of thyroid hormone (T\textsubscript{3}) partially restored these age-related decreases in beta-adrenoceptor activity of the cardiovascular system (12), suggesting that the effects of thyroid hormone and age-related alterations of cardiovascular responsiveness to beta-adrenoceptor stimulation are interrelated. In contrast to the extensive studies on the beta-adrenoceptor system, relatively little is known about the possible effects of these variables on the cardiovascular alpha-adrenoceptor system.

There is now a large body of evidence implicating the existence of postjunctional alpha\textsubscript{1}- and alpha\textsubscript{2}-adrenoceptors in vascular smooth muscle, both of which can produce pressor response in pithed animals (13, 14). The subdivision of alpha-adrenoceptors can be made on the basis of the potencies of a series of agonists and antagonists. Very recently, further different features of alpha\textsubscript{1}- and alpha\textsubscript{2}-adrenoceptors have been studied.
with regards to the susceptibility to pH changes and calcium entry blockers (15) and their receptor reserve (16). However, there is still little information about the possible effects of aging on each subtype-mediated vasoactivity; and especially, the modulation of them by thyroid hormone is unknown. In the heart, previous work showed that alpha-adrenoceptor-mediated cardiac functions decline with increasing age (for review, see Ref. 3). Also, these reduced functions seem to correlate well with the loss of ventricular alpha<sub>1</sub>-adrenoceptor sites as measured by radioligand binding assay (17). However, it is still uncertain whether and to what extent this age-related alteration of alpha<sub>1</sub> receptors can be modified by thyroid hormone.

The present study was undertaken to determine whether the cardiovascular alpha-adrenoceptor system in young and older rats is differentially modulated by thyroid hormone. We have compared the postsynaptic alpha<sub>1</sub>- and alpha<sub>2</sub>-adrenoceptor-mediated vasopressor responses in young and older pithed rats both in the presence and absence of triiodo-L-thyronine (T<sub>3</sub>)-induced hyperthyroidism. Also, in an effort to determine the effects of thyroid hormone on the age-related decline of cardiac alpha-adrenoceptors, alpha<sub>1</sub> receptor sites were examined by direct radioligand binding assay.

### Materials and Methods

**Male, 6–8 week-old (42 to 60 days old, 170–250 g) and 12 month-old (320 to 370 days old, 490–570 g), Sprague-Dawley rats were treated with (-)-3,5,3'-triiodothyronine (T<sub>3</sub>, 500 μg/kg body weight) by intraperitoneal injection once a day in a volume of 1 ml/kg for 6 consecutive days according to the protocol described by Nagel-Hiemke et al. (18). A similar set of animals was treated with vehicle (5 mN NaOH in 0.9% NaCl).**

**Alpha-adrenoceptor-mediated vasopressor responses in pithed rats:** Animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.), and skin temperature was kept constant (37°C) with a heating pad. After administering atropine (2 mg/kg, i.p.), rats were pithed by inserting a stainless-steel rod (1.5 mm in diameter) through the right orbit and foramen magnum down through the spinal cord as described by Gillespie and Muir (19). Immediately after pithing, the tracheal cannula was attached to a rodent respirator (Shinano, model SN-480-7, Japan), and the rat was artificially ventilated with room air at a frequency of 60 cycles/min with a tidal volume of 1.5 ml/100 g body weight. Pithed rats were bilaterally vagotomized in the neck, and d-tubocurarine (3 mg/kg, i.v.) and heparin (150 I.U./kg, i.v.) were injected. Systemic arterial blood pressure was monitored with a right femoral artery catheter. Pressures were measured via Nihon Kohden TP-200T pressure transducers and recorded on a Nihon Kohden model WS-6416 recorder, and heart rates were measured with a Nihon Kohdencardiotachometer triggered by the electrocardiogram (ECG).

The right femoral vein was cannulated for intravenous administration of drugs in a volume of less than 1.0 ml/kg. After administering the beta-adrenoceptor antagonist (+)-propranolol (1.0 mg/kg, i.v.), the preparations were allowed to equilibrate for at least 30 min. Then, the increase in diastolic pressure after i.v. administration of the postsynaptic alpha<sub>1</sub>-receptor selective agonist phenylephrine (20, 21) or the postsynaptic alpha<sub>2</sub>-receptor selective agonists clonidine and UK 14,304 (20, 21) were examined in control (saline injected) and hyperthyroid (T<sub>3</sub> injected) young and older rats. Dose-pressor response curves were obtained by administering increasing doses of each agonist at 10 min intervals, which allowed the blood pressure to return to basal levels between doses.

**Membrane preparation of left ventricles:** Rat left ventricular membranes were prepared by the method of Minneman et al. (22) with minimal modification (23). Briefly, the left ventricle was cleaned of adherent tissue and homogenized with a Brinkmann Polytron (Brinkmann Instruments, Westbury, NY; setting 8 for 20 sec) in ice-cold buffer (0.25 M sucrose, 5 mM Tris-HCl, and 1 mM MgCl<sub>2</sub>, pH 7.4). The homogenate was first centrifuged at 900×<i>g</i> for 10 min to remove unbroken tissue and nuclei, and the supernatant was filtered through four layers of cheesecloth and then centrifuged at 10,000×<i>g</i>
for 30 min at 4°C. The resultant pellet was resuspended in ice-cold buffer containing 50 mM Tris-HCl (pH 7.5) and 10 mM MgCl₂. This resuspending process was repeated at least three times, and the final pellet was directly used for the binding assay.

[³H]Prazosin binding assay: For the identification of myocardial alpha₁-adrenoceptors, ventricular membranes were incubated with [³H]prazosin in buffer (50 mM Tris-HCl and 10 mM MgCl₂, pH 7.5) for 50 min at 25°C, and bound and free radioactivity were separated by filtration and washing over glass fiber filters (Whatman GF/C). From preliminary experiments, specific binding showed kinetics, stereospecificity, and rank order of potency of agonists and antagonists that were characteristic of ligand binding to alpha₁-adrenoceptors. Specific [³H]prazosin binding was experimentally determined from the difference between counts in the absence and presence of 10 nM phentolamine. Specific binding of this radioligand was routinely 80–85% of the total binding at a concentration near its Kᵅ.

Protein concentrations were determined by the method of Lowry et al. (24) with bovine serum albumin as standard.

Data analysis: The means of the pressor responses were obtained, and the resulting dose-response curve from each group was analyzed simultaneously using the four parameter logistic equation (25) on an APPLE IIe system. The resulting ED₅₀'s and maximal responses (Eₘₐₓ) were analyzed for significant difference using the ALLFIT program. The ALLFIT program is a modification of the DeLean et al. program (25) by Martin H. Teicher, and was obtained from the Biomedical Computing Technology Information Center, Nashville, TN.

The data from saturation curves of radioligand binding studies were analyzed using non-linear regression on an NEC 9801F computer. These data were fit based on the law of mass action using a general program for the analysis of data in terms of models (26).

The experimental data given in the text and figures are the mean±S.E.M. of ‘n’ experiments as indicated. Overall changes between groups were analyzed statistically by two-way analysis of variance (factor 1=animal age, factor 2=thyroid treatment). Student’s t-test for unpaired observations was also used to determine the significance of difference between values of the controls and those of T₃-treated animals within the same age group. Criterion for statistical significance was a P value of less than 0.05.

Materials: Furoyl-5-[³H]prazosin (specific activity, 19.8 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Phentolamine (Regitine mesylate; Ciba-Geigy Corp., Summit, NJ), UK-14,304 tartrate (Pfizer, Sandwich, U.K.) and clonidine HCl (C.H. Boehringer Ingelheim, Ltd., Elmsford, NY) were generously supplied by each company. (±) Propranolol HCl and (-) phenylephrine HCl were purchased from Sigma Chemical Co. (St. Louis, MO). d-Tubocurarine chloride and atropine sulfate were obtained from Yoshitomi Pharmaceutical (Osaka, Japan) and Fuso Pharmaceutical (Osaka, Japan), respectively. All other chemicals and reagents used were from standard commercial sources.

Results
As a result of 6-day intraperitoneal injection of T₃ (500 μg/kg), pronounced physical changes occurred. Detailed data for the effects of T₃-treatment on body weight change, heart weight, and plasma concentrations of thyroid hormone and hemodynamic parameters in pithed young and older rats were already described elsewhere (12). Briefly, the circulating T₃ levels measured 17 hr after the last injection in T₃-treated groups of both young and older animals were approximately 8-fold greater than each of the age-matched control groups. However, there was no age-related changes in circulating T₃ levels (87.6±5.3 ng/dl, n=7 in young controls vs. 94.9±13.7 ng/dl, n=7 in older controls). Corresponding to the elevated plasma T₃ levels, in both young and older T₃-treated groups, there was a marked reduction in the mean of body weight gain, and a significant (P<0.05) increase in weight of the left ventricle was found (34% in young and 22% in older rats); however, there was no significant alteration in the protein concentration of the left ventricles in the four groups.
suggesting that the heart underwent a true hypertrophy as opposed to becoming edematous.

Basal blood pressure and heart rate for each group of rats were measured 20 min after pithing. In both young and older pithed rats, T₃-treated groups showed a significantly (P<0.01) elevated basal heart rate compared with euthyroid controls (319±11 in young controls, n=6; 498±14 in young T₃-treated rats n=6; 300±10 in older controls, n=6; 466±18 in older T₃-treated rats, n=6). Analysis of variance, however, indicated no significant difference between young and older T₃-treated animals with respect to heart rate. On the other hand, while there was no significant age-related difference in either basal diastolic and systolic blood pressure, systolic blood pressure was found to be significantly (P<0.05) elevated in the young T₃-treated rats as compared with that in older T₃-treated rats; systolic blood pressures were 76±4 mmHg, n=6 and 55±3 mmHg, n=6 for young and older T₃-treated rats, respectively.

Effects of animal age and T₃ treatment on the postsynaptic vascular α₁- and α₂-adrenoceptor-mediated pressor responses in pithed rats: When phenylephrine, which is a specific α₁-adrenoceptor agonist, was used as a pressor agent, dose-response relationships were almost identical for all groups (Fig. 1). On the other hand, Eₘₐₓ values of dose-response curves for the α₂-selective adrenoceptor agonists clonidine and UK-14,304 in T₃-treated pithed rats were found to be significantly suppressed compared to each of the age-matched control groups; however, there was no significant difference in either ED₅₀ values in the four groups or Eₘₐₓ values between young and older control animals (Table 1, Fig. 2A, B).

Influence of animal age and T₃ treatment on myocardial α₁-adrenoceptor sites: The number of ventricular α₁-receptors was found to be slightly but significantly (P<0.05) decreased in the older control group as compared to that in the young control group (Table 2). Furthermore, the number of α₁-receptor sites measured in the same membrane preparation was significantly decreased in both young and older hyperthyroid groups when compared with age-match controls (Table 2). In young animals, the number of [³H]prazosin binding sites was reduced 58%, while in the older rats, the decrease was 33%. However, there was no significant difference in α₁-receptor density between young and older T₃-treated animals. Analysis of variance

![Fig. 1. Dose-response curves for the increases in diastolic pressure produced by i.v. injection of (-)-phenylephrine in control (open symbols) and T₃-treated (closed symbols) groups of 2 month-old (left) and 12 month-old (right) pithed rats. Symbols represent the mean values±S.E.M. (n=6 in each group). Results do not differ at the P<0.05 level of significance at any point in responsiveness to phenylephrine in the four curves.](image-url)
Table 1. Effects of triiodo-L-thyronine (T₃)-induced hyperthyroidism on vasopressor responses of young and older pithed rats to clonidine and UK-14,304

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<tr>
<th></th>
<th>Clonidine</th>
<th>UK-14,304</th>
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<tr>
<td></td>
<td>Eₘₐₓ</td>
<td>ED₅₀</td>
</tr>
<tr>
<td>2 month-old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>92±9</td>
<td>24±2</td>
</tr>
<tr>
<td>T₃-treated</td>
<td>43±6*</td>
<td>20±4</td>
</tr>
<tr>
<td>12 month-old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>90±6</td>
<td>16±2</td>
</tr>
<tr>
<td>T₃-treated</td>
<td>56±7*</td>
<td>23±4</td>
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As described in "Data analysis", the means of the pressor responses were calculated (six separate experiments in each group), and the resulting dose-response curve was analyzed simultaneously using the four parameter logistic equation (25). The resulting Eₘₐₓ (mmHg) and ED₅₀ (µg/kg) values (the means±S.E.M.) were analyzed for significant difference using the ALLFIT program. *Significantly different from the age-matched control animals at P<0.05.

Fig. 2. Dose-response curves for the increases in diastolic pressure produced by i.v. injection of clonidine (A) and UK-14,304 (B) in control (open symbols) and T₃-treated (closed symbols) groups of 2 month-old (left) and 12 month-old (right) pithed rats. Symbols represent the mean values±S.E.M. (n=6 in each group). Results above the concentration of 30 µg/kg of clonidine and 10 µg/kg of UK-14,304 differ at the P<0.05 level of significance between the control and T₃-treated group at each point in both 2 month-old and 12 month-old rats.
Table 2. Number of alpha-adrenergic receptors in rat left ventricles

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<th>2 month-old rats</th>
<th>12 month-old rats</th>
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<tr>
<td></td>
<td>Control</td>
<td>Hyperthyroid</td>
</tr>
<tr>
<td>Alpha receptors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(fmol/mg protein)</td>
<td>85.9±7.2</td>
<td>36.2±6.0**</td>
</tr>
<tr>
<td>$K_d$ of [3H]prazosin</td>
<td>0.21±0.04</td>
<td>0.24±0.05</td>
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The membrane preparations were made as described in Methods. The number of alpha receptors was determined with [3H]prazosin. The results represent the mean±S.E.M. of six experiments. *Significantly different from the age-matched control animals at $P<0.05$. **Significantly different from the age-matched control animals at $P<0.01$. +Significantly different from the 2 month-old control animals at $P<0.05$.

indicated that both factors of animal age and thyroid hormone treatment are independently responsible for the loss of myocardial alpha$_1$-adrenoceptor density. The affinities of the alpha$_1$-adrenoceptors for [3H]prazosin in the left ventricular membranes were not significantly different in all groups (Table 2).

Discussion

As outlined in the introductory material, it is now established that vascular postjunctional alpha-adrenoceptor-mediated responses can be subclassified into at least two subtypes, alpha$_1$ and alpha$_2$ receptors. However, still very little is known about the physiological regulation of each receptor subtype. Our present study indicates that T$_3$-induced hyperthyroidism causes selective alterations in postjunctional alpha$_1$-adrenoceptor-mediated but not alpha$_1$-adrenoceptor-mediated pressor response irrespective of animal age. Also, the receptor binding data show that the diminished myocardial alpha$_1$ receptors in older rats were further decreased by thyroid hormone treatment.

We found no significant effects of animal age on either postjunctional alpha$_1$- or alpha$_2$-adrenoceptor-mediated pressor response. Recently, Docherty and Hyland (27) have also examined the effects of aging on vascular alpha$_1$- and alpha$_2$-adrenoceptor activity in the pithed young (3–7 months) and senescent (21–24 months) Sprague-Dawley rats utilizing amidephrine and xylazine as an alpha$_1$ and alpha$_2$ agonist, respectively. They found that the pressor effects of the alpha$_1$-adrenoceptor agonist amidephrine were not significantly altered between 3 and 24 months, an observation which agrees well with our present results, although we examined pithed rats between 2 and 12 months of age using phenylephrine as an alpha$_1$-selective agonist. As to the alpha$_2$-adrenoceptor responsiveness, they found that the pressor effects of xylazine were not significantly altered between 3 and 6 months, but that they were significantly reduced in 21–24 month-old pithed rats. Since we used the same strain of rats as they did (male, Sprague-Dawley strain), our results extended and more fully characterized their study. Thus, the pressor effects of alpha$_2$-adrenoceptor agonists in pithed rats seem to be not significantly altered between 2 and 12 months, and then decrease between 12 and 21–24 months, a period which is a senescent stage in this strain of rats. Taken together, it may be concluded that the alpha$_1$-adrenoceptor-mediated vascular responsiveness is not significantly altered between 2 to 21–24 months, while the alpha$_2$-adrenoceptor-mediated one is not altered up to 12 months of age, but can be reduced between 12 to 21–24 months.

It is notable that thyroid hormone specifically attenuates the pressor effects of alpha$_2$- but not alpha$_1$-adrenoceptor agonists irrespective of animal age. The specificity for the alpha$_2$ receptors suggests that the basis for this alteration is receptor-related. However, vascular alpha$_2$-adrenoceptors were not directly examined in the present study mainly because in rat vascular tissue they have thus far proven difficult to detect in a limited number of materials (28, 29), and also there exists possible contamination of the plasma
membrane fraction by presynaptic neural elements as previously reported (28). Although this different susceptibility of $\alpha_1$ and $\alpha_2$ response to thyroid hormone is clearly another different feature of these subtype receptors, the physiological significance and the underlying explanation for these changes are uncertain from the present study.

In the heart, the positive inotropic and chronotropic responses to catecholamines have been traditionally assumed to be primarily due to their effects on cardiac beta$_1$-adrenoceptors. Recent pharmacological evidence, however, has suggested the existence of cardiac alpha-adrenoceptors, which presumably play a role at least in the production of the positive inotropic effect on the myocardium of some animal species including the rat (30, 31). Thyroid dysfunctions have been known to influence these alpha-adrenoceptor-mediated cardiac functions. Thus, Nakashima and coworkers found that propylthiouracil pretreatment sensitizes rat atria to the positive inotropic and chronotropic actions of alpha-adrenoceptor agonists (32). On the other hand, hyperthyroidism has been reported to have opposite effects (33). However, the effects of thyroid state on the alpha-adrenoceptors in the aging heart are not yet well known. In the present study we used $[^3]$H]prazosin as a radioligand, since alpha$_1$-adrenoceptors have been conclusively demonstrated in cardiac tissue with the radioligand assay (34). Our present study confirmed the observation of Partilla et al. (17) that there was an age-related reduction in the number of ventricular alpha$_1$-adrenergic receptors. This age-associated alteration in cardiac alpha$_1$ receptor density may not be due to the altered thyroid state during aging, since there was no age-related change in circulating thyroid hormone levels (12). With thyroid hormone treatment, the ventricular alpha$_1$-adrenergic receptor density further decreased even in older rats. Analysis of variance indicated that thyroid hormone is independently responsible for this loss of myocardial alpha$_1$ adrenoceptor density in the older rats, suggesting that myocardial alpha$_1$ receptors can be dynamically regulated by two different variables of aging and thyroid hormone. In addition, the impaired capacity of receptors in aged animals to be dynamically regulated (e.g., "down-regulation" or "up-regulation") has been considered as one of the possible explanations for the reduced catecholamine responsiveness associated with aging. Our receptor binding data, however, suggest that this possibility is considered unlikely in the heart, since down-regulation of myocardial alpha$_1$ receptors in response to hyperthyroidism is unaltered with age.

In conclusion, the present study provides an important insight into the influence of thyroid hormone and aging on the alpha-adrenoceptor system in the cardiovascular system. In contrast to the beta-adrenoceptor system where effects of thyroid hormone and age-related alterations are known to be interrelated, the data emphasize that these two physiological variables have independent influence on the cardiovascular alpha-adrenoceptor system.

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