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European Journal of Pharmacology 294 (1995) 593–599

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Alterations of α_1-adrenoceptor subtypes in the hearts of thyroxine-treated rats

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Received 2 February 1995; revised 20 September 1995; accepted 22 September 1995

Abstract

Alterations in the cardiac α_1-adrenoceptor and its subtypes in thyroxine-treated rats were studied by means of radioligand binding assays, measurement of contractile response and reverse transcription-polymerase chain reaction (RT-PCR). The results showed that in thyroxine-treated rats the cardiac α_1-adrenoceptor density (B_{max}) was reduced from 51.6 ± 6.0 fmol/mg in control to 40.9 ± 3.7 fmol/mg ($P < 0.01$); and the percentage of high affinity sites for 5-methyl-urapidil decreased from $23.3 \pm 2.0\%$ in control to $10.8 \pm 2.0\%$ in thyroxine-treated rats ($P < 0.05$). The data indicated that the high-affinity sites for 5-methyl-urapidil (α_{1A}-adrenoceptor) were reduced (from 12.0 to 4.4 fmol/mg), but the low-affinity sites for 5-methyl-urapidil (α_{1B}- plus α_{1D}-adrenoceptor) were not changed (from 39.6 to 36.5 fmol/mg). RT-PCR showed that steady-state levels of mRNA for α_{1A}- and α_{1B}-adrenoceptors were decreased, while that for α_{1D}-adrenoceptor was raised in thyroxine-treated rats. In the isolated electrically driven left atria the phenylephrine-induced maximal contractions were reduced from 258 ± 17 mg in control to 188 ± 24 mg in thyroxine-treated rats ($P < 0.05$). The pA_2 values of 5-methyl-urapidil were reduced from 8.89 ± 0.36 in control to the hyperthyroidism of 7.87 ± 0.43 in thyroxine-treated rats ($P < 0.05$). Chlorethylclonidine preincubation shifted concentration-response curves for phenylephrine to the right and reduced the maximal response to a lesser extent in thyroxine-treated rats than in control rats. Thus we concluded that the total number of cardiac α_1-adrenoceptors is reduced in thyroxine-treated rats. The change is subtype selective, with α_{1A}- and α_{1B}-adrenoceptors being reduced in number and α_{1D}-adrenoceptor being increased.

Keywords: α_1-Adrenoceptor, subtype; mRNA; Heart; Thyroxine treatment

1. Intruduction

Cardiovascular manifestations are frequent findings in hyperthyroidism. The relationship between the levels of circulating thyroid hormones and the cardiac response to adrenergic stimulation has been studied for a long time. The results have clearly shown that the altered responses are due, to a large extent, to changes at the level of the adrenoceptors in the heart (Polikar et al., 1993). While most attention has been given to β-adrenoceptors, some investigators reported in hyperthyroid rats that either cardiac α_1-adrenoceptor density or α_1-adrenoceptor agonist-mediated cardiac response was decreased (Williams and Lefkowitz, 1977; Kunos et al., 1974, 1980; Kunos, 1977; Fox et al., 1985; Kupfer et al., 1986; Limas and Limas, 1987; Hanft and Gross, 1990). However, little is known about the changes of subtypes of the α_1-adrenoceptor in hyperthyroidism.

α_1-Adrenoceptors were initially divided into two subtypes – α_{1A} and α_{1B} – according to different affinities for the competitive antagonists WB 4101, 5-methyl-urapidil, (+)-niguldipine, etc., and different susceptibilities to the site-directed alkylating agent chlorethylclonidine (Morrow and Creese, 1986; Han et al., 1987a, b; Minneman et al., 1988; Minneman, 1988; Gross et al., 1988; Boer et al., 1989). Based on the profile of subtype classification, Hanft and Gross (1990) found that in rat myocardium, hyperthyroidism reduced the total density of α_1-adrenoceptors with no change in the proportion of α_{1A} vs. α_{1B} subtypes.

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SSDI 0014-2999(95)00607-9

Soon after establishment of the two subtypes pharmacologically, three molecular clones of α_1-adrenoceptors – α_{1B}, α_{1C} and α_{1D} ($\alpha_{1A/D}$) – were found (Cotecchia et al., 1988; Schwinn et al., 1990; Lomasney et al., 1991; Perez et al., 1991). Based on many subsequent studies, the IUPHAR Adrenoceptor Committee on Receptor Nomenclature and Drug Classification has now agreed on a classification scheme consisting of α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors (TIPS – Receptor and Ion Channel Nomenclature, 1995). In this scheme, α_{1A} refers to the receptor previously identified as α_{1A} by pharmacological criteria and is encoded by the gene previously labeled 'α_{1C}'; α_{1B} refers to the receptor previously classified unequivocally as such by both pharmacological and molecular biological data; and α_{1D} refers to the receptor encoded by cDNA previously labeled 'α_{1D}' or '$\alpha_{1A/D}$' which shares some pharmacological characteristics with both the α_{1A}- and α_{1B}-adrenoceptor. Although the α_{1D} subtype also exists in the heart (Rokosh et al., 1994; Price et al., 1994), the former examinations of α_1-adrenoceptor subtype changes in hyperthyroidism did not consider the α_{1D} subtype. Therefore, in this study we examined the alterations in cardiac α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors by reverse transcription-polymerase chain reaction (RT-PCR), radioligand binding assays and contractile response measurement in thyroxine-treated rats.

2. Materials and methods

2.1. Materials

The drugs used were obtained from the following sources: (±)-propranolol · HCl, (−)-phenylephrine · HCl, yohimbine · HCl, desmethyl-imipramine · HCl, (±)-normetanephrine · HCl, chlorethylclonidine, 5-methyl-urapidil (Research Biochemicals, Natick, MA, USA); BE2254 ((2-β(4-hydroxyphenyl)-ethylaminomethyl)-tetralone) (Beiersdorf, Hamburg, Germany); phentolamine mesylate (Ciba-Geigy, Summit, NJ, USA); [^{125}I]Na^+ (Beijing Institute of Atomic Energy, Chinese Academy of Science). Avian myeloblastosis virus reverse transcriptase (AMV-RT), oligo dT, Taq polymerase (Promega, Beijing, People's Republic of China), and [α-^{32}P]deoxycytidine triphosphate ([α-^{32}P]dCTP, Furui, Beijing, People's Republic of China).

2.1.1. Preparation of hyperthyroidism rat

Male Wistar rats weighing 200–220 g were given a daily injection for 10 days of 200 μg/100 g of *l*-thyroxine (in 50 aqueous dimethyl sulfoxide) or vehicle alone. The levels of serum thyroid hormones were determined by radioimmunoassay.

2.2. Radioligand binding assays

The rats were killed by cervical dislocation and the hearts were removed. The left atria were dissected and ventricular crude particulate fractions were made as described previously (Minneman et al., 1988). Briefly, tissue was homogenized with a Polytron in 20 ml of 20 mM phosphate buffer solution (PBS, pH 7.6) containing 154 mM NaCl, centrifuged at 20000 × *g* for 10 min, and resuspended in the PBS, where specified to the appropriate tissue concentration.

BE2254 was radioiodinated to a theoretical radioactivity of 2200 Ci/mmol as described by Engel and Hoyer (1981) and stored at −20°C in methanol. Measurement of specific ^{125}I-BE2254 binding was performed by incubating tissue preparations with ^{125}I-BE2254 in PBS in a final volume of 250 μl for 20 min at 37°C in the presence or absence of competing drugs. After 20 min, the incubation was terminated by adding 10 ml of 10 mM Tris-HCl (pH 7.4) and the mixture was filtered over a glass fiber filter under vacuum. Each filter was washed with 10 ml of 10 mM Tris-HCl (pH 7.4), dried and its radioactivity (cpm, counts per minute) was measured. Nonspecific binding was determined in the presence of 10 μM phentolamine. In the experiments, the nonspecific binding was less than 15%. Protein concentrations of the preparation were measured by the Coomassie Brilliant Blue method (Bradford, 1976), using bovine serum albumin as standard.

To determine the affinity (K_D) and the maximal binding capacity (B_{max}) of ^{125}I-BE2254 to α_1-adrenoceptors, saturation curves were determined by incubating membrane preparations with increasing concentrations of ^{125}I-BE2254 (12–360 pM, 15000–500000 cpm) and the data were analyzed by the method of Scatchard (1949). To determine the affinity of 5-methyl-urapidil for α_1-adrenoceptors, the potency of 5-methyl-urapidil in competing for the specific ^{125}I-BE2254 binding sites was determined by incubation of one concentration of ^{125}I-BE2254 (35–40 pM, around 50000 cpm) in the presence or absence of 14 concentrations of the antagonist. IC_{50} values were determined as the *x* intercept on a Hill plot, and K_i values were calculated by the method of Cheng and Prusoff (1973). The best two-site fit and one-site fit for a competitive inhibition curve were calculated by computer (NONLIN and BY HAND programs which were kindly provided by Dr. K.P. Minneman). Whether the two-site fit was significantly better than the one-site fit was determined by *F* test.

2.3. Inotropic responses

After the rats were killed by cervical dislocation, the hearts were exposed and the left atria were rapidly removed. The tissues were placed in Krebs solution (composition in mM: NaCl, 120; KCl, 5.5; $CaCl_2$, 2.5;

NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $NaHCO_3$, 20; dextrose, 11; and $CaNa_2EDTA$, 0.029) equilibrated with 95% O_2-5% CO_2 and maintained at 37°C. The Krebs solution, in an organ bath with a volume of 10 ml, contained 1 μM propranolol to block the β-adrenoceptor response, 0.1 μM yohimbine to block the α_2-adrenoceptor response, and 0.1 μM desmethylimipramine and 1 μM normetanephrine to block the uptake of norepinephrine by nerve endings and cardiac tissues. A resting tension of 0.5 g was applied to all the preparations. The basal tension was not changed significantly by the blockers (354 ± 20 mg vs. 348 ± 17 mg, $n = 10$). The tissues were attached to force-displacement transducers for measurement of isometric tension. Inotropic responses of left atria were measured by placing the tissue between two platinum electrodes and stimulating the atria electrically (1 Hz, 5 ms, 2 times threshold voltage). Preparations were allowed to equilibrate for at least 60 min.

In the experiments examining the effect of chlorethylclonidine the cumulative concentration-response curves for phenylephrine were generated first. After washing and 40 min equilibration, preparations were incubated with or without 50 μM chlorethylclonidine for 40 min. Preparations were then washed and equilibrated for a further 40 min, and cumulative concentration-response curves for phenylephrine were repeated.

pA_2 values for 5-methyl-urapidil were determined by the method of Arunlakshana and Schild (1959). Concentration-response curves for phenylephrine in the absence and presence of 10 nM, 30 nM and 100 nM 5-methyl-urapidil were generated consecutively with an interval of 40 min between each two curves to allow 5-methyl- urapidil to equilibrate with the tissues (solution in baths was changed every 8 min). Our preliminary experiments showed that there was no significant difference among four consecutively generated concentration-response curves for phenylephrine (including the basal tension) in the absence of antagonists.

2.4. RNA preparation and RT-PCR

Total RNA of rat heart was isolated from fresh tissues by acid guanidium thiocyanate-phenol-chloroform extraction (Chomzynski and Sacchi, 1987). The purified RNA collected was confirmed by visualization of the 28S and 18S ribosomal RNA bands after electrophoresis of RNA through a 1% agarose-formaldehyde ethidium bromide gel.

The oligonucleotides for three distinct α_1 subtypes were synthesized on a DNA synthesizer (Applied Biosystems, model 39A). The oligonucleotides were constructed from the cDNA sequences of cloned rat α_{1A} (Rokosh et al., 1994), α_{1B} (Voigt et al., 1990) and α_{1D} (Lomasney et al., 1991) cDNA. The sequences of the primers were 5′-CGAGGCCTCAAGTTCCGGCCT-3′ (coding sense) and 5′-TCTCGGGAAAACTTGAGCAG-3′ (coding antisense) for α_{1A} (α_{1C}); 5′-ATCGTGGCCAAGAGGACCAC-3′ (coding sense) and 5′-CGGGAGAGCGATGAAGAAGG-3′ (coding antisense) for α_{1B}; 5′-CGTGTGCTCCTTCTACCTACC-3′ (coding sense) and 5′-GCAGTGCTCCTTCTACCTACC-3′ (coding antisense) for α_{1D}, 5′-GAGACCTTCAACACCCCAGCC-3′ (coding sense) and 5′-TCGGGGCATCGGAACCGCTCA-3′ (coding antisense) for β-actin. The PCR amplification cycle consisted of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and extension at 72°C for 1 min. Negative control reactions without template were routinely included in PCR amplifications with both primer sets. PCR amplifications for the receptors were carried out by using 28 cycles, whereas for β-actin only 20 cycles were used. The reverse transcription reaction was conducted by using 5 μg total RNA, 0.1 μg oligo dT, 5 units of AMV reverse transcriptase in a total volume of 25 μl. In each PCR reaction, 10 μCi [α-^{32}P]dCTP was added to a total volume of 30 μl. The PCR reaction buffer contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 0.1% gelatin, 1% Triton X-100, 20 pmol primers, 100 μM dNTP and 1 unit of DNA polymerase. The PCR products were purified after polyacrylamide electrophoresis and visualized by autoradiography after an exposure of 24 h at −80°C. The radiation intensities of the signals were quantified by a laser densitometer, which expressed densities in arbitrary units, i.e. ratios of the sample intensities to an inner standard intensity. Then the values for bands were normalized by the value for the β-actin (internal standard) band in the same experiment and the final results were expressed as ratios of them.

2.5. Statistics

The results shown in the text and tables were expressed as means ± S.E.M. Statistical analysis of the data was done by Student's t-test, or Student's paired t-test when data were for the same preparation. Two groups of data were considered to be significantly different when $P < 0.05$.

3. Results

3.1. The changes in serum thyroid hormone levels in the thyroxine-treated rats

In the thyroxine-injected rats ($n = 5$), the serum concentration of 3,5,3′-triiodothyronine (T_3) was increased from 0.59 ± 0.16 pg/ml in controls ($n = 5$) to > 9.0 pg/ml ($P < 0.05$), and the serum concentration

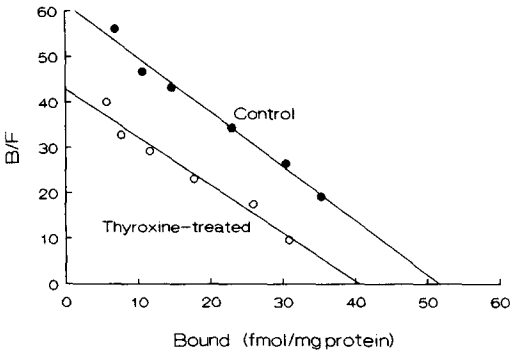

Fig. 1. Scatchard analysis of ^{125}I-BE2254 specific binding in membrane preparations from rats with different thyroid status. Each point is the mean of duplicate determinations from five experiments.

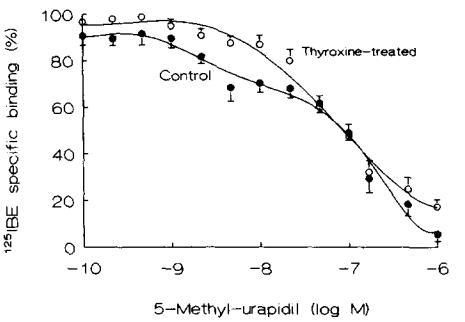

Fig. 2. Inhibition of ^{125}I-BE2254 specific binding by 5-methyl-urapidil in heart membrane preparations from thyroxine-treated or control rats. Each point is the mean of duplicate determinations from five experiments.

of thyroxinine (T_4) was increased from 64 ± 10 pg/ml in controls to > 355 pg/ml ($P < 0.05$).

3.2. Scatchard analysis of ^{125}I-BE2254 binding sites and two-site analysis for 5-methyl-urapidil competitive inhibition curves

The Scatchard analyses for the saturation curves of ^{125}I-BE2254 binding to the crude membrane preparations of ventricles yielded a B_{max} of 51.6 ± 6.0 fmol/mg protein and a K_D of 86.6 ± 8.9 pM ($n = 5$) for the control, and a B_{max} of 40.9 ± 3.7 fmol/mg protein ($P < 0.01$ vs. control) and a K_D of 93.8 ± 13.0 pM for the thyroxine-treated rats (Fig. 1).

^{125}I-BE2254 binding was competitively inhibited by 5-methyl- urapidil in a concentration-dependent manner. The inhibition curves for the control and thyroxine-treated rat hearts were both relatively shallow and the Hill coefficients of the two groups were both significantly less than 1.0. Computer analysis showed that the curves were better fitted by a two-site model than a one-site model ($P < 0.01$). The percentage of high-affinity sites for 5-methyl-urapidil was significantly lower in the thyroxine-treated rats than in the control rats (10.8 ± 2.0% vs. 23.3 ± 2.0%, $P < 0.05$) (Table 1, Fig. 2).

3.3. Changes of inotropic response mediated by α_1-adrenoceptors in isolated electrically driven left atria

There was no difference in basal tension between the control and thyroxine-treated rats (344 ± 20 vs. 364 ± 41 mg). Phenylephrine produced positive inotropic responses in a concentration-dependent manner in both groups. The pD_2 values were similar in the two groups, but the maximal contraction induced by phenylephrine in the thyroxine-treated group was only 73% of that in the control group ($P < 0.05$, Table 2).

Chlorethylclonidine preincubation significantly diminished the phenylephrine-induced contractions in both groups. However, compared with the control group, the concentration-response curves for phenyl-

Table 1
Inhibition by 5-methyl-urapidil of ^{125}I-BE2254 specific binding to membranes of myocardium from control or thyroxine-treated rats

	n	n_H	pK_i	pK_{low}	pK_{high}	% of R_{high}
Control	5	0.44	7.44 ± 0.25	6.82 ± 0.41	9.40 ± 0.20	23.3 ± 2.0
Thyroxine treated	5	0.69	7.33 ± 0.12	6.89 ± 0.09	8.86 ± 0.52	10.8 ± 2.0 [a]

$pK_i = -\log[K_i]$; $pK_{low} = pK_i$ of low-affinity sites; $pK_{high} = pK_i$ of high-affinity sites; R_{high} = high affinity binding site. Data are expressed as means ± S.E.M. [a] $P < 0.05$ compared with control.

Table 2
Effect of chlorethylclonidine preincubation on the phenylephrine-induced contractions in isolated left atria from control and thyroxine-treated rats

	n	pD_2		R_{max} (mg)	
		− CEC	+ CEC	− CEC	+ CEC
Control	5	5.22 ± 0.07	4.43 ± 0.07 [a]	258 ± 17	112 ± 13 [a]
Thyroxine-treated	5	5.18 ± 0.08	4.81 ± 0.08 [a]	188 ± 24 [b]	100 ± 18 [a]

R_{max} = maximal contractile response; − CEC = in the absence of chlorethylclonidine; + CEC = in the presence of chlorethylclonidine. Data are expressed as means ± S.E.M. [a] $P < 0.001$, compared with − CEC (paired t-test); [b] $P < 0.05$, compared with control (t-test).

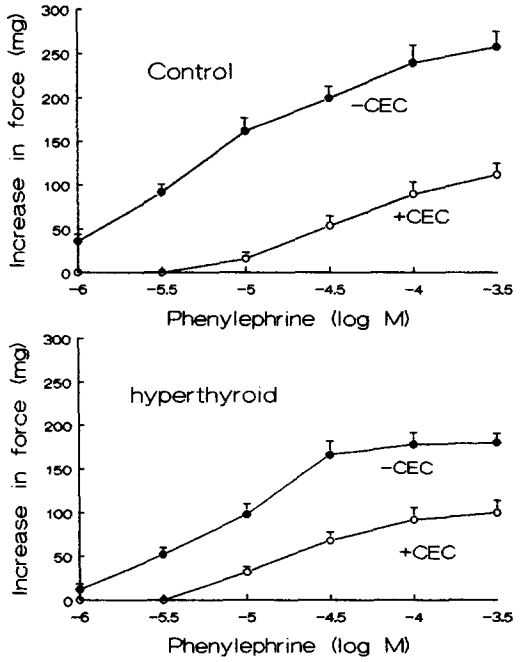

Fig. 3. Effect of chlorethylclonidine preincubation on phenylephrine-induced positive inotropic responses in isolated electrically driven left atria from control (top) and thyroxine-treated (bottom) rats. Each point is the mean ± S.E.M. of five experiments.

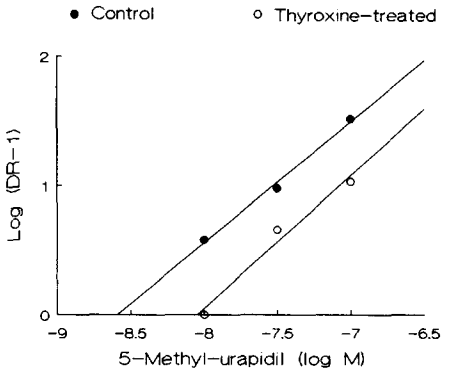

Fig. 4. Schild plots for competitive inhibition of phenylephrine-induced contraction by 5-methyl-urapidil in isolated electrically driven left atria of rats. Each point is the mean from five experiments.

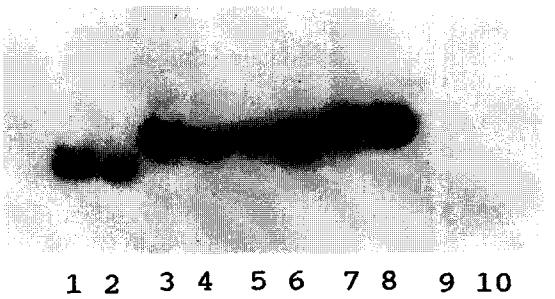

Fig. 5. RT-PCR assay of α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor mRNA expression in hearts from control (lanes 1, 3 and 5) and thyroxine-treated rats (lanes 2, 4 and 6). β-Actin was taken as a control for the quality and amount of mRNA samples applied, which was not different between the two groups (lanes 7 and 8). Lanes 9 and 10 are the negative controls conducted as described in the text. The PT-PCR was performed as described under Materials and methods. This radioautograph picture is representative of four experiments, all of which showed similar results.

ephrine were shifted less to the right (2.4 ± 0.3- vs. 7.2 ± 0.9-fold, $P < 0.001$) and the maximal contractions induced by phenylephrine were reduced less (by 46.8 ± 3.4% vs. 57.5 ± 3.0%, $P < 0.05$) in the thyroxine-treated group (Table 2, Fig. 3).

5-Methyl-urapidil shifted the concentration-response curve for phenylephrine parallel to the right without causing a significant change in the maximal contraction in both groups. The pA_2 value was 7.87 ± 0.20 in the thyroxine-treated group, which was significantly less than the 8.89 ± 0.19 ($P < 0.05$) in the control group. The slopes were not significantly different from the unity of the control (0.94) and the thyroxine-treated (1.09) groups (Fig. 4).

3.4. Results of RT-PCR

In hearts from thyroxine-treated rats, the PCR products for both α_{1A} and α_{1B} subtypes were significantly decreased, whereas the α_{1D} product was significantly increased (Table 3). The above data were from four experiments. Fig. 5 shows a typical result from one experiment.

4. Discussion

We have observed in the present study that the density of α_1- adrenoceptors in ventricular preparation

Table 3
Changes in the cardiac steady-state levels of mRNA for α_{1A}, α_{1B}- and α_{1D}-adrenoceptors in thyroxine-treated rats

	n	α_{1A}	α_{1B}	α_{1D}
Control	4	0.77 ± 0.09	0.82 ± 0.13	0.62 ± 0.05
Thyroxine-treated	4	0.59 ± 0.11 [a]	0.59 ± 0.07 [a]	1.08 ± 0.11 [a]

The steady-state levels of mRNA for the three subtypes of α_1-adrenoceptor were determined by RT-PCR as described in Materials and methods. The radiation intensities of the signals were quantified with a laser densitometer. Values for bands of adrenoceptors were normalized to the value of the β-actin (internal standard) band in the same experiment, and the final results were expressed as ratios of them. [a] $P < 0.05$ compared with the control.

prepared from thyroxine- treated rats was decreased significantly, which was in parallel to the reduction of the agonist-induced maximal contraction in isolated atrium. These results were consistent with the former reports (Williams and Lefkowitz, 1979; Sharma and Banerjee, 1978; Fox et al., 1985; Limas and Limas, 1987; Hanft and Gross, 1990), and suggest that the decline in receptor density would account for, at least in part, the diminished inotropic responsiveness of the heart.

The experiment of inhibition of ^{125}I-BE2254 specific binding by 5-methyl-urapidil indicated that the percentage of high affinity sites decreased from 23.3% in the control rats to 10.8% in the thyroxine-treated rats. Since the B_{max} value was decreased in thyroxine-treated rats (from 51.6 to 40.9 fmol/mg), the calculated high- and low-affinity sites were 12.0 and 39.6 fmol/mg in control rats, and 4.4 and 36.5 fmol/mg in thyroxine-treated rats. This result indicates that only the density of high affinity sites for 5-methyl-urapidil was changed after thyroxine treatment. The latest research data on the pharmacological characteristics of subtypes show that the α_{1A}-adrenoceptor has high affinity for 5-methyl-urapidil, while α_{1B}- and α_{1D}-adrenoceptors have a similar low affinity for 5-methyl-urapidil (Faure et al., 1994; Ford et al., 1994). So the binding results of the present study suggest that after thyroxine treatment (hyperthyroidism) the density of α_{1A}-adrenoceptors is reduced significantly, but the density of α_{1B}- together with α_{1D}-adrenoceptor is not changed, which does not necessarily mean that neither α_{1B}- nor α_{1D}-adrenoceptors are changed. Our functional experiments showed that preincubating preparations with the irreversible α_1-antagonist chlorethylclonidine blocked the phenylephrine-induced contraction to a lesser extent in the thyroxine-treated group than in the control group. The use of chlorethylclonidine as a tool to distinguish the three α_1-adrenoceptor subtypes has become controversial recently (Faure et al., 1994; Ford et al., 1994). It is, however, generally agreed that the α_{1B}-adrenoceptor is most susceptible to be irreversibly inactivated by chlorethylclonidine. Thus the α_{1B}-adrenoceptor, according to the data from experiments with chlorethylclonidine, is likely to be reduced in hyperthyroidism.

Since available drugs are not selective enough to distinguish the three subtypes of α_1-adrenoceptor pharmacologically, the best way to determine the distribution of the subtypes in tissues is to detect the mRNA levels concomitantly with binding and functional experiments. Our RT-PCR data showed that the steady-state level of mRNA for α_{1A}-adrenoceptors declined, which was coincident with the reduction of α_{1A}-adrenoceptors in the binding experiment. The steady-state level of mRNA for α_{1B}-adrenoceptors was decreased while that for α_{1D}-adrenoceptors was increased, which suggests an opposite direction of changes in receptor protein level for the two subtypes. Since the total sum of these two subtypes (α_{1B} and α_{1D}) was not changed and they have similar affinity for 5-methyl-urapidil, the density of low affinity sites for 5-methyl-urapidil may not be altered, as also indicated by the results of binding and functional experiments.

The mechanisms by which thyroid hormones regulate the mRNA levels for adrenoceptors and the nature of the subtype-selective regulation are not clear. It is generally known that thyroid hormones regulate the transcription of certain genes by binding to specific DNA sequences called hormone-response elements. In addition, thyroid hormones may also affect the stability of certain kinds of mRNA (Evans, 1988; Hadcock and Malbon, 1991). These mechanisms may be involved in the changes in the present experiments.

Previous studies suggest that both α_{1A}- and α_{1B}-adrenoceptors mediate positive inotropic responses (Takanashi et al., 1991; Endoh et al., 1992a, b), and that the mechanisms underlying the response to stimulation of the respective subtypes may be different (Yu and Han, 1994). However, there have been no studies of the roles of α_{1D}-adrenoceptors in the agonist-induced inotropic response. Therefore, from our results we cannot infer which subtype(s) of α_1-adrenoceptor is responsible for the decreased positive inotropic response to phenylephrine observed in this study. The possibility of altered post-receptor cascades in myocardium of thyroxine-treated rats cannot be excluded either.

Acknowledgements

This study was supported by grants from the Natural Science Foundation of China and by grant No. 93-591 from the China Medical Board of New York Inc.

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