The influence of experimental hyperthyroidism on responsiveness in rabbit aortic smooth muscle

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Abstract

This study investigated the responsiveness of hyperthyroid and control (euthyroid) rabbit aortic rings to vasoconstrictor agents. There was no significant difference between the responsiveness of hyperthyroid and control tissues to KCl. Maximum responses ($E_{\max}$) to noradrenaline and serotonin were reduced in hyperthyroid rabbit aortic rings compared to those euthyroid rings. The sensitivity (i.e., $EC_{50}$) of hyperthyroid aortic rings to noradrenaline compared to euthyroid controls was unchanged. The $K_b$ value of prazosin against $10^{-6}$ M noradrenaline-induced contractions in eu- and hyperthyroid states was $1.97 \pm 0.59 \times 10^{-9}$ M and $1.92 \pm 0.79 \times 10^{-9}$ M, respectively. The $K_b$ value of methisergide against $10^{-6}$ M serotonin-induced contractions in eu- and hyperthyroid states was $1.46 \pm 0.80 \times 10^{-9}$ M and $1.10 \pm 0.16 \times 10^{-9}$ M, respectively. These data indicate that vascular reactivity is altered in experimental hyperthyroidism and this alteration, if any, do not occur at the receptor level. © 1997 Elsevier Science B.V.

Keywords: Hyperthyroidism; Vascular smooth muscle; Noradrenaline; Serotonin; Rabbit

1. Introduction

The relationship between the levels of circulating thyroid hormones and the response of smooth muscles and heart to adrenergic stimulation has been studied for many years. Many of the clinical manifestations of hyperthyroidism resemble those observed with increased adrenergic tone, including tachycardia, tremor and enhanced thermogenesis, lipolysis and glycogenolysis (Harrison, 1964; Waldstein, 1966). It has been suggested that an altered responsiveness to catecholamines may be primarily responsible for many of the symptoms of thyrotoxicosis and thyroid insufficiency.

Studies on the effects of hyperthyroidism on catecholamine sensitivity have been carried out mainly in the heart. In hyperthyroidism the number of $\beta$-adrenoceptors and thus the responses to $\beta$-agonists are increased (Scarpace and Abrass, 1981; Stiles and Lefkowitz, 1981; O'Donnell and Wanstall, 1986) or unchanged (Banarjee and Kung, 1977). Conversely, the density of $\alpha$-adrenoceptor binding sites has been shown to decrease in hyperthyroid animals (Sharma and Banerjee, 1978; Kunos et al., 1980). In vascular smooth muscle tissues the effect of thyroid hormones on adrenoceptors are controversial. Experiments on isolated vascular tissue derived from hyperthyroid animals have shown an increase (Grassby and McNeill, 1983), a decrease (Coville and Telford, 1970), or no change in sensitivity (Rahman et al., 1987, Fox et al., 1985). In this background, the present study, the effect of hyperthyroidism on the responsiveness of the rabbit aorta to both noradrenaline and serotonin was examined to understand the underlying possible mechanisms involved.
2. Materials and methods

Albino rabbits of either sex weighing 2.5–3 kg were used. Hyperthyroidism was induced by intraperitoneally injections of 0.25 mg/kg per day L-triiodothyronin (T3) in alkaline saline solution (0.01 N NaOH in 0.9% NaCl) given for 14 days (n = 12). The control rabbits (euthyroid status) were injected with vehicle (0.01 N NaOH in 0.9% NaCl) (n = 12). Blood samples from treated and control animals were collected at the time of death and serum T₃ and T₄ levels were measured by radioimmunoassay (RIA) methods.

Rabbits were anesthetized with ketamine (50 mg/kg i.m.) and xylazine (5 mg/kg i.m.) and the aorta was removed and placed in Krebs-Henseleit solution (composition in millimolar: NaCl 118, KCl 4.7, NaHCO₃ 24.9, KH₂PO₄ 1.2, CaCl₂ 1.6, MgSO₄ 1.2 and glucose 11.1). The isolated aorta was cleaned of surrounding connective tissue. A 3–4 mm ring segment was taken from the aorta then cut longitudinally to form rectangles which were mounted using small tissue clips in a 20 ml chamber containing Krebs-Henseleit maintained at 37°C and aerated with 95% O₂-5% CO₂. Aortic rings were allowed to equilibrate for 90 min under resting tensions of 1.5 g. During the equilibration period the tissue bathing solution was changed every 15 min. Isometric contractions of aorta were recorded using a Grass FT 03 Force-Displacement transducer (Grass Medical Instruments, Quincy, MA) and displayed on a Grass 79 E polygraph (Grass Medical Instruments, Quincy, MA).

2.1. Agonist-induced contractions

Tissues were exposed to 80 mM KCl for 5 min to test the viability of the preparation. Tissues were then washed, and thereafter bathing fluid was changed every 15 min except during cumulative agonist administration. After 30 min, agonists were added cumulatively in 0.5 log unit increments beginning with 10 nM (noradrenaline and serotonin).

2.2. Antagonist-induced relaxations

Submaximal concentration of (10⁻⁶ M) noradrenaline or serotonin was administered and when the contraction had reached a plateau, the antagonist was added cumulatively in 1 log unit increments beginning with 0.1 nM (prazosin or methysergide). Since prazosin was dissolved in 0.01 N HCl containing absolute methanol, the vehicle was also used to observe its effect, if any, on the contractile responses of the blood vessels.

2.3. Analysis of the data

The observations were expressed in terms of maximum response (Eₘ) and concentration of agonist producing half maximum contraction (EC₅₀). Potency of agonists was expressed as a Kb value.

Since one aim of the study was to compare the contractile responsiveness of eu- and hyperthyroid arterial smooth muscle, normalization of the active developed force for variation in wall thickness was essential. In this study, the maximum tension generated by an individual agonist was normalized in terms of cross-sectional area. At the end of experiments, tissues were blotted lightly, measured and weighed. The cross-sectional area of each preparation was calculated from length and weight, assuming a tissue density of 1.05 mg/mm³, and developed tensions were expressed per cross sectional area.

EC₅₀ values were calculated by linear regression of the points between 20 and 80% of the maximum response to each agonist.

The concentration-response data of an individual experiment was plotted as the response/concentration against the response (x). This produced a straight line relationship in each experiment as predicted from the Scatchard equation for drug-receptor interaction:

\[ \text{response/concentration} = \frac{1}{EC_{50}} \times \text{response} + \frac{\text{maximum response}}{EC_{50}} \]

In the present study, Kb values for prazosin or methysergide were determined against noradrenaline or serotonin, respectively using the following formula:

\[ K_b = \frac{IC_{50}}{1 + C/EC_{50}} \]

C: concentration of agonist; IC₅₀: concentration of antagonist producing half maximum inhibition; EC₅₀: concentration of agonist producing half maximum contraction.

Briefly, the artery preparations were exposed to a submaximally effective concentration of agonist. After a stable plateau tension had developed, the increasing concentration of antagonist was added to the bath. Differences between groups were assessed by unpaired Student's t-test. Level of significance was \( P < 0.05 \).

2.4. Drugs

The following drugs were used: 5-hydroxytryptamine hydrochloride (Sigma), noradrenaline bitartrate (Sigma), prazosin hydrochloride (Pfizer), methysergide hydrogenmaleat (Sandoz). Drugs were dissolved in dis-
Table 1
Effects of $T_3$-treatment of rabbits on body weight and total serum $T_3$ and $T_4$ concentration

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>$T_3$ (pg/ml)</th>
<th>$T_4$ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ($n = 12$)</td>
<td>2.90 ± 0.45</td>
<td>1.34 ± 0.53</td>
</tr>
<tr>
<td>Hyperthyroid ($n = 12$)</td>
<td>1.85 ± 0.50*</td>
<td>294 ± 117*</td>
</tr>
</tbody>
</table>

Values shown are means ± S.E.M.
$n$, number of animals.
*Significantly different from control group by Student’s t-test, $P < 0.05$.

Tilled water except for prazosin and dilutions were made up in distilled water. Prazosin was dissolved in 0.01 N HCl containing absolute methanol. All stock solutions were prepared just prior to use.

3. Results

The effect of $T_3$-administration on animal weight and serum $T_3$ and $T_4$ levels is shown in Table 1. Pretreatment with $T_3$ produced a significant reduction in the animal weight gain and elevation in serum levels of thyroxine and triiodothyronine above controls ($P < 0.05$).

The concentration-response relationship of the aortae from eu- and hyperthyroid rabbits to various concentrations of noradrenaline and serotonin is shown Figs. 1 and 2. In the hyperthyroid state, the noradrenaline and serotonin concentration-response curve was shifted to the right and the maximum amplitude ($E_{\text{max}}$) of contraction evoked by noradrenaline and serotonin was inhibited compared with that observed in euthyroid rabbits ($P < 0.05$) (Table 2). The $EC_{50}$ values of noradrenaline and serotonin were unchanged (Table 2).

The responses of aortic rings from hyperthyroid rabbits to 80 mM KCl were not significantly different from those obtained with aortic rings from euthyroid rabbits (not shown).

To investigate further, the action of thyroid hormones on responses to noradrenaline and serotonin receptors, the effects of prazosin and methisergide on noradrenaline-and serotonin-induced contractions were investigated, respectively (Figs. 3 and 4). Prazosin and methisergide completely inhibited $10^{-6}$ M noradrenaline- and $10^{-6}$ M serotonin-induced contraction in the two different thyroid states, respectively. There was no significant difference between the $K_b$ values of both prazosin and serotonin in the aortae from either hyperthyroid or euthyroid rabbits (Table 3).

Table 2
Concentration of agonist producing half maximum contraction ($EC_{50}$, M) and maximum response ($E_{\text{max}}$, g/mm²) values for noradrenaline and serotonin in aortic rings

<table>
<thead>
<tr>
<th></th>
<th>$EC_{50}$ ($\alpha = 12$)</th>
<th>$E_{\text{max}}$ ($\alpha = 12$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>5.10 ± 6.50</td>
<td>3.08 ± 0.05</td>
</tr>
<tr>
<td>Prazosin</td>
<td>0.36 ± 0.01</td>
<td>0.40 ± 0.01</td>
</tr>
<tr>
<td>Serotonin</td>
<td>2.60 ± 4.50</td>
<td>3.05 ± 0.07</td>
</tr>
<tr>
<td>Methisergide</td>
<td>0.91 ± 0.01</td>
<td>0.95 ± 0.01</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td></td>
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</tbody>
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Values shown are means ± S.E.M.
$n$, number of animals.
*Significantly different from control group by Student’s t-test, $P < 0.05$. 

Fig. 1. Concentration-response curves for the contractile response to noradrenaline. Significant difference from control response is marked by *($P < 0.05$). Each point represents data from 12 rings. Vertical bar represents standard error of the means (S.E.M.).

Fig. 2. Concentration-response curves for the contractile response to serotonin. Significant difference from control response is marked by *($P < 0.05$). Each point represents data from 12 rings. Vertical bar represents S.E.M.
4. Discussion

Thyroid hormone excess or deficiency, respectively, decreases or increases α-adrenergic receptor sensitivity in some tissues (Rosenqvist and Boreus, 1972; Hashimoto and Nakashima, 1980; Coville and Telford, 1970; Williams et al., 1977). Changes in receptor density caused by thyroid hormones have been suggested to account for changes in tissue sensitivity to catecholamines. Few previous studies have investigated the effects of hyperthyroidism on the sensitivity of vascular tissue to sympathomimetic amines. In this study, in hyperthyroid aortae, noradrenaline and serotonin responses were significantly decreased with no change in agonist potency (EC₅₀ values). Hyperthyroidism did also alter antagonist potency (Kᵦ values) for both prazosin and methisergide. This observation is consistent with previous reports (Coville and Telford, 1970; Gunasekera and Kuriyama, 1990) but it is a controversial with other studies which showed an increase (Grassby and McNeill, 1988; Field et al., 1973) or no change in sensitivity (Fox et al., 1985; Rahmani et al., 1987) in rat vascular tissue after thyroid hormone pretreatment. The mechanism(s) by which hyperthyroid status decreases the responsiveness to noradrenaline or serotonin in rabbit aortic smooth muscle are speculative. A decrease in maximal response to noradrenaline and serotonin in case of aortic rings from hyperthyroid rabbits noted in this study is indicative of a decrease in the number of receptors as well as receptor/post receptor defect. Gunasekera and Kuriyama (1990) found that in hyperthyroid rat within 7 days, α-adrenoreceptor density and basal level of IP₃ were significantly reduced but a normal pattern of stimulated IP₃ synthesis was observed. The results indicate that in hypothyroidism the density of α₁-adrenoceptors is decreased with changes in mechanical responses. Comparatively little is known about the effects of hyperthyroidism on receptors for other agonists (Grassby and McNeill, 1988). In rat, uterine smooth muscle, pretreatment with T₄ increases the sensitivity of this tissue to serotonin and histamine (Coville and Telford, 1970) and in rat mesenteric artery postjunctional supersensitivity was observed to serotonin (Grassby and McNeill, 1988) but in the guinea pig heart responses to histamine are not affected in the hyperthyroid state (MacLeod and McNeill, 1981). Many of the symptoms of hyperthyroidism resemble increased adrenergic tone. Conversely, an apparently decreased peripheral adrenergic function is often found in hypothyroid states (Harrison, 1964; Krishna et al., 1968). In addition it was found that in experimental hyperthyroidism the serotonin levels in blood are increased (Noll et al., 1988). Thus, it is thought that an increase catecholamines or serotonin levels in blood may be primarily responsible for decreased responsiveness to noradrenaline or serotonin. Such elevation of noradrenaline and serotonin levels may down regulate the receptors for noradrenaline and serotonin. This may account for at least part of the decreased responsiveness to noradrenaline or serotonin found in the

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Values shown are means ± S.E.M.

n = number of animals.
present study. However, an inverse relationship has been demonstrated between plasma thyroid hormone levels and catecholamine levels (Staffer et al., 1973) and in the present study, the $K_b$ values for antagonists (prazosin and serotonin) were unchanged in hyperthyroid states, suggesting that the decreased response to agonists do not occur at the receptor level. It was also possible that an increase in vasodilator activity could explain the decrease in the contractile responses to noradrenaline in preparations from $T_3$:treated rabbits, since thyroid hormones have been shown to increase the magnitude of the responses to, and/or the potency of the $\beta$-adrenoceptor agonists, by a mechanism which is specifically associated with the $\beta$-adrenoceptors (O'Donnell et al., 1987). Studies with vascular smooth muscle strips have demonstrated that thyroid hormones may mediate muscle relaxation (Ishikawa et al., 1985). Within 3 h, when heart rate and blood pressure were unchanged, $T_3$:caused a significant decrease in peripheral vascular resistance and a significant increase in stroke volume and cardiac output (Kapitola et al., 1979; Kapitola and Vilimovska, 1981). These data evaluated support a direct role of $T_3$ as a novel vasodilator on peripheral vasculature (Ojamaa et al., 1993). Another possibility is desensitization of medial muscular cells to noradrenaline and serotonin by hyperthyroidism. This possibility was not supported by the data from the present study, that KCl-induced contractile responses were not changed by treatment of $T_3$ in the arterial smooth muscle.

In conclusion, these results indicate that noradrenaline- and serotonin-induced contractions of aortae were significantly reduced but $K_b$ values for antagonists were unchanged in hyperthyroidism. However, no decrease in sensitivity was observed to KCl suggesting that this decreased sensitivity was not due to a decrease in the sensitivity of the contractile apparatus and that the decreased responsiveness related, at least in part, to an alteration in the regulation of postreceptor excitation-contraction coupling.

References

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