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SHORT TERM EFFECTS OF TRIIODOTHYRONINE ON RAT HEART ADRENOCEPTORS

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SUMMARY: Short term effects of triiodothyronine on cardiac adrenoceptors of hypothyroid rats were tested. In vitro incubation of ventricular slices with 10 nM triiodothyronine for 60 min increased the density without affecting the affinity of β-adrenoceptors, as determined from high-affinity binding of [^{3}H]-dihydroalprenolol. In vivo treatment with 0.5 mg/kg triiodothyronine caused a similar, small increase in β-receptor density within 2 hours, and a significant further increase within 36 hours. This increase in β-receptors was associated with a decrease in density of α_1-receptors, identified with [^{3}H]-prazosin. In isolated atria from the same rats, the positive chronotropic response to isoproterenol increased and the response to methoxamine decreased 36 hours after triiodothyronine. These results indicate inverse reciprocal regulation of cardiac post-synaptic α_1 and β-receptors by thyroid hormones.

INTRODUCTION

It has been recognized for a long time that thyroid hormones can influence the adrenergic reactivity of the myocardium (1), but the mechanism of this interaction is not fully understood. Stimulation of the mammalian myocardium by catecholamines is predominantly through β-adrenergic receptors, and several observations indicate a sensitization of β-receptor responses by thyroid hormones (2,3). The results of more recent radioligand binding studies suggest that thyroid hormones can increase the density but not the affinity of cardiac binding sites for radiolabelled β-receptor antagonists (3-6). There are also postsynaptic α-receptors in the heart (7), and thyroid hormones were shown to reduce both the physiological reactivity (8) and the density of these receptors (8,9).

Abbreviations: T_3 = l-triiodothyronine; [^{3}H]DHA = [^{3}H]dihydroalprenolol.

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The rat is a hyperthyroid animal (10), and the above effects of thyroid hormones are more prominent when the animals are first rendered hypothyroid (8,11). In the present experiments we studied the effects of in vitro and short-term in vivo treatment with triiodothyronine (T_3) on cardiac adrenoceptors in thyroidectomized rats.

METHODS

Male Sprague-Dawley rats (300-350 g) were surgically thyroidectomized 6-10 weeks prior to the experiments. Hypothyroidism was ascertained by a cessation of growth, dryness of fur, and a significant reduction of the beat rate of isolated pairs of atria. Some of the thyroidectomized animals were treated with a single dose of T_3, 0.5 mg/kg intraperioneally, 2 or 36 hours before the experiment. The animals were anesthetized with ether, the heart removed, and the atria separated from the ventricles. The spontaneously beating pairs of atria were suspended in 20 ml tissue baths containing Krebs' solution at 31°C and aerated with 5% CO_2 in O_2, and were connected to force-displacement transducers (Grass, FT03C). Contractions were recorded on a polygraph and heart rate in the absence or presence of drugs was counted at high paper speed. The ventricles were placed in ice-cold Krebs' solution and were freed from connective and fat tissue. For in vitro incubation with T_3, ventricles from thyroidectomized rats were cut into 0.5 mm thick slices and were divided into two aliquots, each containing approximately 200 mg tissue slices in 20 ml Krebs' solution in an Ehrlenmeyer flask. The tissue slices were incubated at 37°C in a shaker bath under an atmosphere of O_2:CO_2 95:5. T_3 at a final concentration of 10 nM was added to one of the aliquots, and both were incubated for 60 min. At the end of the incubation slices were collected by centrifugation, they were washed 3 times with fresh buffer and were homogenized to obtain crude membranes for ligand binding assays.

Crude cardiac membranes were prepared either from the ventricular slices described above or from freshly removed ventricles, as described elsewhere (8). β-receptor binding sites were quantitated by [^{3}H]dihydroalprenolol ([^{3}H]DHA, 34-48 Ci/mmole), α-receptors by [^{3}H]prazosin (17.1 Ci/mmole) (NEN) by established methods (12,13). Binding assays were carried out as described elsewhere (8). Briefly, membranes (0.3-0.4 mg/assay tube) were suspended in Krebs' solution and incubated for 15 min in a shaker bath at 31°C with 5-8 different concentrations of the labelled drug. The final incubation volume was 1.0 ml. Specific binding was determined in triplicate as the difference between total binding and binding in the presence of 1 μM propranolol for [^{3}H]DHA, or 2 μM phentolamine for [^{3}H]prazosin. Incubation was terminated by vacuum filtration through Whatman GF/C filters followed by three rapid 5 ml washes (< 15 sec) with Krebs buffer at room temperature. Radioactivity was counted by liquid scintillation spectrometry at 50-55% efficiency. Counting efficiency for each sample was determined by the channels ratio technique. Protein was determined by the method of Lowry et al (14) using bovine serum albumine as standard.

RESULTS

Fig. 1 illustrates the effect of in vitro incubation of hypothyroid heart slices with 10 nM T_3 on β-adrenoceptor binding sites. T_3 caused a small, but

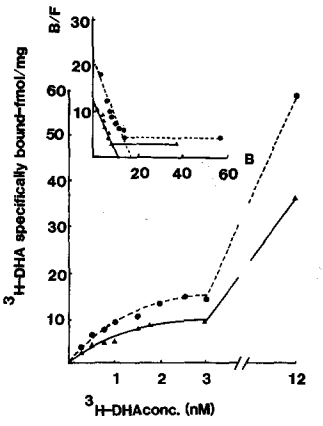

FIG. 1. The effect of in vitro incubation with T_3 on β-receptor binding sites in ventricular slices from hypothyroid rats. Saturation isotherms for [^{3}H]DHA and Scatchard-plots (inset) were constructed from pooled values from 3 separate experiments. In each experiment, heart slices from 2 hypothyroid rats were mixed and divided into 2 aliquots for incubation in buffer only (▲——▲) or in buffer containing 10 nM T_3 (●---●) for 60 min. Linear regression analysis gave a K_d of 0.91 and 0.85 nM and a b_{max} of 11.0 and 18.0 fmol/mg protein for the two groups, respectively.

significant increase in the density of [^{3}H]DHA binding sites without a change in their affinity. Binding was of high affinity and it saturated between 1.5 and 3 nM of the labelled ligand. Measurement of binding at 12 nM of [^{3}H]DHA revealed a second, low affinity binding component and an increased difference between the amount of ligand bound by control and T_3 incubated preparations. However, the significance of this second binding component is unclear and it probably represents nonspecific binding: recent studies in lung (15) and heart tissue (16) indicate that the propranolol suppressible binding of [^{3}H]-DHA has a nonstereoselective component at ligand concentrations above 3 to 5 nM. We also found that the density of the high affinity binding sites was consistently lower in membranes obtained from heart slices incubated in vitro than in those prepared from freshly removed hearts. Addition of a mixture of 19 amino acids (17) or various culture media to the incubation mixture for heart slices did not alleviate this problem, which was probably caused by breakdown of receptors by proteolytic enzymes or dissociation of receptors from membranes during prolonged incubation.

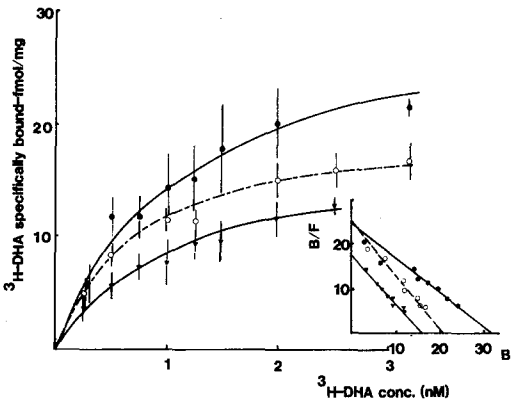

FIG. 2. The effects of in vivo treatment of hypothyroid rats with T_3 on cardiac β-adrenoceptor binding sites. ▼——▼ : untreated, o----o: 2 hours, and ●——●: 36 hours after 0.5 mg/kg T_3. Means and standard errors shown were obtained from 3 to 4 separate experiments. Inset: Scatchard-plots. The mean K_d was 0.92 ± 0.15, 0.85 ± 0.13, and 1.3 ± 0.17 nM and the mean b_{max} 15.3 ± 2.5, 20.2 ± 1.5, and 31.7 ± 4.4 fmol/mg in the three groups, respectively.

The effects of a single in vivo injection of 0.5 mg/kg T_3 on β-receptor binding sites in hearts from thyroidectomized rats are shown in fig. 2. A small, but significant increase in receptor density was apparent in rats sacrificed 2 hours after the administration of T_3, and there was a marked further increase in binding in hearts from rats killed 36 hours after T_3 injection. The K_d of [^{3}H]DHA was not significantly different in the three groups.

Cardiac adrenoceptors in the 36 h T_3-treated and control hypothyroid groups were further analyzed by parallel measurements of ligand binding in membrane fragments and of chronotropic responses of atria from the same hearts. Both α_1 and β-receptor binding sites were measured in the same membrane preparation. The sensitivity of functional β-receptors was assessed from the positive chronotropic response of isolated atria to isoproterenol. Although α-receptors are not involved in rate responses of euthyroid preparations (18), the appearance of such receptors in the hypothyroid myocardium is indicated by a dose-dependent increase in atrial rate produced by the pure α-receptor

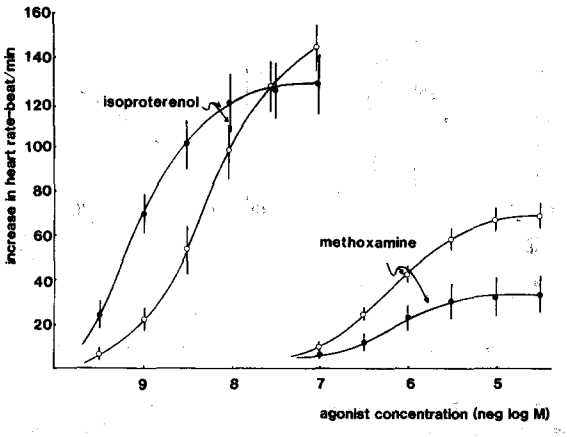

FIG. 3. The effect of T_3 treatment on chronotropic responses of isolated atria to isoproterenol and methoxamine. o——o: untreated hypothyroid (basal rate: 124 ± 7.0 beats/min, n = 8); ●——●: 36 h after T_3 (basal rate: 175 ± 10 beats/min, n = 8).

agonist methoxamine (fig. 3). As illustrated in fig. 3, a single dose of T_3 36 h prior to the experiment increased the sensitivity to isoproterenol, as indicated by a parallel shift to the left of the dose-response curve, whereas the rate response to methoxamine was decreased, as indicated by a downward displacement of the dose-response curve. In association with these changes, the density of β-receptor binding sites in the ventricular myocardium increased and the density of α_1-receptor binding sites decreased without changes in binding site affinities (fig. 4). We found significant day to day variations in receptor binding site densities in both control and T_3-treated preparations. In an attempt to eliminate the effects of such changes on the comparison of the two groups, untreated and T_3-treated rats were paired and killed at the same time. Receptor binding sites in these preparations were assayed simultaneously. The results shown in fig. 4 were replicated two more times.

DISCUSSION

The results presented indicate that triiodothyronine can increase the density of myocardial β-adrenoceptor binding sites in hypothyroid rats without affecting their affinity to dihydroalprenolol. A small but significant increase in β-adrenoceptors was apparent after in vitro incubation of hypothyroid

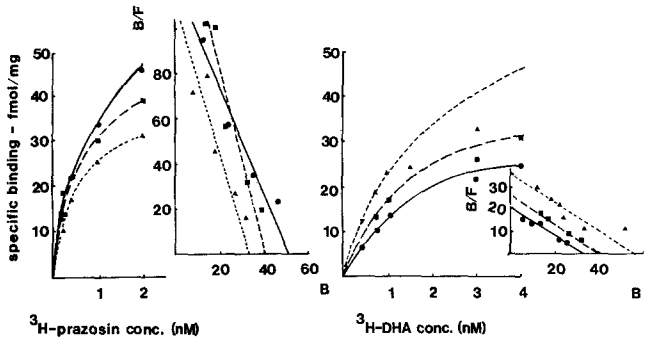

FIG. 4. The effect of T_3 treatment on cardiac α_1 and β-receptor binding sites. ●——●: untreated hypothyroid rat (Tx), ■---■ and ▲·····▲ : 2 hypothyroid rats treated with T_3 36 h before the experiment (Tx + T_3). All 3 animals were from the same operated batch and were sacrificed at the same time. Inset: Scatchard-plots. The density of β-receptor binding sites was 33.0 fmol/mg in Tx and 40.0 and 55.9 fmol/mg in Tx + T_3. K_d's for [^{3}H]DHA were 1.65, 1.5 and 1.52 nM, respectively. For α_1-receptors b_{max} was 49.6 fmol/mg in Tx and 33.2 and 39.9 fmol/mg in Tx + T_3. Respective K_d's for [^{3}H]prazosin were 0.49, 0.30 and 0.33 nM.

heart slices with T_3 or 2 hours following T_3 injection in vivo. These short-term effects of thyroid hormone are probably unrelated to the synthesis of new receptor protein (17) and may represent a direct membrane effect of thyroid hormone. The significant further increase in β-adrenoceptors at 36 hours is probably related to an effect of T_3 on protein synthesis (17). However, it is unclear whether the hormone directly affects the synthesis of the β-receptor binding protein, or whether it induces the synthesis of a cellular factor which would then directly influence both β- and α-receptor binding sites. Although speculative, this second possibility is attractive because it may explain by a single mechanism why thyroid hormone increases the density of β-receptors and at the same time decreases the density of α-receptors.

There has been some controversy as to whether or not thyroid hormone can increase the density of myocardial β-receptor binding sites, and this could be attributed to several factors in experimental design and methodology. Firstly, a study of adrenoceptor mediated mechanical responses of the rat heart indicate that the sensitivity of β-receptors is high in the euthyroid state, and the increase in sensitivity produced by thyroid hormone treatment is much smaller

than the decrease produced by hypothyroidism (10). This indicates that in the rat the regulatory mechanism of β-receptors has a high sensitivity to thyroid hormones, with near maximal 'up-regulation' in the euthyroid state. In accordance with these findings, high affinity [^{3}H]DHA binding sites were increased when hypothyroid rats but not when euthyroid rats were treated with T_3 (6). Thyroid-regulation of myosin ATPase activity in the rat heart shows an analogous pattern (10). Secondly, in some earlier reports the [^{3}H]DHA binding sites detected had a low affinity for the labelled antagonist (K_d: 6 to 19 nM). It has been demonstrated that such sites include a significant non-specific component that does not display stereoselectivity (15,16). The results in fig. 1 indicate that although T_3 can increase the density of high-affinity, β-receptor binding sites, it can increase the density of such low affinity binding sites even more. Therefore conclusions based on the behavior of [^{3}H]DHA binding with a K_d > 2-3 nM has to be regarded with caution. Finally, the present findings indicate that the increase in β-receptor density is associated with a decrease in the density of post-synaptic, α_1 receptors, and that these changes are paralleled by corresponding reciprocal changes in a physiological response mediated by the two receptors.

There is a significant receptor reserve for cardiac β-receptors mediating the rate response (19), which may explain why changes in receptor density are reflected by a parallel shift in the dose-response curve for a strong full agonist. On the other hand, studies with irreversible α-receptor antagonists suggest the lack of significant spare α-receptors in smooth muscle (20,21) as well as in cardiac preparations (11). Thus it is not unexpected that the T_3 induced decrease in the density of α_1-receptors is associated with a reduction of the maximal response to methoxamine rather than a rightward shift of the dose-response curve.

Naturally occurring catecholamines can stimulate both α- and β-receptors, and have higher affinity for the latter. The thyroid induced shift in the balance of these receptors in a tissue where both mediate the same end response

may be a mechanism of regulation of catecholamine sensitivity in a thyroid dependent tissue.

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