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THE INFLUENCE OF HORMONAL AND NEURONAL FACTORS ON RAT HEART ADRENOCEPTORS

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1 The influence of hormonal and neuronal factors on adrenoceptors mediating increased cardiac force and rate of contraction were studied in rat isolated atria. The pharmacological properties of these receptors were deduced from the relative potencies of agonists and from the effects of selective α- and β-adrenoceptor antagonists. The numbers and affinities of α- and β-adrenoceptors were also determined by radioligand binding to ventricular membrane fragments.

2 Hypophysectomy reduced the inotropic potency of isoprenaline and increased the potency of phenylephrine and methoxamine in left atria. The effect of phenylephrine was inhibited by propranolol less effectively and by phentolamine or phenoxybenzamine more effectively in hypophysectomized than in control rats. The difference in block was smaller at low than at high antagonist concentrations. Similar but smaller changes were observed for chronotropic responses of right atria.

3 The decreased β- and increased α-receptor response after hypophysectomy was similar to that observed earlier in thyroidectomized rats (Kunos, 1977). These changes developed slowly after hypophysectomy (>2 weeks), they were both reversed within 2 days of thyroxine treatment (0.2 mg/kg daily), but were not affected by cortisone treatment (50 mg/kg every 12 h for 4 days).

4 Treatment of hypophysectomized rats for 2 days with thyroxine increased the density of [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) binding sites from 27.5 $\pm$ 2.7 to 45.5 $\pm$ 5.7 fmol/mg protein and decreased the density of [^{3}H]-WB-4101 binding sites from 38.7 $\pm$ 3.1 to 18.7 $\pm$ 2.5 fmol/mg protein. The affinity of either type of binding site for agonists or antagonist was not significantly altered by thyroxine treatment and the sum total of α_1- and β-receptors remained the same.

5 Sympathetic denervation of thyroidectomized rats by 6-hydroxydopamine increased the inotropic potency of isoprenaline and noradrenaline and the blocking effect of propranolol, and decreased the potency of phenylephrine and the blocking effect of phenoxybenzamine to or beyond values observed in euthyroid controls. The density of [^{3}H]-DHA binding sites was higher and that of [^{3}H]-WB-4101 binding sites was lower in the denervated than in the innervated hypothyroid myocardium. Depletion of endogenous noradrenaline stores by reserpine did not significantly alter the adrenoceptor response pattern of the hypothyroid preparations and did not influence the density or affinity of [^{3}H]-DHA and [^{3}H]-WB-4101 binding sites.

6 These results indicate that thyrotropin or steroids do not contribute to the reciprocal changes in the sensitivity of cardiac α_1- and β-adrenoceptors in altered thyroid states. These thyroid hormone-dependent changes are probably due to a parallel, reciprocal change in the numbers but not the affinities of α_1- and β-adrenoceptors. Reciprocal regulation of cardiac α_1- and β-adrenoceptors by thyroid hormones requires intact sympathetic innervation but not the presence of normal stores of the neurotransmitter.

Introduction

Hormones can not only directly influence tissue functions but can also modulate tissue responsiveness to other hormones. Thyroid-catecholamine interactions have been recognised for a long time. Recent studies in rats have shown that the thyroid state can differentially affect α- and β-adrenoceptor mediated responses of the heart; thyroidectomy increased the α- and decreased the β-component in inotropic and chronotropic responses to sympathomimetic agents, and thyroid hormone treatment had opposite effects (Kunos, 1977). More recent studies have shown that thyroid hormones can change the numbers of binding sites of radioligands for α- and β-adrenoceptors in the heart (Ciaraldi & Marinetti, 1977; Banerjee & Kung, 1977;

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Williams, Lefkowitz, Watanabe, Hathaway & Besch, 1977; Sharma & Banerjee, 1978), although the relationship between low affinity binding sites detected in some of these studies and functional adrenoceptors in cardiac muscle is unclear (see Discussion).

Manipulations leading to altered thyroid hormone levels are associated with complex changes in endocrine functions as well as with changes in the activity of the sympathetic nervous system. The possible contribution of such factors to the changes in receptor response pattern induced by altered thyroid states is not clear. Thyroidectomy enhances and thyroxine treatment suppresses serum thyrotropin (TSH) levels. In some thyroid related changes, TSH rather than thyroid hormone levels appear to be the critical factor; it has been reported that the catecholamine content of mouse and rat adrenal medulla was decreased after treatment with exogenous thyroxine, but was increased either by treatment with TSH or propylthiouracil or by surgical thyroidectomy (Hopsu, 1960). One of the objectives of the present experiments was to determine whether reduced thyroid hormone levels or an increase in TSH is responsible for the shift from β- to α-receptor responses after thyroidectomy. Thus, we tested the adrenoceptor characteristics of isolated atria from hypophysectomized rats. Hypophysectomized rats are hypoadrenocorticoid as well as hypothyroid, and thyroidectomy can also lead to atrophy of the adrenal cortex (Labrie, Pelletier, Labrie, Ho-Kim, Delgado, MacIntosh & Fortier, 1968). The possible contribution of adrenocortical deficiency to altered myocardial responses in the hypophysectomized rats was therefore tested by *in vivo* treatment of such animals with a corticosteroid. Since thyroxin was found rapidly to reverse the altered α- and β-receptor sensitivity of the hypophysectomized heart, an attempt was made to correlate these changes with possible changes in the number and affinity of α- and β-adrenoceptors, as measured by binding of radioligands to cardiac membrane fragments.

Altered thyroid states can also influence the activity of the sympathetic nervous system. There appears to be an inverse relationship between thyroid state and both catecholamine synthesis and turnover rate in the rat myocardium (Axelrod, 1975). Therefore it seemed of interest to test the possibility that some factors related to sympathetic neuronal activity may have a role in the effects of altered thyroid states on cardiac adrenoceptor responses. This was done by treating thyroidectomized rats with either 6-hydroxydopamine to produce sympathetic denervation, or by reserpine to deplete endogenous stores of noradrenaline. An abstract of the results obtained has been published (Kunos & Mucci, 1975).

The results indicate that (a) thyroid hormones and not TSH or corticosteroids are the factors responsible for the shift from β- to α-receptor responses in the hypothyroid myocardium; (b) this change requires intact sympathetic innervation but not the presence of normal stores of the neurotransmitter; and (c) the rapid, reciprocal changes in α- and β-receptor sensitivity induced by thyroid hormones are associated with and probably due to similar, reciprocal and matching changes in the numbers of α_1- and β-receptors.

Methods

Experiments were done on isolated, electrically driven left and spontaneously beating right atria of male Sprague-Dawley rats. Rats, surgically hypophysectomized, weighing 80 to 200 g, were obtained from the Canadian Breeding Farms, Ltd. Practically all operated animals survived; their growth stopped completely. The success of the operation was also demonstrated by a drop of serum thyroxine (T4) levels to below measurable concentrations (Table 1) as determined by radioimmunoassay. The rate of beat of isolated right atria was also significantly lower in hypophysectomized than in age-matched control rats (see Figure 3). Most of the experiments on atria of hypophysectomized rates were done 4 to 6 weeks after the operation but some rats were tested 2 weeks, or 3 to 4 months post-operatively. Groups of hypophysectomized rats were treated with intraperitoneal injections of T4, 0.2 mg/kg daily for 2, 5 or 8 days, and another group was treated with cortisone acetate, 50 mg/kg every 12 h, for 4 days before the experiment.

Surgical thyroidectomy of normal rats weighing 180 to 200 g was performed as described earlier (Kunos, 1977). Hypothyroidism was ascertained by a reduction of growth, dryness of fur and a significant decrease in serum T4 levels (Table 1). Experiments on atria of thyroidectomized rats were done 4 to 6 weeks after the operation.

A group of thyroidectomized rats was chemically denervated by 6-hydroxydopamine (6-OHDA) treatment: four weeks following thyroidectomy, rats were given two intravenous doses of 6-OHDA, 50 mg/kg within 24 h, followed a week later by 2 × 100 mg/kg. Experiments were performed one week after the last dose of 6-OHDA. Control rats were denervated by a similar 6-OHDA treatment protocol. This treatment schedule was shown to reduce the noradrenaline content of the rat heart by more than 90%, and the resulting supersensitivity was qualitatively the same as that after other forms of sympathetic denervation (Haeusler, Haefely & Thoenen, 1969).

Another group of thyroidectomized rats was treated with reserpine to deplete noradrenaline stores without destruction of sympathetic nerve endings: reserpine was given intraperitoneally in a single dose of

5 mg/kg 24 h before the experiment, or in daily doses of 0.5 mg/kg for 7 days before the experiment. The timing of reserpine treatments was such that rats were killed exactly 6 weeks following thyroidectomy. Responses of atria were almost identical after the two reserpine treatment schedules and the results were pooled. Similar treatment schedules were shown to deplete more than 90% of cardiac noradrenaline stores (Paasonen & Krayer, 1957) although a small but functionally important store of noradrenaline was shown to persist after treatment with somewhat smaller doses of reserpine (Antonaccio & Smith, 1974).

Experiments on isolated atria

Animals were anaesthetized with ether and the hearts removed. Preparation of isolated left and right atria and recording of their isometric contractions at 31°C were done as described earlier (Kunos, 1977). Both basal tension and rate decreased initially, but there was little further change after 90 min of equilibration. Cumulative concentration-response curves for force (left atria) and rate responses (right atria) were then determined for different adrenoceptor agonists. Responses are expressed as the percentage of the maximal control increase in beat rate or amplitude. Absolute force and rate values are also given in some cases. Each preparation was used for several agonists but for only one adrenoceptor antagonist. Agonist concentration-response curves were determined after a standard 40 min exposure to the antagonist. Competitive antagonists remained in the medium, whereas phenoxybenzamine was washed out at the end of the 40 min incubation period. In the absence of an antagonist, repeated determination of agonist concentration-response curves did not alter either the concentration required to produce a half-maximal response (EC_{50}) or the maximal developed tension or the maximal rate. Statistical analysis of the data was made by Student's unpaired, or, where justified, the paired *t* test. Differences with a *P* value of less than 0.05 were considered significant.

Ligand binding in ventricles

A crude membrane preparation was obtained from ventricles of untreated hypophysectomized rats (3 to 4 months after the operation) and from similar animals treated with two injections of 0.2 mg/kg (−)-thyroxine (T4), 48 and 24 h before the experiment. The heart was removed under ether anaesthesia and the ventricles were separated from the atria. The ventricles were minced and homogenized in 40 volumes of Krebs solution at 4°C in a Polytron homogenizer driven at high speed for two 10 s bursts. The homogenate was filtered through 8 layers of cheese-cloth to remove connective and fat tissue, and was centrifuged at 22,000 *g* for 20 min in a Sorvall RC5-B centrifuge at 4°C. The pellet was resuspended in cold Krebs solution and recentrifuged (22,000 *g*, 20 min). The resulting pellet was then resuspended in Krebs solution and rehomogenized to give a final protein concentration of 0.5 to 0.8 mg/ml or 0.4 to 0.64 mg/assay tube, as determined by the method of Lowry, Rosebrough, Farr & Randall (1951). One mg protein was equivalent to 16.4 ± 0.5 mg initial wet tissue weight, and this ratio was not significantly different in any of the experimental groups tested.

[^{3}H]-dihydroalprenolol ([^{3}H]-DHA, specific activity 48.3 Ci/mmol) and [^{3}H]-(2-[(2′6′-dimethoxy)-phenoxyethylamino]methylbenzodioxan ([^{3}H]-WB-4101, specific activity 24.4 Ci/mmol) were used to label myocardial β- and α-adrenoceptor binding sites, respectively (Williams & Lefkowitz, 1978; Winek & Bhalla, 1979; Raisman, Briley & Langer, 1979). The ligands were obtained from New England Nuclear and their radiochemical purity was checked at frequent intervals by thin layer chromatography and radioscanning. Dilutions of the ligands were made in Krebs solution at 4°C immediately before use. Membranes were incubated with the radioactive and competing ligands in glass tubes for 15 min at 31°C, in a total volume of 1.0 ml. In aliquots removed from assay tubes before the end of the incubation, the total radioligand concentration measured was identical to the concentration calculated from the dilution, indicating that, in contrast to [^{3}H]-dihydroergocryptine used in some studies to label α-adrenoceptors, [^{3}H]-DHA and [^{3}H]-WB-4101 are not adsorbed to glass. Incubations were ended by rapid vacuum filtration of the assay mixture through Whatman GF/C glass fibre filters, presoaked in Krebs solution. Filters were washed with 15 ml Krebs solution (24°C) within a period of less than 15 s, and were air-dried overnight. After the addition of a toluene-based fluor, the radioactivity was measured in the Intertechnique liquid scintillation spectrometer at 50 to 55% efficiency. Counting efficiency was determined for each sample by using the channels ratio method and a quench calibration curve.

Specific binding for [^{3}H]-DHA was defined as the difference in radioactivity in the presence and absence of 1 μM (±)-propranolol, determined in duplicate for each data point. After subtraction of the filter blank, specific binding of [^{3}H]-DHA represented 60 to 90% of total binding. Membrane-bound label was 0.7 to 1.8% of the total radioactivity in the assay tube at 0.5 nM [^{3}H]-DHA and 0.5 to 1.1% at 4 nM. Binding isoterms for [^{3}H]-DHA were determined in a concentration range from 0.5 to 3 to 4 nM, and consisted of 5 to 8 points. In this concentration range, binding suppressible by propranolol was identical to binding suppressible by 2×10^{-4} M (−)-isoprenaline. In agree-

ment with recent results of Winek & Bhalla (1979) and Nahorski & Richardson (1979), we found that propranolol-suppressible binding of [^{3}H]-DHA above this concentration range had a non-specific component not suppressible by isoprenaline.

Specific binding of [^{3}H]-WB-4101 was obtained as the binding suppressed by 1 μM prazosin, and it was 40 to 70% of the total. Membrane-bound label was 1.5 to 5% of the total radioactivity in the assay tube at 0.25 nM and 0.5 to 1.2% at 4 nM. The density and affinity of binding sites were obtained in individual experiments from Scatchard plots (Scatchard, 1949) determined by linear regression analysis.

The following drugs were used: (−)-noradrenaline bitartrate (NA) (Calbiochem; arterenol); (±)-isopropylnoradrenaline hydrochloride (K & K; isoprenaline); (−)-isopropylnoradrenaline bitartrate (Sigma); (−)-phenylephrine hydrochloride (Sigma); methoxamine hydrochloride (Burroughs Wellcome); phenoxybenzamine hydrochloride (Smith, Kline & French, Pbz); phentolamine methanesulphonate (Rogitine, Ciba); prazosin hydrochloride (Pfizer); (±)-propranolol and (+)-propranolol (Ayerst); 6-hydroxydopamine hydrobromide (Regis, 6-OHDA); reserpine (Serpasil, Ciba); β-oestradiol-3-benzoate (Sigma). All drugs were freshly diluted before each experiment in 0.9% w/v NaCl solution (saline) containing 0.01 N HCl. Oestradiol was dissolved in 1/3 absolute ethanol + 2/3 0.1 N HCl in saline. Concentrations are expressed as M in the bath.

Results

Effects of hypophysectomy on cardiac adrenoceptors

Atria Hypophysectomy produced selective and highly significant changes in the potencies of α- and β-receptor agonists and in the blocking effectiveness of receptor antagonists. The data in Table 1 show that the inotropic potency of isoprenaline decreased and the potency of phenylephrine increased after hypophysectomy, which resulted in a decrease in the potency ratio of the two agonists. Thyroxine treatment reversed these changes to control levels, whereas cortisone treatment did not. Similar but smaller changes occurred for chronotropic responses. Figure 1 illustrates the inotropic effect of isoprenaline and of the pure α-stimulant, methoxamine, in left atria. Hypophysectomy decreased the basal contractile amplitude, reduced the potency of isoprenaline ($P < 0.01$) and increased the potency of methoxamine ($P < 0.05$); the relative intrinsic activity of the two agonists was also reversed. Treatment of hypophysectomized rats with T4 0.2 mg/kg daily for 2 days completely reversed the changes in agonist potencies but did not significantly influence intrinsic activities or baseline contractile amplitude.

To test whether the change in potency of isoprenaline could be due to a change in its disposition, concentration-response curves to isoprenaline in normal and hypophysectomized atria were determined in the

Table 1 Serum thyroxine levels and agonist potencies in atria from control, hypophysectomized (Hx), hypophysectomized, thyroxine-treated (Hx + T4) and hypophysectomized, cortisone-treated rats

	Control	*Hx*	*Hx + T4*	*Hx + Cortisone*
Serum T4 levels (ng/ml):	58 ± 5 (8)	<5[a]** (8)	112 ± 18* (4)	<5[a]** (3)
Inotropic responses of left atria				
Isoprenaline	9.05 ± 0.21	8.00 ± 0.10**	9.18 ± 0.10	8.04 ± 0.04**
Phenylephrine	5.51 ± 0.10	5.88 ± 0.08*	5.46 ± 0.14	6.34 ± 0.16**
Log potency ratio (iso/phenyle)	3.54 ± 0.21 (13)	2.12 ± 0.11** (18)	3.69 ± 0.04 (4)	1.70 ± 0.20** (3)
Chronotropic responses of right atria				
Isoprenaline	9.20 ± 0.25	8.60 ± 0.13*	8.93 ± 0.13	8.91 ± 0.03
Phenylephrine	5.24 ± 0.11	5.55 ± 0.09*	5.27 ± 0.13	6.00 ± 0.19**
Log potency ratio	3.96 ± 0.21 (13)	3.05 ± 0.15** (21)	3.66 ± 0.05 (4)	2.91 ± 0.22** (3)

Agonist potencies are expressed as pD_2 values ± s.e. (neg log of EC_{50}). Asterisks indicate significant difference from values in corresponding control preparations: *$P < 0.05$; **$P < 0.005$. Numbers of experiments are in parentheses. Serum T4 levels were determined by radioimmunoassay, on the day of the experiment. Experiments on the isolated atria were performed 4 to 6 weeks after hypophysectomy. T4 treatment (0.2 mg/kg daily i.p.) was for 8 days, cortisone treatment (50 mg/kg every 12 h) for 4 days.

[a] Serum T4 levels were below measurable levels in all animals tested in these groups.

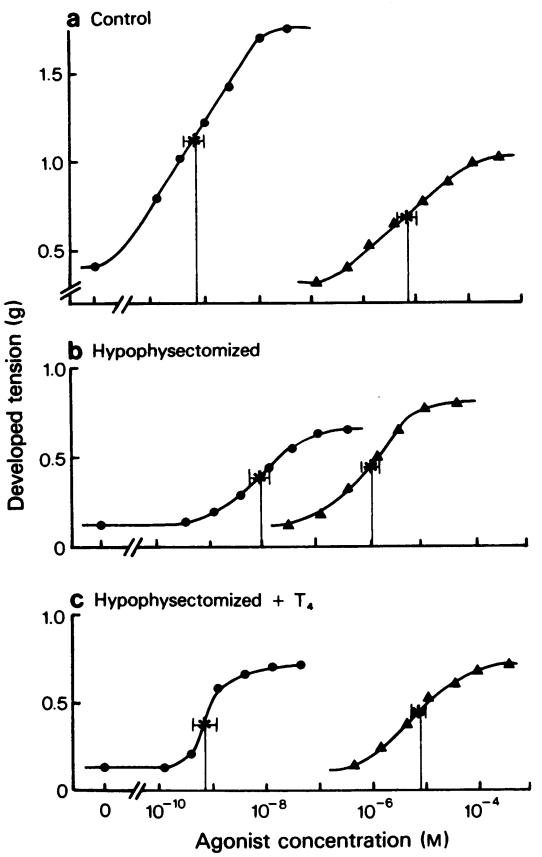

Figure 1 Inotropic responses of left atria to isoprenaline (●) and to methoxamine (▲) in normal (a), hypophysectomized (b), and hypophysectomized rats treated for 2 days with thyroxine 0.2 mg/kg daily (c). Number of experiments: 4 (a); 4 (b); 3 (c). The asterisks on the dose–response curves and the horizontal bars indicate the mean $EC_{50} \pm 2 \times$ s.e. The vertical lines were drawn to illustrate mean EC_{50}s on the abscissae.

absence and in the presence of 20 μM oestradiol, a potent inhibitor of extraneuronal uptake of catecholamines (Iversen & Salt, 1970). Table 2 shows that oestradiol did not reduce the potency difference for isoprenaline either in left or in right atria, although it slightly increased potencies both in control and in hypophysectomized preparations.

In association with the altered potencies of agonists, there were also reciprocal changes in the blocking effectiveness of α- and β-adrenoceptor antagonists when tested against agonists that can activate both α- and β-receptors in the heart. Figure 2 shows that in left atria of control rats (a), inotropic responses to noradrenaline were potentiated and responses to phenylephrine were moderately inhibited after a 40 min exposure to 0.7 μM phenoxybenzamine (Pbz). In atria from hypophysectomized rats (b), a similar exposure to Pbz partially inhibited responses to noradrenaline: the reduction of the maximal response was statistically significant ($P < 0.05$) and there was no potentiation. The effect of phenylephrine was almost completely blocked by Pbz. Baseline contractile amplitude was increased by Pbz but it returned to or below control after washout of the antagonist, when the agonists were tested. Thus, the decrease in maximal responses to agonists was entirely due to a decrease in maximal developed tension. The increased blocking effect of Pbz was reversed toward control in atria of hypophysectomized rats treated with T4 (c), whereas cortisone treatment (d) caused no such reversal.

Hypophysectomy also significantly increased the inhibitory effect of the reversible α-receptor antagonist, phentolamine, and the difference was greater at a high than at a lower concentration of the antagonist. The logarithm of the rightward shift of the phenylephrine inotropic concentration-response curves by 0.1 and 1.0 μM phentolamine was 0.28 ± 0.14 and 0.36 ± 0.16 in controls and 0.86 ± 0.10 and 1.37 ± 0.13 in hypophysectomized rats ($P < 0.005$ for

Table 2 The effect of β-oestradiol on the inotropic and chronotropic potency of isoprenaline

			Control ($n = 3$)	P	*Hypophysectomized* ($n = 3$)
Force	(left atria)				
	before	oestradiol (20 μM)	9.21 ± 0.02	**	7.55 ± 0.04
	after		9.40 ± 0.08	**	7.90 ± 0.10
Rate	(right atria)				
	before	oestradiol (20 μM)	9.15 ± 0.15	**	8.40 ± 0.07
	after		9.28 ± 0.17	*	8.82 ± 0.06

Values shown are pD_2s for isoprenaline (neg log of EC_{50}), means with their standard errors. Hypophysectomized rats were operated 3 months before the experiment. Asterisks indicate significant difference between adjacent values; $*P < 0.05$; $**P < 0.005$.

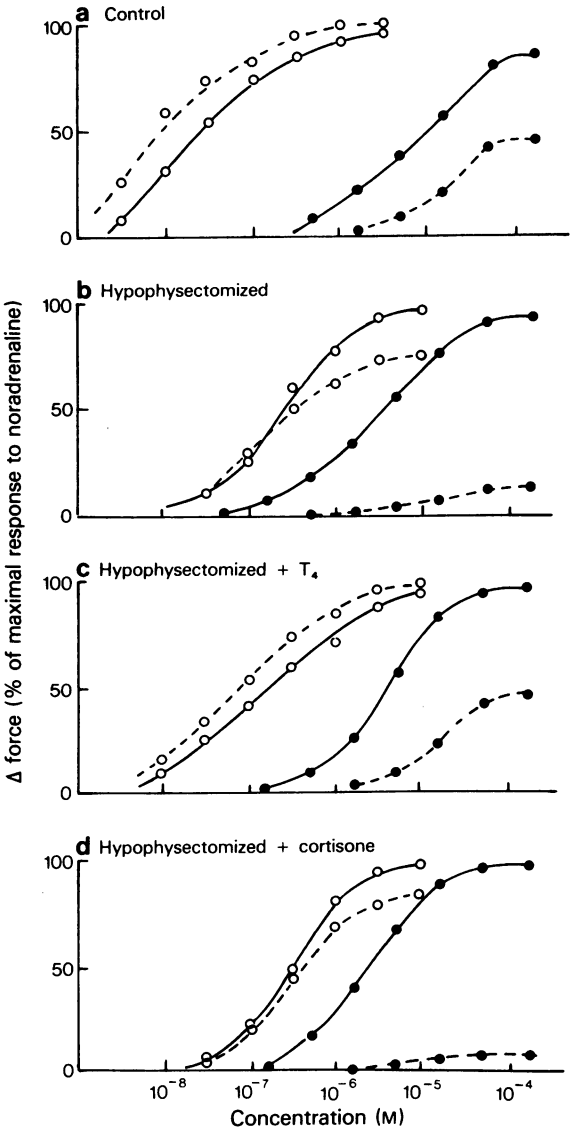

Figure 2 Effects of hypophysectomy (b) and subsequent treatment with thyroxine (c) or cortisone (d) on the phenoxybenzamine block of left atrial inotropic responses. Mean concentration–response curves for noradrenaline (O) and phenylephrine (●) are indicated by solid lines. Concentration–response curves for both agonists were redetermined after a 40 min exposed to and subsequent washout of 0.7 μM phenoxybenzamine (dashed lines). Number of experiments: 8 (a); 12 (b); 4 (c); 3 (d).

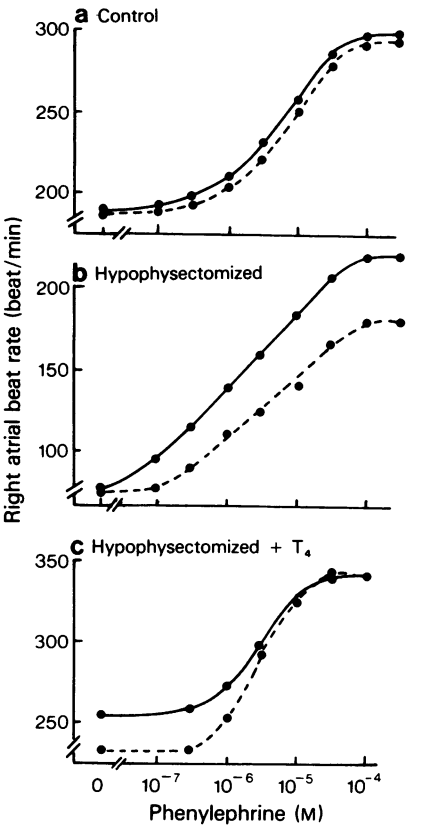

Figure 3 The effect of phenoxybenzamine on chronotropic responses of right atria to phenylephrine in control (a), hypophysectomized rats (b) and hypophysectomized rats treated with thyroxine (c). Solid lines: control; dashed lines: after a 40 min exposure to 0.7 μM phenoxybenzamine. Number of experiments: 8 (a); 8 (b) 3 (c).

both concentrations). Chronotropic responses to phenylephrine did not have an α-adrenoceptor component in controls or in hypophysectomized rats treated with T4, whereas in right atria from hypophysectomized rats Pbz significantly inhibited the rate response to phenylephrine (Figure 3).

In contrast to the increased blocking effectiveness of α-adrenoceptor antagonists after hypophysectomy, the effect of propranolol in inhibiting inotropic responses to phenylephyrine was reduced by hypophysectomy (Figure 4). In agreement with previous observations (Govier, 1968), the maximal inotropic response of euthyroid atria to phenylephrine was consistently reduced by propranolol. Although this effect is difficult to explain in terms of classical competitive antagonism, it may be related to a non-specific cardiodepressant effect of phenylephrine at concentrations above 10^{-4} M which could counteract the positive inotropic response of β-receptor stimulation through 'functional' antagonism. Such a cardiodepres-

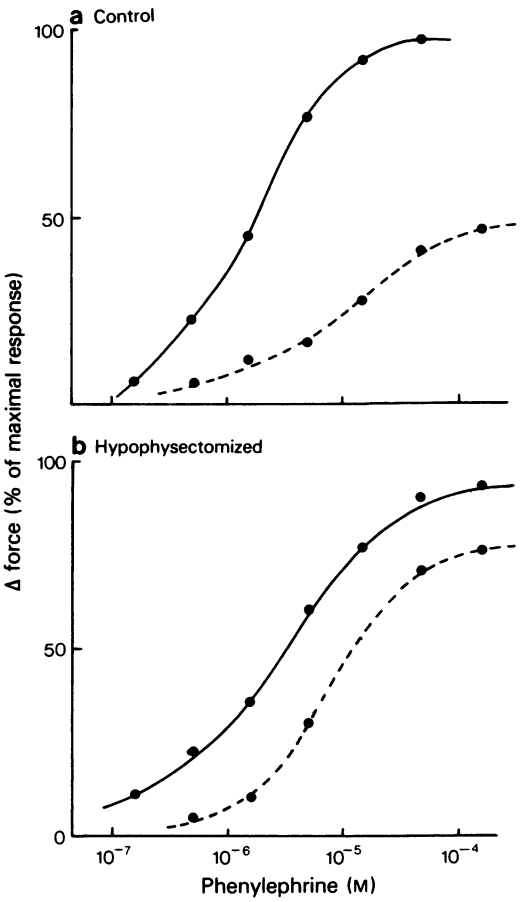

Figure 4 The effect of hypophysectomy on inhibition of left atrial inotropic responses to phenylephrine by propranolol. Control responses (solid lines) and responses obtained in the presence of 34 nM of (±)-propranolol (40 min exposure, dashed lines) are shown. Number of experiments: 4 (a); 4 (b).

sant effect of phenylephrine in the above concentration range has recently been demonstrated (Endoh, Shimizu & Yanagisawa, 1978); (+)-propranolol, which is devoid of β-blocking activity in similar concentrations, did not significantly alter the phenylephrine response. The above observations indicate an increase in the α- and decrease in the β-component of cardiac adrenoceptor responses after hypophysectomy or during treatment of hypophysectomized rats observed earlier in atria of thyroidectomized rats (Kunos, 1977).

To study the time course of these changes, the inotropic potency of isoprenaline and phenylephrine was determined at various times following hypophysectomy or during treatment of hypophysectomized rats with T4 (Table 3). The reduced potency ratio developed slowly after hypophysectomy: two weeks after the operation there were no significant changes in agonist potencies, although serum T4 levels were below 5 ng/ml. Four to six weeks after the operation the potency of isoprenaline was reduced and that of phenylephrine increased, and there was a further reduction in the potency ratio of the two agonists at 3 to 4 months after hypophysectomy. The reversal of these changes was much faster; when T4 treatment, 0.2 mg/kg daily was started 4 months after hypophysectomy, the potency ratio increased to near normal levels after 2 days and no further increase was seen after 5 days of T4 treatment. The time course of the reciprocal changes in the absolute potency of isoprenaline and phenylephrine was parallel within the time range studied.

Ventricles In order to test whether the parallel and reciprocal changes in α- and β-receptor responses are associated with changes in the density or affinity of receptor binding sites, ligand binding studies were done. The specificity of binding of low concentrations (<4 nM) of [^{3}H]-DHA has been recently demonstrated (Williams & Lefkowitz, 1978; Winek &

Table 3 Time course of development of changes in agonist potencies after hypophysectomy and during T4 treatment of hypophysectomized rats

	Control	*Hypophysectomized*			*Hypophysectomized + T4*[a]	
		2 weeks	*4–6 weeks*	*3–4 months*	*2 days*	*5 days*
Isoprenaline	9.23 ± 0.23	9.60 ± 0.40	8.10 ± 0.13**	7.66 ± 0.10**	9.12 ± 0.14	9.09 ± 0.27
Phenylephrine	5.66 ± 0.11	5.70 ± 0.31	6.03 ± 0.12*	6.48 ± 0.05**	5.87 ± 0.20	5.87 ± 0.19
Log potency ratio	3.57 ± 0.28	3.90 ± 0.27	2.07 ± 0.09**	1.18 ± 0.12**	3.25 ± 0.12	3.22 ± 0.16
	(4)	(3)	(4)	(5)	(4)	(4)

Means and standard errors of pD_2 values for inotropic responses of left atria are shown. Numbers of experiments are in parentheses. Asterisks indicate significant difference from corresponding value in controls: *$P < 0.05$; **$P < 0.005$.

[a]Thyroxine treatment (0.2 mg/kg daily) was started 4 months after hypophysectomy.

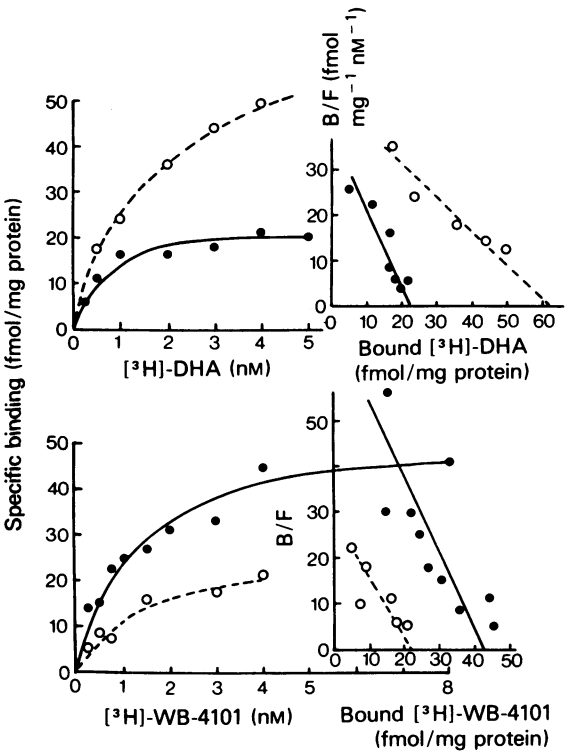

Figure 5 The effect of thyroxine treatment of hypophysectomized rat on the specific binding of [^{3}H]-WB-4101 and [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) to cardiac membrane fragments. (●) Hypophysectomized rat; (○) hypophysectomized rat treated with T4 0.2 mg/kg daily for 2 days. Insets are Scatchard-plots.

Bhalla, 1979; Nahorski & Richardson, 1979) and justified the use of propranolol-suppressible binding of this ligand to label cardiac β-adrenoceptors (see Methods). The high post-synaptic selectivity of both [^{3}H]-WB-4101 and prazosin (Raisman *et al.*, 1979; Cambridge, Davey & Massingham, 1977) ensured selective labelling of post-synaptic α-adrenoceptors of the heart in the present study.

Thyroidectomy leads to atrophy and prolonged treatment with thyroid hormones leads to hypertrophy of the myocardium. Such changes may alter the amount and composition of cell membranes and the membrane surface to tissue mass ratio, which could lead to errors in estimating changes in receptor density. In order to minimize such errors it was decided to assess the direct effect of thyroid hormones on receptors by comparing conditions in hypophysectomized rats before and after a short term treatment with T4. Changes in physiological responses mediated by α- and β-receptors were maximal after two daily injections of 0.2 mg/kg T4 (Table 3), yet the marginal increase in body weight:heart weight ratio indicated the absence of significant cardiac hypertrophy (Table 4).

A second important factor is the conditions under which ligand binding is measured. In contrast to all previous studies, in the present experiments ventricular membranes were prepared and ligand binding assayed in the same medium (Krebs solution at pH 7.4) and at the same temperature (31°C) as used in experiments with intact atria. Although binding was studied in ventricular preparations, the results of Wagner & Brodde (1978) indicate that reciprocal changes in α- and β-receptor responses are qualitat-

Table 4 The effect of thyroxine treatment of hypophysectomized rats on the numbers and affinities of α_1- and β-adrenoceptor binding sites

	[^{3}H]-*WB-4101 binding* (α_1)			[^{3}H]-*DHA binding* (β)			*Total number* (α_1 + β)	*Heart weight/ body weight ratio* (mg/g)
	Density (*fmol/mg prot.*)	*Affinity* (K_d, nM)	K_d:*methoxamine* (μM)	*Density*	*Affinity*	K_d:*isoprenaline* (μM)		
Hypophysectomized	38.7 ±3.1(4)	0.48 ± 0.08	6.5	27.5 ± 2.7(6)	1.59 ± 0.27	0.45	66.2	2.20 ± 0.12(12)
Hypophysectomized treated with T4	18.7** ± 2.5(4)	0.60 ± 0.18	8.9	45.5* ± 5.7(5)	1.89 ± 0.28	0.32	64.2	2.44 ± 0.11(12)

† The K_d of methoxamine was determined at 2 nM[^{3}H]-WB-4101, and the K_d of isoprenaline at 2 nM[^{3}H]-DHA. Values shown are means from 2 separate experiments and are calculated as

$$K_d = \frac{IC_{50}}{1 + (2/K_d \text{ labelled ligand})}.$$

Treatment with (−)-thyroxine was 0.2 mg/kg daily for 2 days. Asterisks indicate significant difference from corresponding value in untreated hypophysectomized rats; $*P < 0.01$; $**P < 0.001$. Number of experiments in parentheses. Binding site densities and affinities were obtained from Scatchard plots (5 to 8 points), as described in Methods.

ively similar in contracting atrial and ventricular preparations from rat heart. Figure 5 illustrates [^{3}H]-DHA and [^{3}H]-WB-4101 binding isotherms obtained in ventricular membranes from a hypophysectomized rat and from a hypophysectomized rat treated daily with T4 0.2 mg/kg for 2 days. T4 treatment increased the density of [^{3}]-DHA binding sites (upper panel) and decreased the density of [^{3}H]-WB-4101 binding sites (lower panel), without significantly altering the binding affinity. Mean values from a large number of similar experiments are shown in Table 4. It can be seen that the increase in the number of β- and decrease in the number of α-adrenoceptors were matching and the sum total of α plus β receptors did not change after T4 treatment. The affinity of binding sites for the labelled antagonists or for the agonists isoprenaline and methoxamine also remained unchanged.

Effects of sympathetic denervation and reserpine treatment of thyroidectomized rats on cardiac adrenoceptors.

Atria Thyroidectomy was previously shown to enhance the α- and reduce the β-adrenoceptor component of inotropic responses in rat heart (Kunos, 1977). A decreased blocking effect of propranolol and increased blocking effect of phenoxybenzamine were also observed when atria from euthyroid rats were tested at low temperature. These temperature-dependent changes were absent, however, when tested in rats previously denervated by treatment with 6-hydroxydopamine (6-OHDA) but not with reserpine (Kunos & Nickerson, 1977). In the present experiments, the interaction of thyroid state and sympathetic innervation was tested on the adrenoceptor response pattern of left atria. Thyroidectomized rats were either denervated by 6-OHDA or their endogenous noradrenaline stores were depleted by reserpine (see Methods). A group of euthyroid rats were also denervated by 6-OHDA to serve as controls in some experiments. The data in Table 5 show that, in agreement with previous observations, thyroidectomy significantly reduced the inotropic potency of isoprenaline and noradrenaline, agonists acting predominantly on β-adrenoceptors in the heart, and increased the potency of phenylephrine. These changes in agonist potencies were prevented in left atria from hypothyroid rats treated with 6-OHDA within 4 weeks after thyroidectomy: the potency of isoprenaline increased and the potency of phenylephrine decreased toward control levels, whereas the increase in the potency of noradrenaline was significantly beyond the control value. In a few rats, where denervation was performed 3 to 4 months after thyroidectomy, these changes were less marked (data not shown). In euthyroid rats, denervation increased the potency of noradrenaline only; and the potency of all three agonists was not significantly different from that in thyroidectomized, denervated rats (Table 5).

To determine whether the absence of the neurotransmitter or some other factor related to sympathetic innervation is responsible for the reversal of the effects of hypothyroidism by 6-OHDA treatment, agonist potencies were determined in left atria of thyroidectomized rats treated with either a single large dose or repeated smaller doses of reserpine (see Methods). As shown in Table 5, reserpine pretreatment did not significantly modify agonist potencies in thyroidectomized rats, although a small sensitization for noradrenaline and isoprenaline may be noticed.

Results obtained with receptor antagonists showed a similar reversal of the effects of thyroidectomy by denervation but not by reserpine pretreatment. Figure 6 illustrates the effect of Pbz on inotropic responses to noradrenaline and phenylephrine in normal and thyroidectomized rats and in thyroidectomized rats treated with 6-OHDA or reserpine. A 40 min exposure to 7.3 μM Pbz potentiated responses to noradrenaline and only moderately inhibited responses to phenylephrine in control atria (a). In thyroidectomized rats (b), a similar exposure to Pbz partially inhibited responses to noradrenaline: the potentiation

Table 5 The effects of denervation by 6-hydroxydopamine or pretreatment with reserpine on the inotropic potencies of agonists in left atria from normal and thyroidectomized rats

	Control		*Thyroidectomized*		
Treatment:	*None*	*6-OHDA*	*None*	*6-OHDA*	*Reserpine*
Isoprenaline	9.29 ± 0.12	9.50 ± 0.22	8.63 ± 0.13	9.40 ± 0.14**	8.88 ± 0.20
Noradrenaline	7.67 ± 0.09	8.65 ± 0.12**	7.12 ± 0.10	8.86 ± 0.16**	7.48 ± 0.16
Phenylephrine	5.52 ± 0.10	5.90 ± 0.16	6.31 ± 0.06	5.98 ± 0.16*	6.26 ± 0.17

Values are means and standard errors from 8 to 22 experiments. Asterisks indicate significant difference from the potency of the same agonist in untreated normal or untreated thyroidectomized rats: *$P < 0.01$: **$P < 0.001$. Potency is expressed as pD_2.

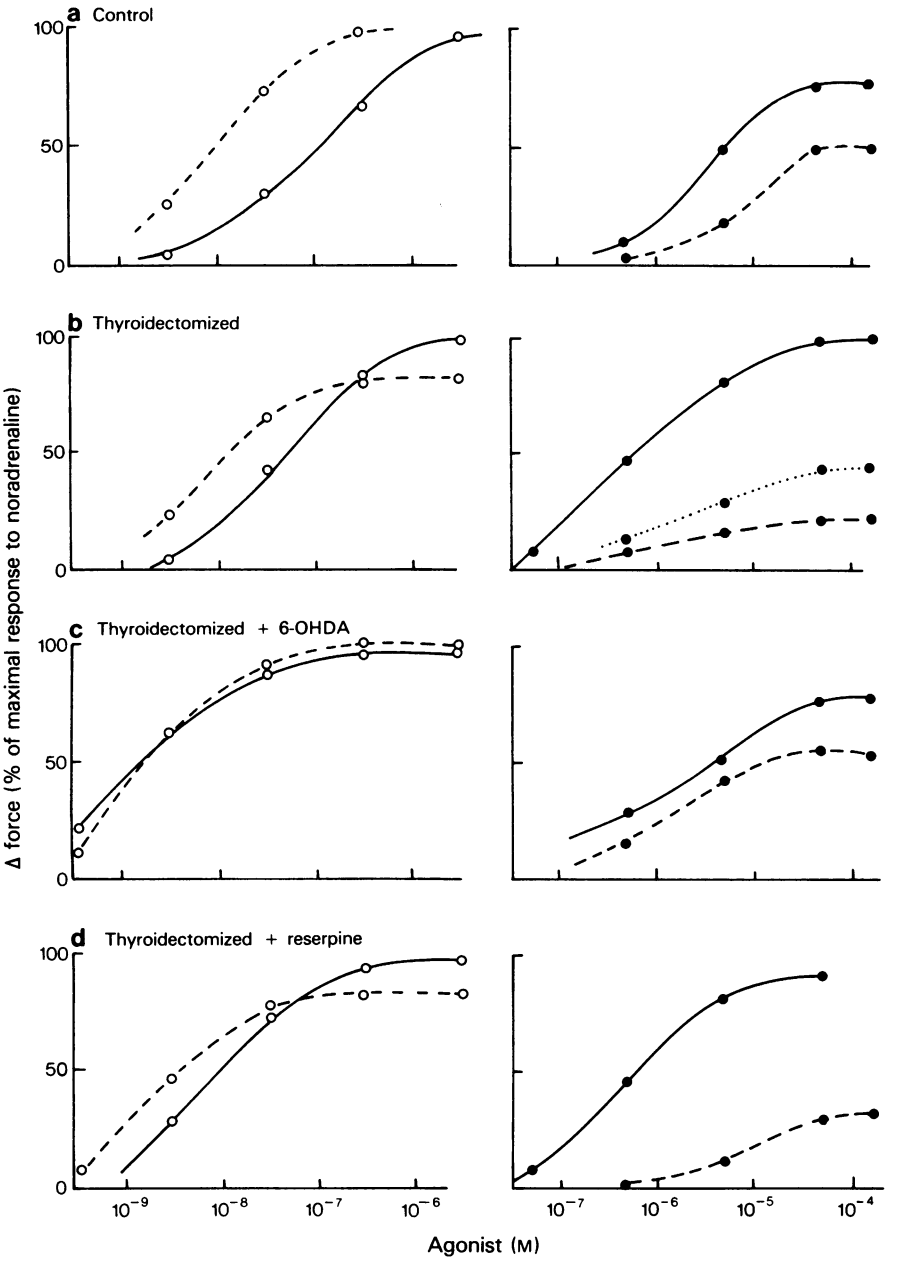

Figure 6 The effects of thyroidectomy and subsequent treatment with 6-hydroxydopamine (6-OHDA) or reserpine on phenoxybenzamine blockade of inotropic responses of left atria. Mean concentration-response curves for noradrenaline (left panels) and phenylephrine (right panels) are indicated by solid lines. Responses to agonists were redetermined after a 40 min exposure to 7.3 μM phenoxybenzamine (dashed lines). The dotted line in (b) shows the blockade by 0.7 μM phenoxybenzamine of the effect of phenylephrine. Number of experiments: 11 (a); 14 (b); 8 (c); 8 (d).

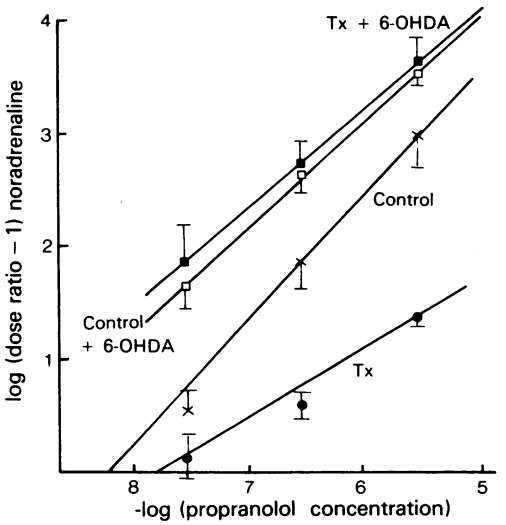

Figure 7 Inhibition by propranolol of left atrial inotropic responses to noradrenaline in control and thyroidectomized (Tx) rats; effects of denervation by 6-hydroxydopamine. Schild-plots shown represent the mean slope and mean intercept (pA_2) calculated from values in individual experiments, where they were obtained by linear regression analysis. Points are means of 4 to 5 experiments; vertical lines show s.e. mean. Mean slopes are 1.09 ± 0.05 (control, ×), 0.65 ± 0.05 (Tx, ●), 0.92 ± 0.07 (control + 6-OHDA, □), 0.90 ± 0.08 (Tx + 6-OHDA, ■).

observed at lower agonist concentrations was less than in controls and the maximal effect was significantly reduced. In the same preparations, the effect of phenylephrine was blocked almost completely, and even 0.7 μM Pbz produced more block that 7.3 μM Pbz did in controls. 6-OHDA treatment of thyroidectomized rats (c) not only reversed the decrease in noradrenaline potency, but increased it significantly beyond control levels. Pbz did not further potentiate the noradrenaline response but did not block it either, and its block of the effect of phenylephrine was also significantly reduced. In contrast to the effects of denervation, reserpine treatment of thyroidectomized rats (d) did not alter the blocking effects of Pbz.

Denervation of thyroidectomized rats also reversed the decrease in the blocking potency of propranolol. Figure 7 shows concentration-response relations for the propranolol block of inotropic responses to noradrenaline in innervated and 6-OHDA denervated euthyroid and hypothyroid rats. Noradrenaline EC_{50}s were determined in each preparation in the absence and in the presence of sequentially increased concentrations of propranolol, and Schild-plots were constructed (Schild, 1947). At high concentrations, propranolol was less effective in atria from thyroidectomized rats than in controls but the difference in block decreased with decreasing antagonist concentrations and there was no significant difference in the pA_2 value of propranolol between the two groups (8.13 ± 0.34 in controls and 7.80 ± 0.20 in hypothyroid rats). Denervation of control rats increased the degree of apparent block by all three concentrations of propranolol used. Denervation of thyroidectomized rats caused a greater increase in block at high than at low propranolol concentrations and the difference in slope of Schild-plots between control and thyroidectomized rats disappeared after 6-OHDA treatment. These observations indirectly indicate that denervation but not reserpine treatment prevented or reversed the increase in α- and decrease in β-receptors associated with hypothyroidism.

Ventricles Direct binding studies using ventricular membranes from untreated, denervated and reserpine-treated hypothyroid rats confirmed this conclusion.

Table 6 The effects of sympathetic denervation and reserpine treatment on cardiac α_1 and β-receptors in thyroidectomized rats

Treatment:	*n*	[^{3}H]-*WB-4101 binding* (α_1)		[^{3}H]-*DHA binding* (β)		*α:β ratio*
		Density[1]	*Affinity*[2]	*Density*	*Affinity*	
Thyroidectomy	6	29.8 ± 2.6	1.09 ± 0.25	25.3 ± 2.8	1.96 ± 0.28	1.44 ± 0.12
Thyroidectomy + 6-OHDA	6	21.0 ± 1.7**	0.64 ± 0.10	41.6 ± 6.0*	2.92 ± 0.57	0.58 ± 0.12**
Thyroidectomy + reserpine	3	32.5 ± 4.3	1.04 ± 0.10	25.2 ± 4.7	1.29 ± 0.49	1.47 ± 0.27

[1] fmol/mg protein; [2] K_d, nM.

All experiments were done 6 weeks after thyroidectomy. For details of binding assays and treatment schedule for 6-OHDA see Methods. Reserpine, 5 mg/kg i.p., was given 24 h before the experiment. Asterisks indicate significant difference from corresponding value in untreated thyroidectomised rats: *$P < 0.05$; **$P < 0.005$. *n* indicates number of experiments. Each heart was assayed for both ligands.

Binding isotherms for [^{3}H]-DHA and [^{3}H]-WB-4101 were determined in membranes from each heart. The data in Table 6 show that the density of postsynaptic α_1-receptor sites decreased and the density of β-receptor sites increased after denervation of thyroidectomized rats, resulting in a reversal of the ratio of α- to β-receptors from 1.44 ± 0.12 to 0.58 ± 0.12. The affinities of binding sites were not significantly influenced. In contrast to denervation, reserpine treatment did not alter either the density or the affinity of α- and β-receptors, and the α:β ratio also remained unchanged at the level of untreated thyroidectomized rats.

Discussion

Alterations of thyroid state in rats result in reciprocal changes in the potencies of drugs acting on α- or β-adrenoceptors in the heart (Nakashima & Hagino, 1972; Kunos, Vermes-Kunos & Nickerson, 1974; Kunos, 1977; McNeill & Simpson, 1978; Hashimoto & Nakashima, 1978). On the basis of indirect evidence it has been suggested that such changes reflect a reciprocal change in the number of available receptors, possibly by an interconversion mechanism (Kunos, 1977). The present results confirm that thyroid hormones play a part in these changes which are revealed as matching, reciprocal changes in the numbers of α- and β-receptor binding sites without significant changes in their affinities. The results also suggest a 'trophic' influence of sympathetic nerves on the conformational state of cardiac adrenoceptors: sympathetic denervation but not the depletion of endogenous stores of noradrenaline prevented the shift from β- to α-adrenoceptors in the hypothyroid myocardium.

In tissues where both α- and β-adrenoceptors mediate the same end response, a shift in the balance between the two may be indicated by a change in the relative potencies of selective α- and β-adrenoceptor agonists and by opposite changes in the effective blockade produced by α- and β-receptor antagonists against mixed α–β agonists (Kunos, 1978; 1980). The changes observed in the physiological responses of atria from hypophysectomized rats, or earlier in hypothyroid rats (Kunos, 1977), are compatible with a change in the relative numbers of α- and β-adrenoceptors. As discussed below, they cannot be satisfactorily explained by non-specific drug effects, changes in pre- or postreceptor mechanisms or by major changes in the affinity of receptors.

Indirect effects of agonists or changes in their disposition cannot explain the changes in their potency after hypophysectomy. Cardiac responses to phenylephrine are not altered by reserpine (Wagner & Reinhardt, 1974) or by sympathetic denervation (Kunos & Nickerson, 1977), which indicates that the effects of phenylephrine are not mediated by release of endogenous noradrenaline. Phenylephrine is taken up into nerve endings and is metabolized by monoamine oxidase (MAO). Methoxamine, however, is not a substrate for either MAO, catechol-*O*-methyltransferase (COMT) or neuronal uptake (Kalsner & Nickerson, 1969), and the increase in its potency after hypophysectomy can only result from increased α-receptor sensitivity. Possible changes in the disposition of isoprenaline can be also ruled out from causing the reduction in its potency after hypophysectomy. Isoprenaline is taken up into cardiac muscle cells and is metabolized by COMT. Oestradiol, a potent inhibitor of extraneuronal uptake of catecholamines in the rat heart (Iversen & Salt, 1970), did not reduce the difference in isoprenaline sensitivity between normal and hypophysectomized rat atria. Since inhibition of uptake$_2$ prevents the access of the agonist to COMT, this finding eliminates both processes as possible causes for altered isoprenaline sensitivity. Altered post-receptor mechanisms are also unlikely to account for the change in agonist potencies, as the inotropic potency of a non-adrenergic agent such as calcium was found not to be influenced by thyroid state (Hashimoto & Nakashima, 1978).

Some change in post-receptor events may be suggested by the change in the relative intrinsic activities of isoprenaline and methoxamine after hypophysectomy (Figure 1). The selectivity of this change could suggest that the event affected is close to receptor activation, such as the coupling of α- or β-receptors to effector systems; change in a more distant event should have similarly influenced both agonists. However, the finding that short-term thyroxine treatment completely reversed the reciprocal changes in agonist potencies without significantly correcting the changes in intrinsic activity (Figure 1c) indicates that the direct effect of thyroid hormones is on receptors and that post-receptor mechanisms may be indirectly influenced by some effect of prolonged thyroid deficiency on the myocardium.

The possibility that the observed changes in the potencies of α- and β-receptor agonists are related to changes in the relative numbers of receptors is supported by experiments with receptor antagonists. If the affinity of β-adrenoceptors were altered by hypothyroidism, one would expect a change in the dissociation constant of propranolol. However, the pA_2 value for propranolol was minimally affected after thyroidectomy, whereas the slope of the Schild-plot was reduced (Figure 7). The latter may be accounted for by an increased proportion of α-adrenoceptors in the hypothyroid myocardium, whose contribution to the net response to noradrenaline progressively increases as the proportion of unblocked β-receptors is decreased. In denervated preparations, where the α-receptor component was negli-

gible both in controls and in hypothyroid rats, Schild-plots for propranolol were overlapping and their slopes were close to 1.0. The converse relationship appears to hold for α-adrenoceptors; phentolamine inhibited inotropic responses to phenylephrine more effectively in hypophysectomized than in control rats, and the difference in block was greater at higher than at lower antagonist concentrations. Again, this suggests that the difference in α-blockade was due to the greater contribution of β-receptors to the net effect of phenylephrine in euthyroid than in hypothyroid rats, and not to a change in the affinity of α-receptors. This is in accordance with findings that after elimination of the β-receptor component of the inotropic effect of phenylephrine by pindolol there was no difference in the pA_2 values for phentolamine and yohimbine in left atria of euthyroid and propylthiouracil-treated rats (Wagner & Brodde, 1978).

From the above it follows that differences in the blocking effects of receptor antagonists against mixed α–β agonists are better detected at higher antagonist concentrations where non-specific effects are also more apparent. This could be particularly important for α-receptor antagonists which are known to interact with a number of other receptors and non-specific tissue components. Nevertheless, the specificity of α-blockade in the hypothyroid myocardium is indicated by several findings in the present and in a previous study (Kunos, 1977): (1) phentolamine and Pbz, antagonists with different chemical reactivity, were both more effective blocking agents after thyroidectomy or hypophysectomy; (2) inotropic responses to Ca^{2+} were not affected by α-adrenoceptor blockade; (3) in contrast to the block of α-adrenoceptors, the block of muscarinic receptors of left atria by Pbz was not influenced by thyroid state; (4) the increased block of α-receptors by Pbz in the hypothyroid myocardium was evident at the low concentration of 7 nM.

By exclusion of alternative mechanisms, the altered receptor response pattern may be attributed to a change in the relative number or availability of α- and β-adrenoceptors. The increased potency of the selective α-receptor agonist, methoxamine, and the decreased potency of isoprenaline after hypophysectomy indicate that both α- and β-adrenoceptors are affected, and it is not simply a change in the activity of one receptor that masks or unmasks the unchanged activity of the other. The results of ligand binding studies presented in this paper are compatible with this interpretation: rapidly developing, matching, reciprocal changes in the numbers of α- and β-adrenoceptors occurred without significant changes in their affinities after T4 treatment of hypophysectomized rats.

The effect of thyroid state on binding sites in rat heart for labelled adrenoceptor antagonists was the subject of several recent studies, but the results of some of these have to be interpreted with caution. The K_d of [^{3}H]-DHA used to label cardiac β-receptors was between 5 and 20 nM and saturation was detected at concentrations above 10 to 15 nM (Ciaraldi & Marinetti, 1977; Williams *et al.*, 1977). More recent studies indicate that stereoselective β-receptors in heart and other tissues have higher affinity for [^{3}H]-DHA ($\leq$2 nM) and at ligand concentrations in excess of 3 to 5 nM, propranolol-suppressible binding includes an increasing proportion of non-specific sites (Williams & Lefkowitz, 1978; Nahorski & Richardson, 1979; Winek & Bhalla, 1979). [^{3}H]-dihydroergocryptine ([^{3}H]-DHEC) used to label cardiac α-adrenoceptors in different thyroid states also poses some problems. Recent studies indicate that at ligand concentrations below 3 nM [^{3}H]-DHEC preferentially (but not exclusively) labels post-synaptic, high affinity (K_d < 2 nM) α-receptors in heart and smooth muscle (Guicheney, Garay, Levy-Marchal & Meyer, 1978; Kunos, Hoffman, Kwok, Kan & Mucci, 1979), whereas at higher ligand concentrations a large part of binding is to a second site with lower affinity, possibly representing presynaptic α-receptors (Guicheney *et al.*, 1978; Story, Briley & Langer, 1979). Interpretations of changes in the density of [^{3}H]-DHEC binding sites may therefore be complicated by changes in innervation density, particularly when the effects of prolonged thyroid dysfunction are studied.

When high affinity binding sites for [^{3}H]-DHA and [^{3}H]-DHEC were detected in rat heart membranes suspended in Tris buffer, Banerjee and coworkers found an increased density of β- and decreased density of α-receptor binding sites after treatment of thyroidectomized rats with a total of 1,500 μg/kg T3 over a period of 6 days (Banerjee & Kung, 1977; Sharma & Banerjee, 1978). The present findings confirm and extend these observations in several respects. (1) Physiological responses and binding were measured under identical ionic, pH and temperature conditions. Although rate and force responses were measured in atria and binding in ventricles, thyroid-dependent reciprocal changes in α- and β-receptor responses appear to be qualitatively similar in different parts of the heart (Wagner Brodde, 1978). (2) The possibility that presynaptic α-receptors, not involved in the physiological response measured were included in binding sites was eliminated by the use of postsynaptic selective α-receptor antagonists both as the labelled and the suppressing ligand. (3) The shorter treatment schedule and lower doses of thyroid hormone in the present study allowed measurements before the development of significant cardiac hypertrophy. This reduces the possibility that the observed changes in receptor densities were due to the synthesis of new cell membrane material with higher β- and lower α-receptor density. This possibility is also discounted by the finding that the sum total of α plus β receptors remained unchanged. The observed changes

therefore represent true changes in receptor density in cell membrane material existing at the start of T4 treatment.

Although the changes in binding site numbers and in responses mediated by cardiac α- and β-adrenoceptors may be coincidental, a causal relationship is suggested by two facts. Firstly, the change in physiological response pattern could be best explained by a change in the relative numbers of receptors and this prediction was borne out by results of the binding study. Secondly, the time course of changes in response and binding were parallel. The altered response pattern of the hypothyroid myocardium was completely reversed within 2 days (Table 3); although binding was only tested at 2 days, the changes in receptor numbers observed also must have been maximal, since numerically almost identical changes were observed by Banerjee and coworkers after longer treatment with a higher dose of T3 (see above). The changes in α- and β-receptors were also not dissociated within the time range studied, which is at variance with recent results of Hashimoto & Nakashima (1978). These authors found that the inotropic sensitivity of guinea-pig atria to isoprenaline only increased after 6 to 7 days of T4 treatment, whereas the opposite change in the sensitivity to phenylephrine developed more rapidly. The much faster development of isoprenaline supersensitivity in the present study is in agreement with the rapid development of such changes in cultured heart cells (Wildenthal, 1974; Tsai & Chen, 1978).

The molecular mechanism of the thyroid-dependent inverse reciprocal regulation of cardiac α- and β-receptors is unknown. The parallel time course and the matching changes in receptor numbers strongly suggest a functional coupling of the two receptors in the postsynaptic membrane. However, the possibility that these changes are independent and coincidental has not been excluded.

The slow onset of the altered receptor response patten in the hypothyroid myocardium raised the possibility that these changes may be mediated indirectly, by factors other than thyroid hormones. Noradrenaline turnover rate was shown to increase after thyroidectomy (Tu & Nash, 1975; Tedesco, Flattery & Sellers, 1977) or hypophysectomy (Landsberg & Axelrod, 1968), and an increased tonic release of the neurotransmitter may down-regulate postsynaptic receptors (Raff, 1976). This mechanism, however, cannot explain the present findings. Whereas β-receptor sensitivity decreased, α-receptor sensitivity increased in the hypothyroid myocardium, and depletion of noradrenaline stores by reserpine failed to prevent or reverse these changes. Also, there is no indication of increased myocardial stimulation *in vivo* in hypothyroidism; this may relate to a shift from *O*-methylated to deaminated metabolites of noradrenaline in the hypophysectomized rat heart, which suggests that the increased noradrenaline turnover is intraneuronal, producing inactive metabolites (Landsberg & Axelrod, 1968).

Some effect of sympathetic innervation on cardiac adrenoceptors is indicated, however, by the finding that chemical denervation by 6-OHDA carried out shortly after thyroidectomy prevented the shift from β- to α-type responses, whereas reserpine treatment failed to produce a similar effect. These differential effects were also manifest when binding sites were directly measured. Denervation but not reserpine treatment reversed the ratio of α- to β-receptors. These changes are unlikely to be due to loss of binding sites on nerve terminals, since the density of β-receptors increased after denervation, and the decrease in α-receptors was for α_1 types, believed to represent postsynaptic receptors in peripheral tissues. These observations suggest that some neuronal factors other than normal stores of the neurotransmitter may be involved in the apparent 'conversion' of β- to α_1-adrenoceptors in the hypothyroid rat heart. However, such a 'trophic' influence does not exclude a direct effect of thyroid hormones on receptors, which is suggested by an effect of triiodothyronine on β-receptor reactivity (Wildenthal, 1974) or [^{3}H]-DHA binding (Tsai & Chen, 1978) in isolated, cultured myocardial cells.

The slow onset of the effects of hypothyroidism on adrenoceptors suggests that the thyroid-dependent factors involved have a slow turnover rate. Alternatively, the sensitivity of such factors to thyroid hormones could be high and even small amounts of thyroid hormones remaining in tissues after thyroidectomy or hypophysectomy could prevent their breakdown and the consequent shift from β- to α_1-receptors. This latter possibility is suggested by the rapid reversal of the effects of hypothyroidism by T4 treatment, which could be as fast as 30 min in some cultured cells (Popovic, Brown & Adamson, 1979). Some receptor–related, short-term effects of T3 appear to be independent of protein synthesis (Kempson, Marinetti & Shaw, 1978; Tsai & Chen, 1978) and studies are underway in our laboratory to determine whether the apparent conversion of cardiac α_1- to β-receptors could involve such a rapid effect.

In the present study the effects of hypophysectomy on adrenoceptor responses were very similar to those observed earlier in thyroidectomized rats (Kunos, 1977), which indicates that thyroid hormone and not TSH is the factor involved. The similarity between the two models also eliminates the possible involvement of parathyroid deficiency in the altered receptor response after surgical thyroidectomy. The adrenocorticoid deficiency of the hypophysectomized rat also does not contribute to the change in cardiac receptor responses, since only T4 and not cortisone corrected

these changes. This is different from the situation in the rat liver, where adrenalectomy was shown to change the adrenergic activation of glycogen phosphorylase from an α- to a mixed α-β type response (Chan & Exton, 1977); the increase in β-receptors was reversed by a steroid treatment schedule identical to the one that proved ineffective in the present study on heart (Wolfe, Harden & Molinoff, 1976).

Thyroid state can also affect liver adrenoceptors: thyroidectomy in rats changed phosphorylase activation in hepatocytes from an α- to a predominantly β-type response; this change is similar and even more complete than that after adrenalectomy, but it can be reversed only by thyroxine and not by cortisone treatment (Preiksaitis & Kunos, 1979). These findings indicate that an apparent change in adrenoceptor type can be induced by different stimuli, which possibly affect a final common pathway. It is also evident that the direction of the change produced by the same stimulus can be different in different tissues. The shift from β- to α-receptors in the hypothyroid rat heart is similar to the shift in adrenoceptor responsiveness in human adipose tissue in hypothyroidism (Rosenqvist, 1972), but opposite to the change from α- to β-type response observed in the hypothyroid rat liver. In contrast to the heart, the altered response of the liver is not affected by denervation (Preiksaitis & Kunos, unpublished). Although the reason for these puzzling, tissue-specific differences is not yet clear, their existence could provide new ways for studying the hormonal modulation of adrenoceptors.

Inverse, reciprocal regulation of α- and β-adrenoceptors in tissues where both receptor types mediate the same end response may have biological significance. Cardiac α- or hepatic β-receptors in the rat may represent vestiges of effector systems that were more dominant earlier in ontogenetic or phylogenetic development. Motor responses of vascular smooth muscle are mediated by α-receptors, and cardiac α-receptors may reflect the common embryological origin of the two tissues. Similarly, the greater dominance of hepatic β-receptors in juvenile rats (Blair, James & Foster, 1979) or in adult rats after partial hepatectomy (Hornbrook, 1978) could suggest a change in receptor type with cell differentiation. Shifts in the balance of α- and β-adrenoceptors by hormonal and other factors may be a homeostatic control mechanism that serves to adapt tissue function to altered metabolic conditions.

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