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# THYROID HORMONE-DEPENDENT INTERCONVERSION OF MYOCARDIAL $\alpha$- AND $\beta$-ADRENOCEPTORS IN THE RAT

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**1** The effects of thyroid state on the properties of adrenoceptors mediating inotropic and chronotropic responses of the rat heart were assessed on the basis of the relative potencies of $\alpha$- and $\beta$-adrenoceptor agonists, the effects of $\alpha$- and $\beta$-adrenoceptor antagonists and the tissue uptake of [$^3$H]-phenoxybenzamine ([$^3$H]-PB).

**2** In isolated, electrically driven left atria the ratio of the inotropic potencies of isoprenaline and phenylephrine and the inhibitory potency of propranolol (40nM–4 $\mu$M) were significantly reduced after thyroidectomy and were moderately increased after thyroxine treatment of control rats.

**3** Block of inotropic responses to noradrenaline and to phenylephrine by PB (7.3nM–7.3 $\mu$M) and the tissue uptake of [$^3$H]-PB were significantly greater in preparations from thyroidectomized than in those from control or from thyroxine treated rats. $\alpha$-Adrenoceptor inhibition by phentolamine (0.26–2.6 $\mu$M) also increased after thyroidectomy, and phentolamine effectively protected $\alpha$-adrenoceptors from block by and binding of [$^3$H]-PB.

**4** The $\beta_1$-receptor antagonist H 93/26 (0.1 $\mu$M) significantly potentiated $\alpha$-adrenoceptor blockade by PB in hypothyroid but not in control preparations.

**5** In spontaneously beating right atria the chronotropic potency of agonists and the effects of antagonists were altered in the same way as were inotropic responses and the slope of the agonist concentration-response curves were significantly reduced after thyroidectomy. Effects of agonists and antagonists were not significantly influenced by thyroxine treatment.

**6** Changes in the effects and tissue uptake of sympathomimetic drugs observed after thyroidectomy were reversed to or beyond control levels by thyroid hormone treatment of thyroidectomized animals.

**7** The results presented are interpreted as indicating a thyroid hormone-dependent interconversion of myocardial $\alpha$- and $\beta$-adrenoceptors. It is suggested that this interconversion is similar to that observed earlier in frog hearts at different temperatures, and that both effects may reflect an allosteric transition between two forms of a single basic structure.

## Introduction

The striking similarities between the effects of sympathetic activity and the cardiovascular symptoms of hyperthyroidism have long suggested some relationship. However, there has been considerable controversy in the literature about the influence of thyroid hormones on the adrenergic reactivity of the heart. Classical works suggested that hyperthyroidism increased and hypothyroidism decreased the sensitivity of the myocardium to catecholamines or to sympathetic nerve stimulation, although some of the more recent studies failed to confirm these changes (Harrison, 1964; Waldstein, 1966). Some of this controversy may have arisen from the fact that most studies did not distinguish between $\alpha$- and $\beta$-adrenoceptor responses and did not test the effects of drugs with selective actions on $\alpha$- and $\beta$-adrenoceptors. Although the adrenoceptors mediating inotropic and chronotropic responses of the heart have the pharmacological characteristics of $\beta$-adrenoceptors, there has been evidence that $\alpha$-adrenoceptors can also mediate these responses in both amphibian (Nickerson, 1949) and mammalian hearts (Wenzel & Su, 1966; Benfey & Varma, 1967; Govier, 1968), and the two kinds of receptors may be differently influenced by thyroid hormones. Recent studies with isolated hearts of frogs have shown that the adrenoceptors that mediate inotropic and chronotropic responses can be altered qualitatively by ambient temperature in a way that their characteristics shift from $\beta$ toward $\alpha$ when the temperature of the

isolated hearts is reduced through a critical range of 17–22°C (Kunos & Szentiványi, 1968; Buckley & Jordan, 1970; Kunos, Yong & Nickerson, 1973; Harri, 1973; Kunos & Nickerson, 1976). These results suggested that $\alpha$- and $\beta$-adrenoceptors represent different forms of a single basic structure, whose properties can be influenced by the metabolic activity of the tissue. Although qualitatively similar changes in adrenoceptor characteristics appear to occur in the mammalian myocardium (Kunos & Szentiványi, 1968; Kunos, Vermes-Kunos, Boyd & Nickerson, 1973; Benfey, Kunos & Nickerson, 1974; Amer & Byrne, 1975), the physiological significance of a temperature-induced change in a homeothermic species is obviously limited. Several observations indicate, however, that adrenoceptors can be influenced by factors other than temperature, including the administration of a iodo- or fluoroacetate (Nickerson & Nomaguchi, 1950) or dinitrophenol (Kunos & Szentiványi, 1968; Matheny & Ahlquist, 1975), skeletal muscle activity (Szentiványi, Kunos & Juhász-Nagy, 1970a) and increased vagal influence on the myocardium (Szentiványi, Kunos & Juhász-Nagy, 1970b). Under these conditions, $\alpha$-adrenoceptor properties were always promoted by a decrease and $\beta$-adrenoceptor properties were enhanced by an increase in metabolic activity. Therefore, it seemed possible that changes in thyroid state would also alter adrenoceptor properties. The results presented in this paper indicate a maximal dominance of $\beta$-adrenoceptors mediating inotropic and chronotropic responses in rats after thyroid hormone treatment, and an increased dominance of $\alpha$-adrenoceptors after thyroidectomy. Some of these results have been published in a preliminary report (Kunos, Vermes-Kunos & Nickerson, 1974).

## Methods

Experiments were performed on isolated, electrically driven left and spontaneously beating right atria of male Sprague-Dawley rats. Hypothyroidism was induced by surgical thyroidectomy in rats weighing 180–220 grams. The thyroid glands were removed under ether anaesthesia. In most animals, the parathyroids could not be separated and were also removed. In a few rats, hypothyroidism was induced by a single intravenous injection of 200 μCi of $^{131}I$. The results obtained in preparations from these animals were identical with those in atria from surgically thyroidectomized rats and the two groups were pooled. The experiments on the isolated tissues were carried out 6 weeks after the operation or $^{131}I$ treatment, by which time the animals had become hypothyroid. Hypothyroidism was ascertained by reduction of growth, dryness of fur, a significant decrease in serum thyroxine levels, as determined by the method of Murphy & Pattee (1964), and by a significant decrease in the control rate of beating of right atria (Table 1). Control preparations were from weight-matched, or from sham-operated age-matched animals. Responses of atria from the two kinds of controls were not significantly different and the results were pooled. A third group of animals was first thyroidectomized and then injected daily with 1 mg/kg thyroxine ($T_4$) or 0.25 mg/kg tri-iodothyronine ($T_3$) for 1 week before the experiment. The effects of $T_4$ or $T_3$ treatment on responses of the isolated atria were similar. In the last group of animals, high serum thyroxine levels were maintained (Table 1) by daily intraperitoneal injections of 1 mg/kg $T_4$ for 8 days before the experiment.

All animals were anaesthetized with ether and the

**Table 1** The effect of thyroid state on weight gain, serum thyroxine ($T_4$) levels, basal heart rate and on the ratio of the inotropic potency of isoprenaline and phenylephrine

| | *Weight gain*[a] *(g/week)* | *Serum $T_4$ levels*[b] *(ng/ml)* | *Basal right*[c] *atrial rate (beats/min)* | *Isoprenaline*[e] *phenylephrine molar potency ratio (log)* |
|---|---|---|---|---|
| Control | 28.2 ± 1.4 (40) | 58.5 ± 3.4 (10) | 192.1 ± 4.2 (17) | 3.50 ± 0.14 (30) |
| Thyroidectomized | 3.2 ± 0.6** (40) | 21.2 ± 4.2** (8) | 118.2 ± 3.1** (32) | 2.24 ± 0.16** (18) |
| Thyroidectomized + $T_3$ or $T_4$ | 11.9 ± 5.7* (7) | 91.3 ± 8.1**[d] (6) | 251.2 ± 5.8** (15) | 4.02 ± 0.24 (6) |
| Control + $T_4$ | 20.5 ± 6.8 (8) | 169.3 ± 26.3** (6) | 253.2 ± 8.2** (8) | 4.61 ± 0.21** (12) |

Values are means with their standard errors. Numbers of experiments are given in parentheses. Significance of difference from corresponding control values: *$P < 0.05$, **$P < 0.005$.
[a]during week prior to experiment; [b]determined at the time of the experiment by the method of Murphy & Pattee (1964); [c]at 31°C; [d]measured in animals treated with $T_4$; [e]for inotropic responses of left atria.

hearts quickly removed. The right and left atria were separated and mounted in 20 ml organ baths containing a modified Krebs-Henseleit solution of the following composition (mM): NaCl 115.3, KCl 4.6, $CaCl_2$ 1.8, $MgSO_4$ 1.1, $NaHCO_3$ 22.1, $KH_2PO_4$ 1.1 and glucose 11.1. The medium was maintained at 31°C and aerated with 5% $CO_2$ in $O_2$. The tip of the atrial appendage was attached by a thread to a force-displacement transducer (Grass FT83C) and isometric contractions were recorded on a polygraph (Grass Model 7). Resting tension was set at 0.5 g and the preparations were allowed to equilibrate for 90 min with frequent changes of the medium. Left atria were driven by platinum electrodes delivering square wave pulses of 1 ms duration, at a frequency of 1 Hz and at a voltage slightly over threshold (0.3–1.0 V). Right atria beat spontaneously and the rate was measured by direct counting from the chart paper. Both basal tension and rate decreased initially, but there was little further change after 90 min of equilibration. Cumulative concentration-response curves for various agonists were determined before and after different $\alpha$- or $\beta$-adrenoceptor antagonists. Maximal responses were determined for each agonist and responses are expressed as the percentage of the maximal control increase in beat amplitude or rate. Absolute force and rate values are also given in some cases. Exposure to all of the receptor blocking agents was for 40 minutes. After incubation with phenoxybenzamine (PB), alone, or in combination with phentolamine or H 93/26, the preparations were repeatedly washed for at least 30 min before retesting the agonists. Phentolamine and the $\beta$-blocking agents used remained in the bath during addition of an agonist. Each preparation was used with only one blocking agent, except when the effect of a combined exposure to two blocking drugs was tested.

The validity of the use of $pD_2$ values to express agonist potency was supported by findings in preliminary experiments where neither a significant desensitization nor a significant change in maximal responses occurred when control dose-response curves were repeatedly determined in the same preparation.

*Experiments with [$^3$H]-phenoxybenzamine*

[$^3$H]-PB hydrochloride was prepared from [$^3$H]-benzyl-chloride by the method of Nikawitz, Gump, Kerwin & Ullyot (1952), and had a specific activity of 33.13 mCi/mmol. Its physiochemical and pharmacological properties were identical to those of an authentic sample of PB hydrochloride (Smith, Kline & French), and it was shown to be radiochemically pure (Yong & Nickerson, 1973). Left atria, exposed to [$^3$H]-PB, were taken from the organ bath 2 h after removal of the drug and repeated washing of the preparation with drug-free medium. The preparations were freeze-dried, weighed, and digested in 0.5 ml NCS tissue solubilizer (Amersham/Searle) at 50°C. Counting solution (14.5 ml of Aquasol, New England Nuclear) was added and the radioactivity was measured in a liquid scintillation counter (Nuclear Chicago Mark I). Counting efficiency was assessed by the channels ratio method and a quench calibration curve, and was found to be in the range of 20 to 35%. The results are expressed as concentration of the labelled drug in the dry tissue.

*Drugs*

The following drugs were used: (–)-arterenol bitartrate (Calbiochem; referred to as noradrenaline), (+)-isopropylnoradrenaline hydrochloride (K & K; isoprenaline), (–)-neo-synephrine hydrochloride (Sterling-Winthrop; phenylephrine), phenoxybenzamine hydrochloride (Smith, Kline & French; PB), phentolamine methanesulphonate (Ciba), (±)-propranolol hydrochloride (Ayerst) and 1-isopropylamino-3-[4-(2-methoxyethyl)-phenoxy]-2-propanol (H 93/26, Hässle). Most of the drugs were freshly diluted before each experiment in 0.9% w/v NaCl solution (saline) containing 0.01 N HCl. Stock solutions of PB and [$^3$H]-PB were made in propylene glycol containing 0.01N HCl and were stored at –20°C. Dilutions were made with Krebs solution for each administration of the drug. Concentrations are expressed as the final concentration in M or μM in the bath.

Differences between means were evaluated by Student's two tailed $t$ test for unpaired data or the $t$ test for paired data, as appropriate, and differences with a $P$ value of 0.05 or less were considered significant.

## Results

*Effects of altered thyroid state on adrenoceptors mediating inotropic responses*

Thyroidectomy moderately reduced and thyroid hormone treatment slightly increased the basal contractile force of left atria but the maximal developed tension in response to agonists was similar in all groups. The relative inotropic efficacies of the agonists tested were also not significantly altered by changes in thyroid state; maximal increases in force in response to noradrenaline (NA) and isoprenaline were similar and the efficacy of phenylephrine was 70 to 100% of that of the other two agonists, in any of the groups tested. However, there were significant and selective changes in the potencies of the three amines (Figure 1). Sensitivity to the relatively pure $\beta$-adrenoceptor agonist, isoprenaline, was significantly increased after thyroxine treatment and decreased following thyroidectomy, whereas sensitivity to the $\alpha$-adrenoceptor agonist, phenylephrine, was changed in

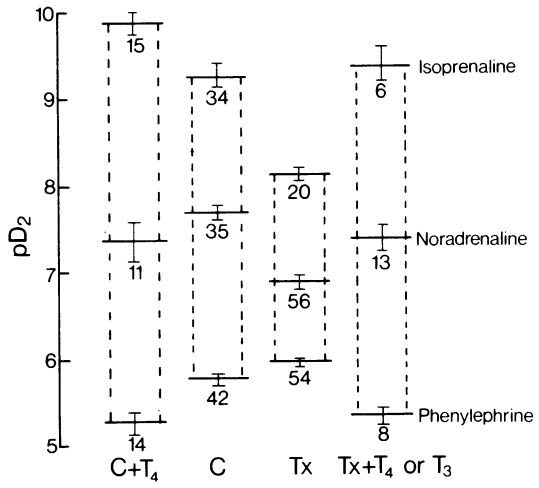


**Figure 1** Inotropic potencies of sympathomimetic agonists on left atria from rats in different thyroid states. Horizontal lines are means and vertical bars are s.e. of $pD_2$, (negative logarithm of the molar concentration that produces half-maximal response). C=control; Tx=thyroidectomized; C+$T_4$=thyroxine-treated controls; Tx+$T_4$ or $T_3$=thyroidectomized, treated with thyroid hormones. Numbers of preparations used are given by small numbers.

the opposite way. NA sensitivity was significantly reduced by thyroidectomy but was not significantly altered by thyroxine treatment. For all three amines, the change in potency observed in the hypothyroid myocardium was reversed to or beyond control levels by thyroid hormone treatment of the thyroidectomized animals. The reciprocal changes in the sensitivity of $\alpha$- and $\beta$-adrenoceptors are reflected by changes in the molar potency ratio of isoprenaline and phenylephrine. The correlation between this parameter and serum thyroxine levels in different thyroid states (see Table 1) suggests that the balance between $\alpha$- and $\beta$-adrenoceptors in the heart is under thyroid control; high thyroid hormone levels enhance the dominance of $\beta$-adrenoceptors and low hormone levels promote $\alpha$-adrenoceptor properties.

Changes in the effectiveness of $\alpha$- and $\beta$-adrenoceptor antagonists were also compatible with this possibility. The data in Table 2 demonstrate that inhibition of responses to NA by propranolol was decreased by thyroidectomy and was moderately increased after thyroxine treatment. It was also noted that the relationship between dose-ratio, and inhibitor concentration, in hypothyroid atria deviated considerably from the relation expected from simple competitive inhibition. The effect of H 93/26, a cardioselective $\beta$-blocking agent (Johansson, 1973), on inotropic responses to phenylephrine also decreased in hypothyroidism. The log dose-ratio for half-maximal responses in the presence of 0.1 μM H 93/26 and in its absence was $0.66 \pm 0.14$ in atria from thyroidectomized and $1.11 \pm 0.11$ in preparations from control animals ($P < 0.05$).

In contrast to $\beta$-adrenoceptor inhibitors, the effectiveness of $\alpha$-adrenoceptor antagonists was increased after thyroidectomy and decreased following thyroid hormone treatment. Figure 2a shows that in control preparations, a 40 min exposure to 7.3 μM PB potentiated responses to NA and moderately inhibited the effect of phenylephrine. In preparations from thyroidectomized animals (Figure 2b), a similar exposure to PB partially inhibited responses to NA and blocked the effect of phenylephrine almost completely. Thyroid hormone treatment reversed the effect of PB on NA to full potentiation and reduced its blockade of responses to phenylephrine below control levels (Figure 2c). In atria from $T_4$-treated control rats (Figure 2d) PB potentiated responses to NA and did not significantly inhibit responses to phenylephrine. In association with its increased blocking effect, significantly more [$^3$H]-PB was bound to the hypothyroid myocardium ($343 \pm 18$ pmol/mg dry tissue,

**Table 2** The effect of thyroid state on the inhibitory potency of propranolol in rat left atria

| | *40 nM* | *Propranolol 0.4 μM* | *4 μM* |
|---|---|---|---|
| Control | $0.70 \pm 0.09$[a] (11) | $1.84 \pm 0.15$ (11) | $3.01 \pm 0.37$ (5) |
| Thyroidectomized | $0.37 \pm 0.07$* (7) | $0.70 \pm 0.10$** (8) | $1.39 \pm 0.07$** (8) |
| Thyroidectomized + $T_3$ or $T_4$ | $0.75 \pm 0.10$ (5) | $1.70 \pm 0.21$ (5) | $2.54 \pm 0.38$ (5) |
| Control + $T_4$ | $1.41 \pm 0.10$** (6) | $2.24 \pm 0.07$* (5) | $3.20 \pm 0.05$ (5) |

[a] Log dose-ratios, calculated at 50% of the maximal control inotropic response to noradrenaline. Values are means and their standard errors. Number of experiments in parentheses.
Significance of difference from corresponding control values: *$P < 0.05$; **$P < 0.005$.

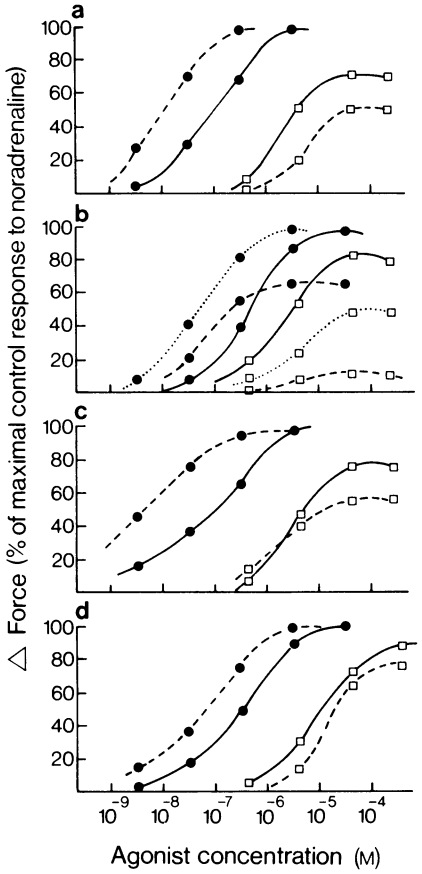


**Figure 2** Effects of phenoxybenzamine on inotropic responses to noradrenaline and phenylephrine in different thyroid states. (●) Noradrenaline; (□) phenylephrine; solid lines: control responses; dashed lines: after a 40 min exposure to phenoxybenzamine (7.3 μM); dotted lines: after exposure to phenoxybenzamine (7.3 μM) in the presence of phentolamine (26.5 μM). (a) Control preparations ($n=18$); (b) thyroidectomized ($n=21$); (c) thyroidectomized + $T_4$ or $T_3$ ($n=8$); (d) control + $T_4$ ($n=8$).

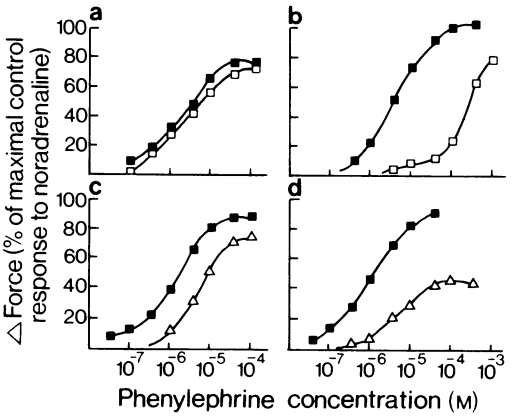


**Figure 3** Effects of low concentrations of phenoxybenzamine on inotropic responses to phenylephrine. (a) and (c) Control; (b) and (d) thyroidectomized; (■) control responses; (□) after a 40 min exposure to phenoxybenzamine (0.07 μM); (△) after exposure to phenoxybenzamine (0.7 μM). Number of experiments: 4 (a), 3 (b), 4 (c), 7 (d).

$n=22$) than to atria from either control ($262 \pm 16$, $n=13$, $P<0.005$) or from thyroidectomized, hormone-treated rats ($223 \pm 10$, $n=8$, $P<0.005$).

The increased blocking effect of PB in the hypothyroid myocardium was not similar to the 'spurious' block that can be observed when baseline contractile amplitude is increased in the presence of PB (Nickerson & Chan, 1961): after washout of the PB, the baseline contractile force was reduced slightly below control and the significant reduction in the maximal developed tension in response to NA and phenylephrine was not accompanied by a significant change in the maximal effect of $CaCl_2$ (Table 3). When hypothyroid atria were exposed to 0.7 μM PB in the presence of 12.8 μM quinidine, a drug known to reduce the rate of transmembrane $Ca^{2+}$ exchange (Silva Graça & Van Zwieten, 1972), the degree of block of responses to phenylephrine was the same as after PB alone. These observations discount the possibility that the increased blocking effect of PB in the hypothyroid myocardium is a non-specific effect on $Ca^{2+}$ movements. The change in the blocking effectiveness of PB in hypothyroidism was also observed at lower concentrations of the inhibitor (Figure 3). In the atria from control rats, 0.07 μM PB was ineffective in inhibiting responses to phenylephrine (Figure 3a) and 0.7 μM PB produced a moderate block (Figure 3c), whereas in atria from thyroidectomized animals, 0.07 μM PB produced significant inhibition (Figure 3d) and reduction of the maximal response by 0.7 μM (Figure 3d) was greater than in control preparations.

Thyroidectomy also increased the blocking effect of the reversible α-adrenoceptor antagonist, phentolamine. The marginal inhibition by 2.6 μM phentolamine of responses of control atria to phenylephrine (log dose-ratio $0.32 \pm 0.09$, $n=5$) was significantly increased in preparations from thyroidectomized rats ($0.80 \pm 0.11$, $n=5$, $P<0.01$). Inhibition by phentolamine was reversible by washing the preparations for 30 to 60 minutes. When left atria, from thyroidectomized animals, were exposed to 7.3 μM [$^3$H]-PB in the presence of 26.5 μM phentolamine and then washed for at least an hour (6

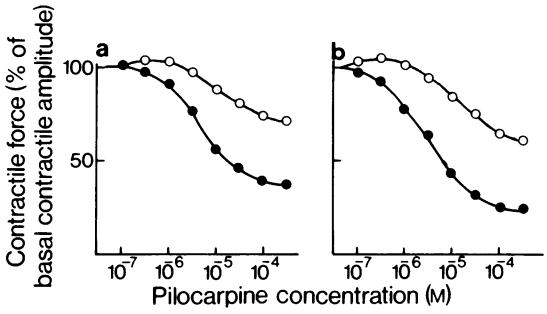


**Figure 4** Inhibition of phenoxybenzamine of the negative inotropic response to pilocarpine. (a) Control ($n=6$); (b) thyroidectomized ($n=8$). (●) Control responses, (○) after a 40 min exposure to phenoxybenzamine (0.7 μM).

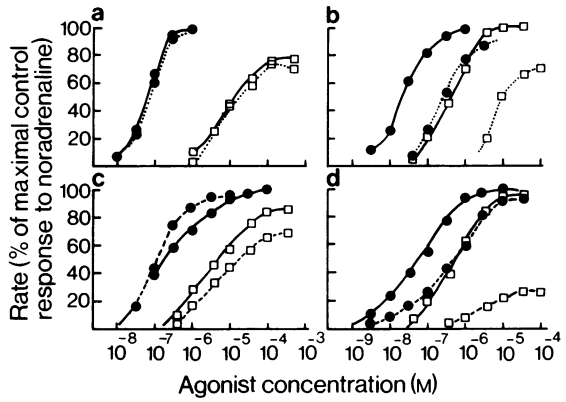


**Figure 5** The effect of combined exposure to β- and α-adrenoceptor blocking drugs on inotropic responses to sympathomimetic drugs in different thyroid states. (a) and (c) Control; (b) and (d) thyroidectomized. (●) Noradrenaline; (□) phenylephrine. Solid lines: control responses; dotted lines (a, b): after a 40 min exposure to phenoxybenzamine (0.007 μM) in the presence of H 93/26 (0.1 μM); dashed lines (c, d): after exposure to phenoxybenzamine (0.07 μM) in the presence of H 93/26 (0.1 μM). Numbers of experiments: 4 (a), 5 (b), 5 (c), 5 (d). For explanation see text.

experiments), protection of α-adrenoceptors was shown by a significant reduction in both the block by (Figure 2b, dotted lines) and in the retention of [$^3$H]-PB (from 343 ± 18 to 247 ± 16 pmol/mg dry tissue, $P<0.005$). In 6 control preparations, a similar combination of the two antagonists resulted in potentiation of responses to NA identical to that caused by PB alone, and retention of [$^3$H]-PB was the same (265 ± 17) as after exposure to [$^3$H]-PB alone (262 ± 16 pmol/mg dry tissue).

In the presence of phentolamine, potentiation by PB of responses of hypothyroid atria to NA (Figure 2b) was not different from the potentiation seen in control preparations (Figure 2a), indicating that this effect was not influenced by the change in thyroid state. Inhibition by PB of the negative inotropic effect of pilocarpine was also not affected by thyroidectomy (Figure 4). In the latter experiments, pilocarpine was used as an agonist, since the use of acetylcholine for quantitative evaluation of cholinoceptor blockade in tissues with high cholinesterase activity is misleading. These observations suggest that the altered reactivity of the hypothyroid tissue to PB is selective to α-adrenoceptors.

Since β-adrenoceptor activity was only reduced but not abolished after thyroidectomy, the effect of α-adrenoceptor antagonists could have been masked by simultaneous activation of β-adrenoceptors by agonists. To reduce the β-adrenergic component of inotropic reactions, some preparations were exposed to PB in the presence of a small concentration of a β-adrenoceptor antagonist (Figure 5). The effect of

**Table 3** The effect of phenoxybenzamine (PB) on basal contractile force and on maximal contractile responses to agonists in left atria from thyroidectomized rats

| | *Baseline contractile force (mg)* | *Maximal increase in contractile force (mg)* | | |
|---|---|---|---|---|
| | | *NA* | *Phenylephrine* | *$CaCl_2$* |
| Control | 341 ± 106 | 743 ± 167 | 537 ± 85 | 623 ± 137 |
| After PB 0.7 μM | 326 ± 101 | 705 ± 170 | 250 ± 101 | 697 ± 141 |
| % change | −4.2 ± 3.2 | −5.9 ± 3.9 | −54.7 ± 7.4* | +13.0 ± 5.1 |
| Control | 293 ± 52 | 660 ± 92 | 538 ± 76 | 514 ± 90 |
| After PB 7.3 μM | 271 ± 36 | 583 ± 107 | 84 ± 21 | 537 ± 85 |
| % change | −0.6 ± 4.8 | −15.0 ± 5.6* | −81.8 ± 4.7** | +0.3 ± 4.1 |

Significant change: *$P<0.05$; **$P<0.005$
Number of experiments is 6 (7.3 μM PB) and 3 (0.7 μM PB).

0.1 μM H 93/26 on responses to phenylephrine was first tested and the preparations were then washed for an hour. This time was sufficient to reverse the observed inhibition almost completely. As in other experiments mentioned above, inhibition was significantly greater in control preparations than in atria from thyroidectomized animals. The preparations were then incubated with the same concentration of H 93/26 and, 10 min later, PB (0.007 or 0.07 μM) was added to the medium. After a 40 min incubation with both drugs, the preparations were washed for an hour with drug-free medium and the effects of the agonists were retested. The results show that in control preparations, simultaneous inhibition of β-adrenoceptors did not significantly enhance the degree of α-adrenoceptor blockade: low concentrations of PB remained practically ineffective in inhibiting responses to either NA or phenylephrine (Figure 5a,c). On the other hand, in atria from thyroidectomized animals the observed inhibition was clearly greater than after PB alone. Exposure to 0.07 μM PB in the presence of 0.1 μM H 93/26 inhibited the effects of NA and blocked responses to phenylephrine almost completely (Figure 5d, compare to results in Figure 3b), and even 0.007 μM PB produced significant inhibition of responses to both agonists (Figure 5b). These experiments indicate that in control preparations the strong dominance of β-adrenoceptors is not significantly reduced after their moderate inhibition, whereas in atria from thyroidectomized rats, an even smaller inhibition produced by the same concentration of the β-blocking drug, significantly enhanced the apparent α-adrenoceptor activity.

*The effect of altered thyroid state on adrenoceptors mediating chronotropic responses*

Although changes in thyroid hormone levels caused significant and predictable changes in basal right atrial rate (Table 1), the maximal increase in rate produced by agonists and their relative efficacies were not significantly altered. The maximal increase in heart rate produced by NA was slightly less than that after isoprenaline and slightly more than that after phenylephrine in all four groups. However, there were selective changes in the potencies of agonists, qualitatively similar to those observed with inotropic reactions in left atria. The data in Table 4 show that thyroidectomy reduced the potency of isoprenaline and increased the potency of phenylephrine, which resulted in a decrease in their molar potency ratio. Unlike inotropic responses, this change was not fully reversed by thyroid hormone treatment and thyroxine treatment of control rats failed to alter significantly the chronotropic potency of agonists. The same trend could be observed when inhibition by propranolol of chronotropic responses to NA was determined in different thyroid states (Table 5). The significant decrease in the effectiveness of propranolol in hypothyroidism was only partially reversed by thyroid hormone treatment and the increase in the inhibitory effect of propranolol in thyroxine-treated control rats was not statistically significant. The conclusion from these experiments, that chronotropic responses of control atria are closer to maximal β-adrenoceptor dominance than inotropic responses, was corroborated by results obtained with α-adrenoceptor antagonists. In contrast to inotropic responses, chronotropic responses to phenylephrine did not have a significant α-adrenoceptor component: PB (Figure 6a) or phentolamine (Figure 6c) failed to inhibit the increase in heart rate produced by phenylephrine. In right atria from thyroidectomized animals, the appearance of α-adrenoceptors mediating chronotropic responses was indicated by the significant inhibition of the effect of phenylephrine by PB (Figure 6b) or by phentolamine (Figure 6d).

Changes in thyroid state were associated with significant changes in the slopes of concentration-response curves for chronotropic effects. Changes in

**Table 4** Chronotropic potency of adrenoceptor agonists in different thyroid states

| | *Isoprenaline* | *Noradrenaline* | *Phenylephrine* |
|---|---|---|---|
| Control | 9.49 ± 0.21 (11) | 7.84 ± 0.11 (17) | 5.64 ± 0.09 (16) |
| Thyroidectomized | 8.68 ± 0.23* (15) | 6.83 ± 0.12** (29) | 6.14 ± 0.16* (12) |
| Thyroidectomized + $T_3$ or $T_4$ | 9.05 ± 0.10 (5) | 7.18 ± 0.16** (15) | 5.25 ± 0.21 (7) |
| Control + $T_4$ | 9.40 ± 0.14 (7) | 8.09 ± 0.16 (8) | 5.76 ± 0.14 (5) |

Values shown are means ± s.e. of $pD_2$ (negative logarithm of molar concentration required to produce half-maximal response). Numbers of experiments are given in parentheses.
Significance of difference from corresponding value in controls: *$P < 0.05$; **$P < 0.005$.

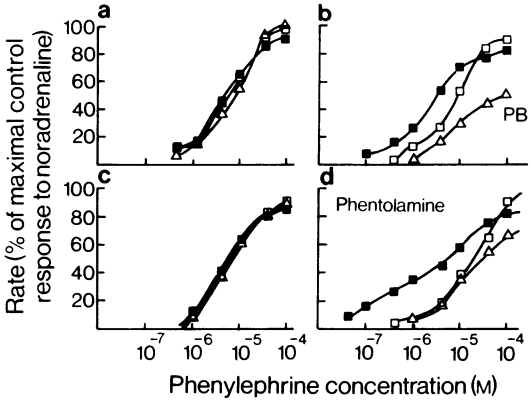


**Figure 6** Effects of α-adrenoceptor antagonists on chronotropic responses to phenylephrine in different thyroid states. (a) and (c) Control; (b) and (d) thyroidectomized. (■) Control responses; (□) after a 40 min exposure to 0.7 μM phenoxybenzamine (a and b) or to 0.3 μM phentolamine (c and d); (△) after a 40 min exposure to 7.3 μM phenoxybenzamine (a and b) or 2.6 μM phentolamine (c and d). Number of experiments: 4 (a), 4 (b), 6 (c), 7 (d).

the slopes of inotropic concentration-response curves were smaller and less consistent. Although the slope of the concentration-response relationship in a complex system can be influenced by a variety of factors and its value in studying quantitative aspects of drug-receptor interactions is limited, the parallel between the observed changes in receptor properties and changes in slopes of the concentration-response curves of chronotropic reactions justifies a limited quantitative analysis of dose-response relationships. A simple way to describe concentration-response curves is to use the ratio of two concentrations causing specified effects (Stephenson, 1956). Table 6 shows the logarithms of the ratios of concentrations that produce 50% and 20% of the maximal chronotropic responses to isoprenaline and to phenylephrine. In control preparations, where both agonists exerted their effects on β-adrenoceptors, steep slopes resulted in small log-dose ratios: the ratio was smaller for isoprenaline than for phenylephrine and both were smaller than the value of 0.60 (log of 4) predicted by a 1 : 1 or non-cooperative drug-receptor interaction. In right atria from thyroidectomized animals, the appearance of an α-adrenoceptor component in the chronotropic effect of phenylephrine was associated with a significant decrease in slope, indicated by the increase in ratio. The increase in ratio for isoprenaline was much smaller. Inhibition of α-adrenoceptors by phentolamine or a low concentration of PB decreased the log-ratio toward that in control preparations as also illustrated by the steeper concentration-response curves in Figure 6b and d whereas inhibition of β-adrenoceptors by H 93/26 produced a further increase in ratio, i.e. decrease in slope. These observations suggest that activation of myocardial β-adrenoceptors result in steeper dose-response curves than activation of α-adrenoceptors, and a change in the balance of α- and β-adrenoceptors results in a corresponding change in slopes.

## Discussion

The results presented establish a strong correlation between thyroid hormone levels and the relative importance of myocardial α- and β-adrenoceptors mediating inotropic and chronotropic responses. In general, thyroidectomy shifted the balance of adrenoceptors toward the dominance of the α component, whereas maximal dominance of β-adrenoceptors was observed in preparations from thyroxine-treated animals. The properties of adrenoceptors in the myocardium of euthyroid rats appeared to be closer to those in hyperthyroid than in hypothyroid animals.

The observed shifts in the balance between the two kinds of receptors can be interpreted as due to changes in the equilibrium between two forms of a single type

**Table 5** Inhibition of chronotropic responses to noradrenaline by propranolol in different thyroid states

| | *40 nM* | *Propranolol 0.4* μM | *4* μM |
|---|---|---|---|
| Control | 1.43 ± 0.17[a] (4) | 2.15 ± 0.23 (4) | 3.09 ± 0.27 (4) |
| Thyroidectomized | 0.84 ± 0.19* (9) | 1.28 ± 0.25* (10) | 1.86 ± 0.29* (10) |
| Thyroidectomized + $T_3$ or $T_4$ | 1.07 ± 0.20 (8) | 1.78 ± 0.22 (8) | 3.07 ± 0.31 (8) |
| Control + $T_4$ | 1.76 ± 0.14 (4) | 2.51 ± 0.18 (4) | 3.38 ± 0.24 (4) |

[a]Log dose-ratios, calculated at 50% of the maximal chronotropic response to noradrenaline. Values are means and their standard errors. Number of experiments is given in parentheses.
Significance of difference from corresponding control values: *$P < 0.05$.

of unit, as proposed earlier (Kunos & Szentiványi, 1968; Kunos & Nickerson, 1976), although they can also be explained by two independent pools of receptors with reciprocal sensitivities to the effects of thyroid hormones. In earlier observations on temperature-induced receptor transformation the first interpretation was favoured by the finding that $\beta$-adrenoceptors did not appear at high temperatures after $\alpha$-adrenoceptors had been alkylated at a low temperature (Kunos *et al.*, 1973; Kunos & Nickerson, 1976), and there are analogies between the two models that suggest that the changes in responsiveness produced by altered thyroid hormone levels reflect a similar interconversion of myocardial $\alpha$- and $\beta$-adrenoceptors.

The metabolic rate of tissues is low both in hypothyroidism and at low temperatures. Similarly to the effect of hypothyroidism in rats, low temperature in frog hearts induced reciprocal changes in the effectiveness of $\alpha$- and $\beta$-blocking agents (Kunos & Szentiványi, 1968; Buckley & Jordan, 1970; Harri, 1973; Kunos & Nickerson, 1976), significantly reduced the potency ratio of isoprenaline and phenylephrine (Harri, 1973; Tirri, Harri & Laitinen, 1974; Kunos, unpublished), and increased the tissue binding of [$^3$H]-PB (Kunos & Nickerson, 1976). The $\alpha$-adrenoceptor component in inotropic responses appears to require intact sympathetic innervation and denervation of rats by 6-hydroxydopamine was shown to stabilize the receptor in the $\beta$-form both at low temperature (Kunos *et al.*, 1973) and in hypothyroidism (Kunos & Mucci, 1975). Both hypothyroidism and low temperature decreased the sensitivity of the myocardium to the natural transmitter adrenaline (frog) or NA (rat), which suggests that $\alpha$- and $\beta$-adrenoceptors may be low- and high-sensitivity forms of the same unit. Adrenoceptor reactivity in frog isolated hearts shows seasonal variations and the greater sensitivity of the winter frog heart to $\alpha$-adrenoceptor blockade could be abolished by treating frogs with anterior pituitary extract, which possibly increased thyroid activity (Nickerson & Nomaguchi, 1950), or by raising the temperature of the isolated heart (Kunos & Szentiványi, 1968). These findings and our unpublished observation that the shift in the properties of myocardial adrenoceptors after thyroidectomy was not significantly increased by lowering the temperature of these preparations, suggest that the mechanisms by which thyroid hormones and temperature can influence receptors are similar.

Although adrenoceptors mediating inotropic and chronotropic responses were affected by changes in thyroid hormone levels in a qualitatively similar way, the equilibrium between the $\alpha$ and the $\beta$ component was different for the two reactions. In contrast to inotropic responses, and in agreement with published observations (Krell & Patil, 1969; Parr & Urquilla, 1972), chronotropic responses of control atria did not have an $\alpha$-adrenoceptor component. This difference was also apparent in preparations from thyroidectomized rats, where propranolol was more effective and PB was less effective in inhibiting chronotropic than inotropic responses. Maximal dominance of $\beta$-adrenoceptors in chronotropic responses of control atria may explain why the potency of agonists for this effect did not significantly change after thyroxine treatment. These observations indicate that the equilibrium between $\alpha$- and $\beta$-adrenoceptors is different for different kinds of responses. Observations on the effects of thyroid state on myosin ATP-ase activity show important species differences in whether conditions in the euthyroid animal are closer to those in the hypothyroid or in the hyperthyroid state (Yazaki & Raben, 1975). Similar differences may exist for receptor equilibrium and may have contributed to the considerable controversy in the literature on the effects of altered thyroid states on

**Table 6** Slopes of chronotropic concentration-response curves in different thyroid states

| *Agonist:* / *Antagonist:* | *Isoprenaline* — | *Phenylephrine* — *Phenoxybenzamine, 0.7 μM or phentolamine, 0.3 μM* | | *Phenylephrine* — *H 93/26, 0.1 μM* | |
|---|---|---|---|---|---|
| Control | 0.45 ± 0.02 (13) | — | | 0.55 ± 0.04 (15) | — |
| Thyroidectomized | 0.60 ± 0.06 (11) | 0.55 ± 0.05 (7) | $P < 0.05$ | 0.84 ± 0.09 (18) | $P > 0.05$ 1.26 ± 0.27 (5) |
| *P* value | < 0.05 | | | < 0.01 | |

Values are means ± s.e. of log dose-ratio (logarithm of the ratio of $EC_{50}$ and $EC_{20}$ for isoprenaline or for phenylephrine). Numbers of experiments are given in parentheses.

catecholamine sensitivity (Harrison, 1964; Waldstein, 1966). Another source of discrepancies may be the inconsistency in the way sensitivity to agonists has been determined; although the conclusion in some recent studies was a lack of change in myocardial sensitivity to noradrenaline in altered thyroid states (Buccino, Spann, Pool, Sonnenblick & Braunwald, 1967; Levey, Skelton & Epstein, 1969), changes similar to those in the present study are apparent when concentrations of noradrenaline producing half-maximal responses are determined from the data presented by these authors.

The specificity of $\alpha$-adrenoceptor blockade in the present study is supported by several observations. Thyroidectomy increased the blocking effectiveness of both PB and phentolamine, antagonists with very different chemical structures and reactivities. PB is known to have a wide range of effects other than inhibition of $\alpha$-adrenoceptors, which probably all involve alkylation of various tissue components (Harvey & Nickerson, 1954). However, potentiation of responses to noradrenaline after protection of $\alpha$-adrenoceptors by phentolamine (Figure 2b) and inhibition of the negative inotropic effect of pilocarpine by PB (Figure 4) were unaffected by thyroidectomy, which indicated a selective increase in the interaction of PB with $\alpha$-adrenoceptors. The absence of a significant change in the reactivity of PB with nonspecific tissue components is also suggested by the finding that after protection of $\alpha$-adrenoceptors by phentolamine, binding of [$^3$H]-PB was reduced to a level not significantly different from that in controls. PB also did not influence the inotropic response to $CaCl_2$ and its inhibition of responses to phenylephrine was not altered by the presence of quinidine, which suggested that the effect of PB on $\alpha$-adrenergic responses was not related to a nonspecific inhibition of transmembrane $Ca^{2+}$ movements that can occur with a concentration of PB (0.1 $\mu$M) much higher than those used in the present study (Shibata, Carrier & Frankenheim, 1968).

The mechanism by which threshold blocking concentrations of a $\beta$-adrenoceptor antagonist during exposure to PB significantly potentiate the apparent blocking effect of the latter in hypothyroid preparations (Figure 5) or in frog hearts at low temperatures (unpublished) is not readily explained. Persistence of the increased blocking effect of PB, after the $\beta$-blocking drug had been washed out, suggests that this potentiation is not due to an 'occult' block of $\alpha$-adrenoceptors, masked by activation of $\beta$-adrenoceptors. If this were so, potentiation of the blocking effect of PB should have been reversed after removal of the $\beta$-blocking drug, but it was not. Instead, it may be proposed that inhibition of the $\beta$ form of the receptor shifted the equilibrium in favour of the $\alpha$ form, which was then 'trapped' by the irreversible antagonist. This effect would be similar to the shift in equilibrium, induced by antagonists, between conformational states of an allosteric cholinoceptor (Karlin, 1967). The above mechanism might also account for observations that in euthyroid preparations PB can increase the inhibition of inotropic responses to NA and phenylephrine by a high concentration of pronethalol or propranolol, if given after but not before the $\beta$-blocking drug (Govier, 1968; Kaumann, 1970). It is also interesting to note that changes in the relative dominance of $\alpha$- and $\beta$-adrenoceptor responses in the present study were associated with changes in the slopes of agonist concentration-response curves, although the mechanism underlying these changes is not clear.

Observations of a temperature-induced interconversion of histamine $H_1$- and $H_2$-receptors in guinea-pig ileum (Kenakin, Kreuger & Cook, 1974) and of a thyroid hormone-dependent change in the concentration of œstrogen receptors in the rat pituitary gland (Cidlowski & Muldoon, 1975) may suggest that metabolic control of receptors may be a general phenomenon affecting not only adrenoceptors, but other hormone receptors as well.

The present findings on the pharmacological activity of drugs acting on adrenoceptors in preparations from thyroidectomized animals are in good agreement with previous reports of an increased effectiveness of phenylephrine and phentolamine and decreased effectiveness of isoprenaline and propranolol in hearts from propylthiouracyl-fed rats (Nakashima, Maeda, Sekiya & Hagino, 1971; Nakashima & Hagino, 1972). The observation of an increased binding of [$^3$H]-PB to the hypothyroid myocardium in the present study suggests that the change in receptor response is due to a change in the number of available binding sites. Although PB can bind to a number of nonspecific tissue components, the parallel decrease in block and binding after protection of $\alpha$-adrenoceptors by phentolamine, *in vitro*, or thyroid hormone treatment *in vivo*, indicates that the difference in the binding of PB is associated with $\alpha$-receptors and that its nonspecific binding is not affected by changes in thyroid state.

Interconversion of $\alpha$- and $\beta$-adrenoceptors due to altered thyroid hormone levels could account for a number of other published observations. A selective increase in adrenergic stimulation of the aorta of hypothyroid rabbits (Rosenquist & Boréus, 1972), a diminished calorigenic response to noradrenaline in thyroid hormone deficient newborn rats (Steele & Wekstein, 1973) and a decreased glycogenolytic response to isoprenaline in $^{131}$I-treated rats (Dominguez, Catanzaro, Fernandez & Vidal, 1973) suggest that the hormone-induced change in adrenoceptor properties may not be limited to the myocardium. Other observations, including a selective increase in isoprenaline sensitivity of cultured mouse heart cells exposed to triiodothyronine (Wildenthal, 1974) and a decreased adrenergic vasoconstriction,

reversed by pronethalol, in the hind limb of thyroxine-treated dogs (Zsoter, Tom & Chappel, 1964) are compatible with a shift in receptor properties from $\alpha$ to $\beta$ in hyperthyroidism. Thyroid hormones may also modulate their own release by influencing the properties of adrenoceptors located on cells of the thyroid gland; isoprenaline was shown to increase the *in vitro* synthesis of thyroid hormones in isolated calf thyrocytes, an effect blocked by phentolamine, or increase the *in vivo* secretion of thyroid hormones in thyroxin pretreated mice, an effect blocked by propranolol (cited by Melander, Ranklev, Sundler & Westgren, 1975).

Although inotropic and chronotropic responses of the myocardium can be mediated by both $\alpha$- and $\beta$-adrenoceptors, the underlying mechanisms may differ. Stimulation of $\beta$-adrenoceptors is associated with a rise in cyclic AMP levels both in intact hearts (Robison, Butcher, Øye, Morgan & Sutherland, 1965) and in subcellular membrane preparations (Murad, Chi, Rall & Sutherland, 1962). Preliminary observations in our laboratory showed that $\beta$-adrenoceptor stimulation increased cyclic AMP levels both in contracting and in quiescent myocardial preparations, whereas an $\alpha$-adrenoceptor mediated elevation of cyclic AMP levels in hypothyroid left atria required not only intact cells but contractile activity as well (Kunos, 1975). It has also been reported that stimulation of $\alpha$-adrenoceptors in the dog heart is not associated with an 'oxygen-wasting' effect (Privitera & Rosenblum, 1967) and it is interesting to note that both low temperature in frogs (Korb, Poupa & Carlsten, 1973) and hypothyroidism in rats (Chappel, Rona & Gaudry, 1959) appear to prevent isoprenaline-induced myocardial necrosis. Therefore, it is tempting to speculate that changes in adrenoceptor characteristics play a role in the adaptation of mechanical responses of the myocardium to its metabolic state. Such as adaptation might contribute to the reported therapeutic effect of lowering thyroid activity in patients with angina pectoris (Segal, Silver, Yohalem & Newburger, 1958) and to the increased sensitivity of the hyperthyroid and decreased sensitivity of the hypothyroid myocardium to arrhythmias (Freedberg, Papp & Vaughan Williams, 1970).

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