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# The effects of altered thyroid state upon responses mediated by atrial muscarinic receptors in the rat

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**1** Untreated rats, and rats treated with methimazole (0.05% w/v in drinking water) or thyroxine (1 mg/kg, s.c., three times weekly) for 4–6 weeks to induce hypo- and hyperthyroidism respectively, were used to study the influence of thyroid hormone upon negative chronotropic and inotropic responses mediated by cardiac muscarinic receptors, and upon the affinity of these receptors for atropine.

**2** Negative chronotropic effects of methacholine were investigated by establishing partial concentration-response curves for isolated preparations of right atria. Methacholine was least potent in tissues from thyroxine-treated rats.

**3** Isolated left atria paced at 3 Hz, and spontaneously beating right atria, were used in studies of the negative inotropic effects of methacholine. This agonist was least potent in atria from the thyroxine-treated rats in which it also produced the smallest maximal responses.

**4** The negative inotropic effects of carbachol were examined on left atria paced at 3, 5 and 5.8 Hz to approximate the basal contraction rates of isolated right atria from methimazole-treated, untreated control and thyroxine-treated rats, respectively. At each of these frequencies, carbachol was most potent in atria from methimazole-treated rats, and least potent in atria from thyroxine-treated rats. Maximal responses were smallest in the latter group.

**5** $pA_2$ values for atropine with methacholine as the agonist were obtained by the method of Arunlakshana & Schild (1959) for spontaneously beating right atria (negative chronotropic and inotropic effects) and left atria paced at 3 Hz (negative inotropic effects). Slopes of Schild plots did not differ from minus one in tissues from each of the experimental groups; $pA_2$ values were similar, indicating that thyroid status is without effect upon the affinity of this antagonist for muscarinic receptors mediating both negative inotropic and chronotropic effects.

**6** The results are discussed in the light of reports that hypothyroidism increases, and hyperthyroidism decreases the numbers of high affinity muscarinic receptor binding sites in the rat myocardium.

## Introduction

The literature contains numerous accounts of the effects of thyroid state upon the numbers of adrenoceptor binding sites and upon adrenoceptor-mediated responses in isolated preparations of rat myocardium (e.g. Ciaraldi & Marinette, 1977; Kunos, 1977; McConnaughey, Jones, Watanabe, Besch, Williams & Lefkowitz, 1979; Turner & Shenfield, 1980). Similarly there have been reports of changes in the numbers of muscarinic receptor binding sites in rat myocardial membranes in altered thyroid state (Sharma & Banerjee, 1977; Robberecht, Waelbroeck, Claeys, Nguyen Huu, Chatelain & Christophe, 1982). However, studies of the influence of altered thyroid state upon responses mediated by cardiac muscarinic receptors in this species have been concerned with the effects of vagal stimulation upon heart rate *in vivo* (Hoffmann, Hoffmann & Talesnik, 1947; Cairoli & Crout, 1967; Frazer & Hess, 1969).

In the present study we have used isolated right and left atria from untreated control rats, and from rats treated with methimazole or thyroxine, to induce hypo- and hyperthyroidism, respectively, in an examination of the influence of thyroid state upon negative chronotropic and inotropic responses elicited by muscarinic cholinoceptor agonists. The effect of thyroid state upon the affinity of right and left atrial muscarinic receptors for atropine has also been examined by the $pA_2$ method of Arunlakshana & Schild (1959). A preliminary account of these results was presented to the 8th International Pharmacological Congress (Ishac & Pennefather, 1981).

## Methods

Male Long Evans Hooded rats (180–200 g) were given either methimazole (0.05% w/v in their drinking water) to induce hypothyroidism, or thyroxine ($T_4$, 1 mg/kg s.c. thrice weekly), to induce hyperthyroidism. A further group of rats was concurrently treated with methimazole as above, and in addition received thyroxine (25 μg/kg s.c. thrice weekly) to render them euthyroid. All treatments were maintained for 4–6 weeks before use. Treated rats, and age-matched untreated rats, were caged in groups of six and housed at 22°C with a light-dark cycle of 12 h. Several days before use, rats were weighed, and blood samples (0.75 ml) were taken from the tail vein under light halothane anaesthesia for subsequent radioimmunoassay of circulating $T_4$ and triiodothyronine ($T_3$) levels, using Tetra-Tab RIA and Tri-Tab RIA diagnostic kits, respectively.

### *Isolated atrial preparations*

Rats were killed by a blow to the head, the thorax opened and the heart quickly removed and flushed with Krebs-Henseleit solution of the following composition (mmol/1): NaCl 118.05, KCl 4.69, $MgSO_4$ 0.45, $KH_2PO_4$ 1.18, $NaHCO_3$ 25.00, glucose 11.66 and $CaCl_2$ 2.52. Left and/or right atria were isolated and set up in a 30 ml organ bath containing Krebs-Henseleit solution, bubbled with 5% $CO_2$ in $O_2$, and maintained at 37°C. The initial tension applied to each tissue was 0.5 g; this was adjusted if necessary at the end of the 30 min equilibration period before the addition of drugs. The tissue holders used for left atria incorporated bipolar platinum electrodes to allow field stimulation with rectangular pulses (0.5 ms) and supramaximal voltage (20% above maximal), at 3.0, 5.0 or 5.8 Hz. Isometric contractions were measured by means of Grass FT03 transducers and recorded on a Grass Polygraph (Model 79C). The contractions triggered a Grass tachometer so that the rate of spontaneously beating right atria could also be recorded.

### *Determination of agonist potencies*

*Right atria* This preparation was used in studies of both the negative chronotropic and negative inotropic potencies of methacholine. Concentration-response curves were established in preparations from untreated control, methimazole-treated and thyroxine-treated groups. The agonist was added non-cumulatively, at 4 min intervals. Each dose was allowed to remain in contact with each preparation until the response reached a plateau; after which the preparation was washed with three times the bath volume of Krebs-Henseleit solution. In most experiments at least four doses producing responses within the linear range of the concentration-response curve were used.

Negative inotropic responses were expressed as percentage inhibitions of the pre-dose steady state contraction. The negative logarithms of the molar concentrations of methacholine producing 50% inhibition of the pre-drug force of contraction (neg log $IC_{50}$s), were used as measures of negative inotropic potency. Negative chronotropic responses were expressed both as absolute falls in atrial rate and as percentage decreases in basal atrial rate. Complete log concentration-response curves for negative chronotropic effects of methacholine were not obtained because high doses caused a marked and prolonged decline in force to a level insufficient to trigger the tachometer.

*Left atria* Left atria paced at 3 Hz, were used to study the negative inotropic potencies of methacholine and carbachol. The latter drug was used since it is not a substrate for cholinesterases. Concentration-response curves were constructed and potency determined as described above. Similar experiments were also conducted, in which carbachol was used as the agonist, in the group of rats which concurrently received both methimazole and thyroxine, as described above, to render them euthyroid. In another group of experiments, left atria were paced at frequencies of 3.0, 5.0 and 5.8 Hz, which approximate the spontaneous rates at which right atria from methimazole-treated, untreated control and thyroxine-treated rats beat *in vitro*. The effects of carbachol were examined at each of these frequencies. The order in which trains of stimulation at the three frequencies were applied to any one tissue was randomly varied from experiment to experiment. Doses of carbachol were not added until steady-state contractions (Blinks & Koch-Weser, 1961) in response to stimulation at any given frequency were established. In these experiments negative log $EC_{50}$ values were used as estimates of potency.

### *Determination of $pA_2$ values for atropine*

The effects of atropine were examined upon the negative inotropic response to methacholine in both left and right atria, and upon the negative chronotropic effect in right atria. After a control concentration-response curve for methacholine was obtained as described above, each tissue was allowed to equilibrate with atropine for 40 min prior to repetition of the determination of concentration-response curves to methacholine. Three increasing concentrations of atropine (1 or 2, 10 and 100 nmol/l) were employed. Concentration-ratios were determined for the agonist at each antagonist concentration. The

log (concentration-ratio − 1) values so obtained were plotted against log antagonist concentration in the form of a Schild plot (Arunlakshana & Schild, 1959). A line of best fit and a $pA_2$ value was obtained from this plot for each preparation. For further analysis, results from all experiments with one tissue from each experimental group were pooled, to allow estimation of $pA_2$ values and slopes, together with 95% confidence limits, as described previously (Yoong, Story, Ishac, Pennefather & Handberg, 1982).

*Statistical methods*

Mean log concentration-response curves for each agonist were constructed from results using tissues from each experimental group. Least squares regression lines were fitted to the central portions of these curves and tested for linearity. Comparisons between lines obtained for tissues from treated animals were compared with corresponding lines obtained in tissues from untreated control animals. Each pair of lines was tested for parallelism and co-incidence, and the potency ratio together with 95% confidence limits was determined, using the statistical methods described in Documenta Geigy (Diem, 1962). Comparison of mean values were made by Student's non-paired multiple *t* tests if a one-way analysis of variance revealed a significant difference. In all comparisons $P<0.05$ has been taken as an index of statistical significance.

*Drugs and solutions*

The following drugs were used: methacholine chloride (acetyl-β-methylcholine chloride, Sigma), carbachol (carbamyl-choline chloride, BDH), atropine sulphate (Koch-Light Laboratories), methimazole (1-methyl-imidazole-2-thiol, Sigma) and L-thyroxine, sodium salt (Sigma). Methimazole was made up in tapwater, and thyroxine was suspended in 0.9% w/v NaCl solution (saline). Other drugs were dissolved in distilled water.

## Results

Thyroxine administration caused elevation of circulating $T_3$ and $T_4$ levels, and increases in both left and right atrial weights (Table 1). Methimazole administration produced the opposite effects (Table 1) and in addition, a marked decrease in the rate of gain of body weight, as found previously (Yoong *et al.*, 1982). The administration of thyroxine to methimazole-treated rats prevented the decline in circulating $T_4$ and $T_3$ levels.

Isolated right atria from thyroxine-treated rats beat more rapidly than those from untreated control rats, while hypothyroidism was associated with bradycardia. Higher stimulation voltages (40 V dial setting) were required to elicit maximum contractions in left atria from thyroxine-treated rats compared with corresponding preparations from methimazole-treated and untreated control rats (15 V dial setting). However, the mean steady-state force of contraction developed was similar, at similar frequencies of stimulation, in left atria from each experimental group (Table 2).

**Table 1** Effects of altered thyroid state upon plasma levels of triiodothyronine ($T_3$) and thyroxine ($T_4$), atrial wieght and right atrial rate

| *Treatment group* | $T_4$ (ng/ml) | $T_3$ (ng/ml) | *Atrial weight* (mg) Left | Right | *Right atrial rate* (b/min) |
|---|---|---|---|---|---|
| Untreated control ($n = 6$) | 46 ± 4 | 1.02 ± 0.08 | 16.4 ± 1.1 | 35.4 ± 1.3 | 302 ± 6 |
| Thyroxine-treated ($n = 6$ or 7) | 288 ± 37* | 5.20 ± 0.39* | 22.4 ± 2.5* | 42.7 ± 2.2* | 347 ± 13* |
| Methimazole-treated ($n = 6$) | 4 ± 1* | 0.27 ± 0.04* | 10.2 ± 0.9* | 24.1 ± 2.2* | 175 ± 2* |
| Thyroxine/methimazole-treated ($n = 6$ or 7) | 39 ± 18 | 0.89 ± 0.16 | 15.3 ± 1.6 | 32.9 ± 2.4 | ND |

All values are mean ± s.e.mean.
*Significantly different from corresponding untreated control value (ANOVA, non-paired *t* test, $P<0.05$).
ND = not determined.

**Table 2** Effect of altered thyroid state upon steady-state force of contraction of spontaneously beating right atria and paced left atria

| *Treatment group* | *Steady-state force of contraction* (mg) *Right atria* | *Left atria* 3 Hz | 5 Hz | 5.8 Hz |
|---|---|---|---|---|
| Untreated control ($n = 6$) | 145 ± 19 | 295 ± 30 | 264 ± 28* | 242 ± 28* |
| Thyroxine-treated ($n = 6$ or 7) | 182 ± 22 | 245 ± 22 | 215 ± 20* | 202 ± 18* |
| Methimazole-treated ($n = 5$ or 6) | 123 ± 12 | 270 ± 25 | 268 ± 36 | 250 ± 32 |

All values are mean ± s.e.mean.
*Significantly different from corresponding value at 3 Hz (paired $t$ test, $P < 0.05$).

### *Agonist potencies*

*Right atria* Addition of methacholine caused concentration-related decreases in both the rate and force of contractions in right atria from untreated control, methimazole-treated and thyroxine-treated rats. Figure 1 demonstrates the effects of methacholine upon an untreated control preparation; Figure 2 shows the mean log concentration-response curves for the negative inotropic effects of this agonist, expressed as percentage inhibition of basal force, in preparations from untreated control, methimazole-treated and thyroxine-treated rats. For each dose of the agonist the negative inotropic effect was more marked than the negative chronotropic effect in each of the experimental groups. Tissues from thyroxine-treated rats were less sensitive to methacholine, so that the mean log concentration-response curves for the negative inotropic effect of this agonist lay to the right of those for tissues from untreated animals; the mean maximum percentage inhibition of contractile force was also less than that obtained with tissues from untreated rats (Figure 2). The mean negative log $IC_{50}$ values for the negative inotropic effects of methacholine are given in Table 3.

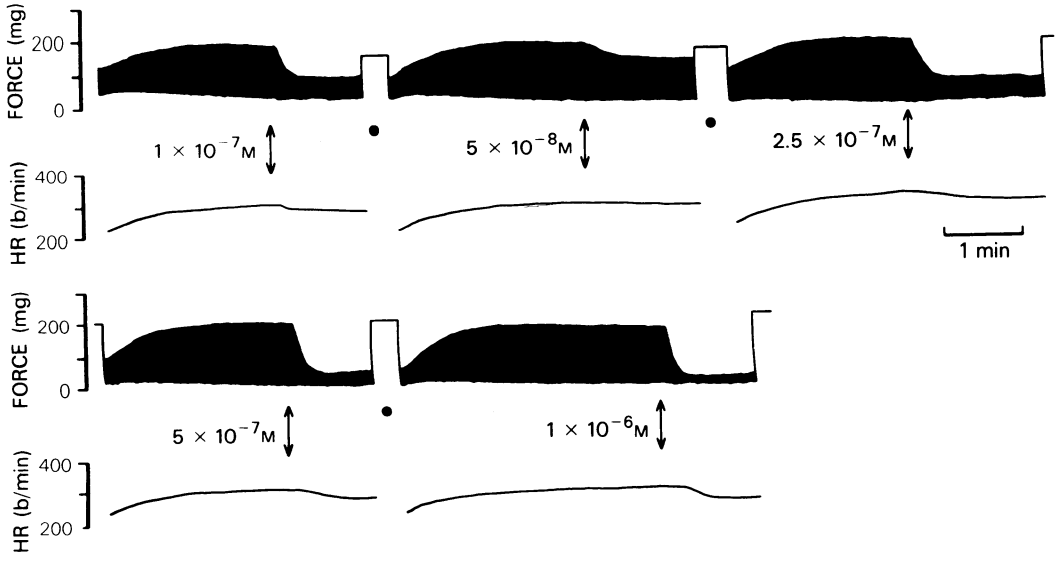


**Figure 1** The effects of methacholine on the force and rate (HR) of spontaneous contraction of a right atrium from an untreated control rat. Concentrations of methacholine were added at (⇕) and washed out at (●). Each concentration of methacholine produced more marked inhibition of atrial force than of atrial rate.

**Table 3** Negative inotropic potencies of agonists on left and right atria from untreated control, thyroxine-treated and methimazole-treated rats

| *Agonist* | *Tissue* | $-log\ IC_{50}$ *Untreated control* | *Thyroxine-treated* | *Methimazole-treated* |
|---|---|---|---|---|
| Methacholine | Right atria | 6.94 ± 0.16 (6) | 6.19 ± 0.21 (7)*† | 6.63 ± 0.12 (6) |
| | Left atria at 3 Hz | 6.94 ± 0.18 (6) | 5.97 ± 0.10 (6)*† | 7.05 ± 0.11 (6) |
| Carbachol | Left atria 3 Hz | 6.64 ± 0.13 (6) | 5.67 ± 0.44 (6)* | 7.06 ± 0.23 (5)* |
| | 5.0 Hz | 6.71 ± 0.05 (6) | 5.83 ± 0.29 (6)* | 7.00 ± 0.13 (5)* |
| | 5.8 Hz | 6.58 ± 0.08 (6) | 6.07 ± 0.11 (6)* | 7.02 ± 0.15 (5)* |

Values are mean ± s.e.mean. Values in parentheses = *n*.
*Significantly different from corresponding untreated contol value (ANOVA, *t* test, $P < 0.05$).
†In some preparations, the maximal inhibition obtained was less than 50% of basal force, the concentration contributing to the mean values in this instance was that producing maximum effect.

The mean concentrations of methacholine producing falls of 30 beats/min were 0.28 ± 0.15 (6), 0.33 ± 0.16 (7) and 0.46 ± 0.19 (6) μmol/l in right atria from untreated control, thyroxine-treated and methimazole-treated animals, respectively. Complete log concentration-response curves for the negative chronotropic effects were not obtained, as explained in the Methods. However when responses were expressed as percentage decrease in atrial rate, the partial curves obtained with atria from the thyroxine-treated group were found to lie to the right of those in atria from untreated control animals.

*Left atria* The effects of methacholine upon the force of contraction of left atria paced at 3 Hz were quantitatively similar to those upon right atria (Figure 2), in that log concentration-response curves obtained in tissues from thyroxine-treated animals lay to the right of those obtained in untreated control tissues, and exhibited lower maxima. However the log concentration-response curves in left atria from the methimazole-treated group lay to the left of those for the control group. The mean negative log $IC_{50}$ values obtained in tissues from each treatment group are shown in Table 3.

Carbachol also produced negative inotropic effects in left atria paced at 3 Hz which, in preparations from thyroxine-treated animals were less marked and had a lower maximum than in preparations from untreated control animals (Figure 2). Concentration-response curves for carbachol in left atria from methimazole-treated rats lay to the left of those obtained with corresponding preparations from untreated control animals; a comparison of the linear portions of the log concentration-response curves revealed that the difference in position was significant. Thus the potency ratio was 2.09 with 95% confidence limits of 1.28 to 3.49.

Left atria, paced at 3 Hz, from a euthyroid group of animals (which had been treated with both methimazole and thyroxine; see Methods section), developed a force similar to that in atria from untreated rats (264 ± 33 mg, $n = 5$ and 295 ± 30 mg $n = 6$ respectively). Carbachol was equipotent and equieffective in producing negative inotropic effects in these two experimental groups. The negative log $IC_{50}$ values were 6.75 ± 0.17 (5) and 6.64 ± 0.13 (6) in thyroxine/methimazole-treated and untreated control, respectively. This indicates that it was the hypothyroid state, and not the administration of methimazole *per se*, which affected the potency of carbachol in atria from methimazole-treated, hypothyroid rats.

The negative inotropic effects of carbachol were examined further in left atria from untreated control, methimazole-treated and thyroxine-treated animals, which were paced at 5.0 and 5.8 Hz. The higher of

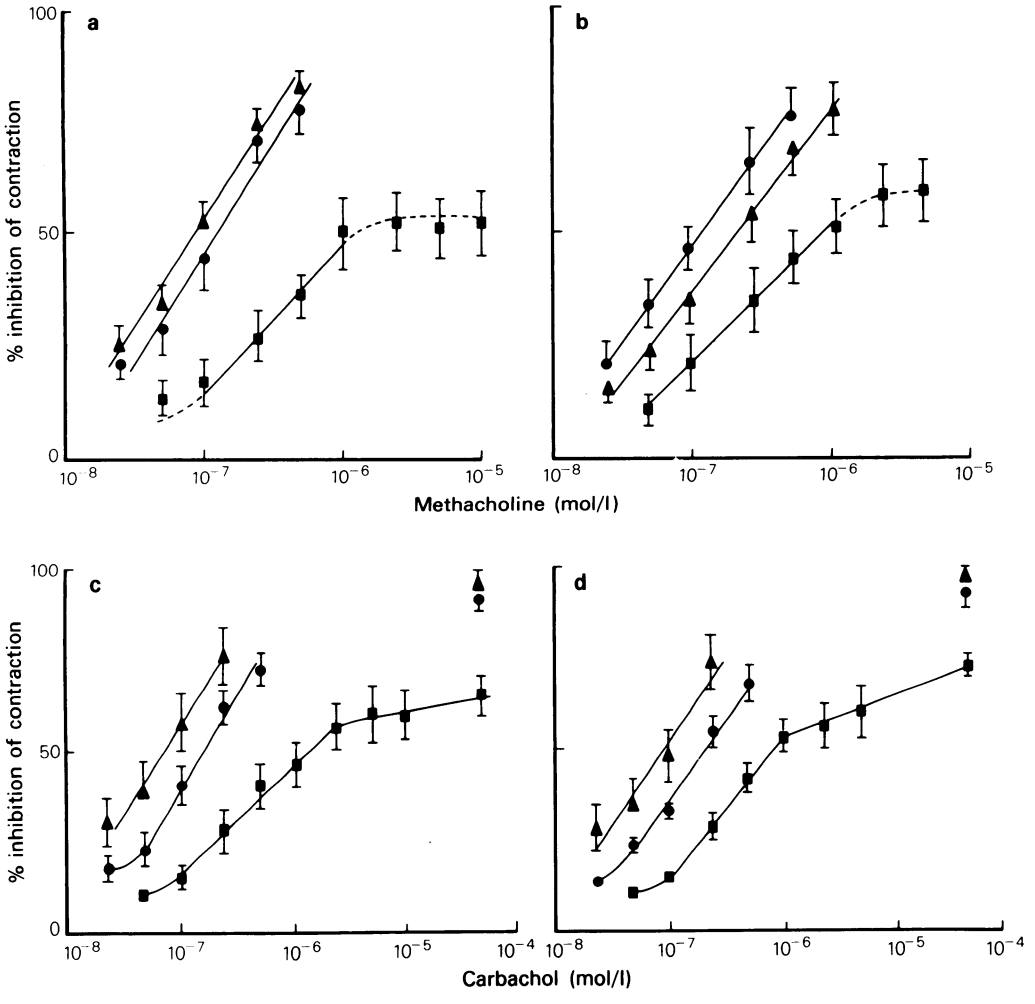


**Figure 2** Graphs (a) and (b) show log concentration-response curves for methacholine on the force of contraction of left atria paced at 3 Hz (a), and upon spontaneously beating right atria (b) from untreated control (●), methimazole-treated (▲) and thyroxine-treated (■) rats. Vertical lines represent s.e.mean of 6–7 experiments. Graphs (c) and (d) show corresponding curves for carbachol on left atria paced at 3 Hz (c) and at 5.8 Hz (d). Points represent the mean of 5–6 experiments.

these two frequencies approximates the basal rate of contraction of isolated right atria from thyroxine-treated animals, the lower that of untreated control animals, whereas the standard frequency of 3 Hz used in the study approximates that of right atria from methimazole-treated animals (Table 1). The mean steady-state force of contraction of atria from each of the three experimental groups are shown in Table 2; the mean negative log $IC_{50}$ and $EC_{50}$ values for carbachol at the three frequencies are shown in Table 3. The log concentration-response curves for atria paced at 5.8 Hz are illustrated in Figure 2. At this frequency, as at each of the lower frequencies, the curves for tissues from thyroxine-treated animals lay significantly to the right of those from untreated control animals, and those from methimazole-treated animals significantly to the left. Mean maximum negative inotropic effects were consistently smallest in tissues from thyroxine-treated animals.

### *$pA_2$ values for atropine with methacholine*

Atropine (1–100 nmol/l) produced parallel, rightward shifts in the positions of log concentration-response curves, for both negative inotropic and chronotropic effects of methacholine, in right atria

from all treatment groups. This was also the case for the effects of atropine upon the negative inotropic effects of methacholine in left atria. Schild plots constructed with data from these experiments yielded slopes of minus one for each type of response. The corresponding mean $pA_2$ values were also unaffected by changes in thyroid state. For example, mean $pA_2$ values (with 95% confidence limits) for atropine against the negative inotropic action of methacholine were 8.62 (8.36, 9.13; $n=6$), 8.78 (8.58, 9.09; $n=7$) and 8.57 (8.33, 8.99; $n=6$) in left atria from untreated control, thyroxine-treated and methimazole-treated rats, respectively. The slopes of the corresponding Schild plots were 1.14 (0.84, 1.44), 1.04 (0.75, 1.33) and 1.12 (0.80, 1.44) respectively.

## Discussion

The present experiments establish that alteration of thyroid state markedly affects the potency and effectiveness of the muscarinic agonists, methacholine and carbachol, upon the atrial myocardium of the rat. In contrast, the affinity of the right and left atrial muscarinic receptors, for the competitive muscarinic antagonist, atropine, remain unchanged in altered thyroid state.

In this study, as in three related studies from this laboratory (Yoong *et al.*, 1982; Ishac & Pennefather, 1983; Ishac, Pennefather & Handberg, 1983) in which the effects of altered thyroid state upon atrial and vascular adrenoceptor-mediated responses were examined, we have used methimazole to induce the hypothyroid state. Methimazole is water soluble and has a longer biological half life than propylthiouracil (Pittman, Beschi & Smitherman, 1971). Moreover it does not inhibit the peripheral deiodination of $T_4$ to $T_3$. (Hershman & van Middlesworth, 1962; Chopra, 1977; Aizawa & Yamada, 1981). Thus we were able, by co-administering methimazole and thyroxine, to establish an additional control group of animals which were chemically and physiologically euthyroid (Yoong *et al.*, 1982; Ishac *et al.*, 1983). We have discussed elsewhere the advantages of the use of either methimazole or propylthiouracil over thyroidectomy (Ishac & Pennefather, 1983; Ishac *et al.*, 1983). In brief, these lie in the absence of an effect of the anti-thyroid drugs on calcium homeostasis. In the present study the negative inotropic potency of carbachol, in atria from a 'euthyroid' control group of animals was virtually identical with that in the corresponding untreated control group, indicating that the administration of methimazole *per se* does not influence the response to muscarinic agonists.

Several workers including Freedberg, Papp & Vaughan Williams (1970), Levey (1971) and Simpson, Rodgers & McNeill (1981) have drawn attention to difficulties in the interpretation of studies of the effects of altered thyroid status upon the inotropic and chronotropic effects of sympathomimetic amines. These difficulties result from the direct and indirect effects of altered thyroid state upon the electrophysiological, metabolic and contractile properties of the myocardium. We have attempted to avoid such difficulties by examining, when possible, the effects of the agonists over a full concentration-response range, as suggested by Kunos (1981) and Gibson (1981).

In our experiments, thyroxine-treatment led to atrial hypertrophy and to increased right atrial rate. The opposite effects were observed in methimazole-treated rats. These findings are consistent with those of other workers (Yazaki & Raben, 1975; Turner & Shenfield, 1980; Simpson *et al.*, 1981). Higher than normal stimulation voltages were required to elicit maximum contraction in left atria from hyperthyroid rats; as described previously by Freedberg *et al.* (1970), using rabbit atria. However, there were no significant differences attributable to changes in thyroid state in the steady-state force developed by right or left atria (Table 2).

In the present study we have directly investigated the effects of carbachol in left atria from methimazole-treated, untreated control and thyroxine-treated rats paced at 3.0, 5.0 and 5.8 Hz to approximate the basal rates of isolated right atria from animals in the three treatment groups. These experiments were prompted in part by reports that the negative inotropic effect of carbachol in normal atria is absent when the interval between stimuli is such that rested-state contractions occur and increases progressively with more rapid stimulation (Koch-Weser, Berlin & Blinks, 1964; Ravens & Ziegler, 1980). Moreover the interval-strength relationship in rat atria is markedly influenced by thyroid status (Handberg, Ishac & Pennefather, 1983). Thus the normal negative 'staircase' effect which occurs in the adult rat myocardium (Forester & Mainwood, 1974; Langer, 1978) is accentuated in hypothyroidism, while in hyperthyroidism a positive 'staircase' effect develops at stimulation frequencies of 1–2 Hz and above. However, the data summarized in Figure 2 and Table 2 show that the decrease in the negative inotropic potency and maximal effect of carbachol in left atria from thyroxine-treated rats, and the increase in potency in methimazole-treated rats occur independently of changes in the interval-strength relationship over the range of frequencies studied. For this reason we also consider it probable that the differences in the negative inotropic effects of methacholine observed in right atria in the three groups of aimals are not solely a consequence of differences in basal atrial rates.

The use of the $pA_2$ method of Arunlakshana & Schild (1959) to study the influence of altered thyroid state upon the affinity of atrial muscarinic receptors for atropine avoids problems arising from differences in basal activity. Analysis of the antagonism of methacholine by atropine by this means has confirmed that the nature of the antagonism is competitive, and that the affinity of muscarinic receptors for this antagonist is unaffected by thyroid status. In binding studies, Sharma & Banerjee (1977) and Robberecht *et al.* (1982) found that neither hyperthyroidism nor hypothyroidism affected the affinity of muscarinic binding sites in rat myocardial membranes for the specific muscarinic receptor antagonist [$^3$H]-quinuclidinyl benzilate ([$^3$H]-QNB).

In contrast, our results show that thyroid state influences the negative inotropic responses to methacholine and carbachol. Thyroid hormone may modify these responses via effects distal to the muscarinic receptors, e.g. by altering action potential duration (Freedberg *et al.*, 1970). However, Robberecht *et al.* (1982) have recently found, as previously reported by Sharma & Banerjee (1977), that hyperthyroidism decreases the number of [$^3$H]-QNB binding sites in cardiac membranes. Thus the decrease in maximal inotropic response which we observed using muscarinic agonists in both left and right atria from thyroxine-treated rats, may result from this decrease in the total receptor number.

The presence of 'high' and 'low' affinity muscarinic binding sites, has now been demonstrated in a radioligand binding study in rat myocardial membranes (Ehlert, Roeske & Yamamura, 1981). Robberecht *et al.* (1982) have shown that hyperthyroidism specifically decreases, and hypothyroidism specifically increases the numbers of high affinity binding sites. Provided that these binding sites mediate the same end-organ effects, the concentrations of agonists required to produce equivalent effects would be greater in tissues from hyperthyroid animals than in those from untreated control animals. This was the effect seen in the present study, in which greater than 5 fold decreases in the negative inotropic potencies of methacholine and carbachol occurred in atria from thyroxine-treated rats.

In our study the effects of methimazole-treatment upon the negative inotropic potency of carbachol were small; only a two fold increase in potency was observed. This may reflect the increase in the number and proportion of high affinity binding sites detected by Robberecht *et al.* (1982).

In our examination of the influence of altered thyroid state upon the chronotropic effects of methacholine, complete log concentration-response curves were not constructed. Nevertheless our results are consistent with those of Frazer & Hess (1969) who examined negative chronotropic effects of carbachol in anaesthetized rats. In our experiments as in theirs, when responses were expressed as absolute decreases in heart rate, no significant influence of thyroid state upon the potency of the agonist emerged. However, if responses to methacholine are expressed as percentage changes in basal atrial rate, a statistically significant reduction in the negative chronotropic potency of methacholine in right atria from thyroxine-treated rats is apparent.

Our findings lead us to suggest that the decreased cardiac responses to vagal stimulation in hyperthyroid animals (Cairoli & Crout, 1967; Hoffmann *et al.*, 1947) may be explained by postjunctional changes in the response to acetylcholine occurring at the receptor level. It remains to be established whether thyroxine treatment acts directly upon cardiac muscarinic receptors, as it does upon cardiac adrenoceptors (Tsai & Chen, 1978; Chang & Kunos, 1981), or indirectly by altering cardiovascular dynamics. However, the effects of thyroid hormone upon muscarinic receptors in the rat are clearly tissue-selective. Muscarinic binding sites in the submaxillary gland are unaffected by thyroid status (Pointon & Banerjee, 1979). Moreover, Gaginella, Wietecha, Hecht & Kerzner (1981) have reported that altered thyroid state affects neither the numbers of muscarinic receptor binding sites in membrane preparations of ileal or colonic muscle, nor the responses of these tissues to muscarinic agonists. Further experiments are required to establish whether this selectivity reflects the existence of distinct muscarinic receptor subtypes in different tissues.

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