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The influence of the thyroid state upon responses to noradrenaline and phentolamine in perfused mesenteric arterioles from the rat

E. J. N. ISHAC, J. N. PENNEFATHER*, *Department of Pharmacology, Monash University, Clayton, Victoria 3168, Australia*

The cardiovascular changes accompanying hyperthyroidism resemble those associated with sympathetic hyperactivity. Both adrenergic neuron and β-adrenoceptor blocking drugs have proved useful in the treatment of thyrotoxicosis. There have been numerous studies indicating that altered myocardial responses mediated via changes in the numbers and or affinity of myocardial adrenoceptors may contribute to the cardiovascular symptoms of altered thyroid state (Williams et al 1977; Ciaraldi & Marinetti 1977; Kunos et al 1980). In contrast, the influence of thyroid hormone upon responsiveness of vascular resistance vessels to adrenoceptor agonists remains controversial. Brown & Pollock (1980) have recently reported that the vasculature of hindlimbs from thyroidectomized rats exhibits a decreased sensitivity, but an increased maximal response to phenylephrine. Responses to vasopressin were unaltered.

Triiodothyronine or thyroxine pretreatment has been reported to decrease the sensitivity of preparations of isolated aorta from rabbits, rats and guinea-pigs to monoamines (MacMillan & Rand 1962; Coville & Telford 1970). In contrast, alterations of the potencies of catecholamines when thyroid hormone was added to the bathing solution of isolated preparations, have been attributed to chelation of copper ions by thyroxine (Lee et al 1965). In the present study we have compared the vasoconstrictor response to noradrenaline of perfused mesenteric arterioles (McGregor 1965) from hyper- and hypothyroid rats, and from control rats. In parallel with this study the potency of the α-adrenoceptor antagonist phentolamine in antagonizing the effect of noradrenaline has been examined by the pA_2 method of Arunlakshana & Schild (1959).

Methods

Male Long Evans rats, 180–210 g were assigned to treatment groups as follows: (a) control: which were untreated, (b) hyperthyroid: which received thyroxine (sodium salt) 1 mg kg^{-1} thrice weekly s.c., and (c) hypothyroid: which received methimazole 0·05% w/v in their drinking water. All animals were treated for 28 days before use.

Growth rates (g/rat per wk) were monitored over the 28 day treatment period. In a group of similarly-treated rats, total circulating levels of thyroxine and triiodothyronine were assayed with specific radioimmunoassay kits (Tetra-Tab-RIA, Tri-Tab-RIA, Nuclear-Medical Lab.).

The isolated perfused mesentery preparation of the rat. Rats were anaesthetized with urethane 25% w/v (6 ml kg^{-1} i.p.).The preparation was excised as described by McGregor (1965) except that the vasculature was gently teased from the intestine. The artery was perfused from a reservoir with Krebs-Henseleit solution of the following composition (mmol $litre^{-1}$): NaCl 118; KCl 4·69; $MgSO_4$ 0·45; KH_2PO_4 1·18; $NaHCO_3$ 25; glucose 11·66; $CaCl_2$ 2·52 and bubbled with 5% CO_2 in O_2. The isolated perfused preparation floated on the surface of a 400 ml organ bath which was also filled with Krebs-Henseleit solution maintained at a temperature of 37 °C. Tissues were perfused at a constant flow rate of 2 ml min^{-1} with a peristaltic roller pump (Cole-Palmer, Masterflex). Perfusion pressure was recorded via a side-arm of the arterial cannula with a Gould Statham P23 pressure transducer connected to a Washington 400 4MD 4C pen recorder.

Drug addition was preceded by an equilibration period of 30–40 min. Vasoconstrictor responses were obtained by perfusing the tissues with various concentrations of noradrenaline for 6 s at the constant flow rate of 2 ml min^{-1}. Infusion of noradrenaline produced a transient vasoconstriction which began within 25 s. Responses were measured as changes in perfusion pressure (mm Hg). Responses were obtained to noradrenaline, first in the absence, then in the presence of three increasing concentrations of phentolamine (5×10^{-9} mol $litre^{-1}$ to 5×10^{-7} mol $litre^{-1}$) added to the perfusion solutions.

The increasing doses of phentolamine were perfused for 20 min to equilibrate with the tissue before redetermination of each log dose-response curve (Coupar & McLennan 1978). The graphical method of Arunlakshana & Schild (1959) was used for pA_2 estimation.

Isolated right atria. After removal of the mesentery preparation the thorax was opened and the right atria was excised and placed in a separate organ bath in Krebs-Henseleit solution maintained at 37 °C. Atrial rate was determined using an FTO3 strain-gauge transducer connected to a Grass polygraph Model 79C.

The drugs used were: (−)-noradrenaline bitartrate (Sigma Chemical Co.); phentolamine mesylate (Regitine, Ciba-Geigy, Aust.); methimazole (Sigma Chemical Co.) and L-thyroxine (sodium salt) (Sigma Chemical Co.).

* Correspondence.

Table 1. Growth rates, right atrial rate and plasma thyroid hormone levels.

	Control $\bar{x} \pm$ s.e.m. (n)	Hyperthyroid $\bar{x} \pm$ s.e.m. (n)	Hypothyroid $\bar{x} \pm$ s.e.m. (n)
Growth rate (g/rat per wk)	27·4 ± 2.3 (7)	28·0 ± 1·5 (7)	11·0 ± 1·0 (9)*
Right atrial rate (b min^{-1})	302 ± 6 (6)	347 ± 13 (6)*	175 ± 2 (8)*
Thyroxine (μg dl^{-1})	6·5 ± 0·9 (6)	26·2 ± 4·1 (6)*	0·4 ± 0·1 (6)*
Triiodothyronine (ng dl^{-1})	133 ± 16 (6)	557 ± 26 (6)*	39 ± 5 (6)*

* Significantly different from control. (P <0·05) ANOVA.

Table 2. Mean x-intercept values and slopes with 95% confidence limits from Schild plots for phentolamine with noradrenaline.

	N,n	Slope	x-intercept†
Control	8,22	− 0·81* (0·63–0·99)	8·70 (8·45–9·07)
Hyperthyroid	7,19	− 0·81* (0·51–0·97)	8.70 (8·34–9·31)
Hypothyroid	9,25	− 0·74* (0·63–0·93)	8·47 (8·30–8·69)

N = number of rats; n = number of data points in each treatment group.

* Indicates slope significantly different (P <0·05) from unity.

† x-intercept = -log [phentolamine] when log (DR-1) = 0.

Statistical analysis. Comparisons of differences between mean values were made by means of the unpaired Student's t-test if a one way ANOVA revealed a significant difference among group mean values. Mean log dose-response curves for noradrenaline in tissues from hyperthyroid and hypothyroid animals were constructed and least squares regression lines fitted to the central portions and were tested for linearity. The lines were compared with the corresponding line obtained from experiments with control preparations, using the statistical tests for parallelism and potency ratio described in Documenta Geigy (6th Ed.). Similar analysis was applied to Schild plots derived from pooled estimates of dose-ratio obtained from individual experiments. In all comparisons the criterion of statistical significance has been taken as P <0·05.

Results

The effects of alterations of thyroid state upon the growth rate of rats, and plasma concentrations of thyroxine (T4) and triiodothyronine (T3) are shown in Table 1. It can be seen from this Table that the treatments with thyroxine and methimazole were effective in rendering animals hyper- and hypothyroid respectively. Differences in right atria rate reflect the direct effect of altered thyroid status upon the heart. Atrial rates of thyroxine-treated rats were found to significantly elevated, and those of methimazole-treated rats, significantly decreased compared to atria from untreated animals (Table 1).

A primary aim of the present study of the influence of thyroid status upon adrenoceptor-mediated responses in a preparation of resistance vessels was to investigate the antagonism of noradrenaline by phentolamine. Therefore maximal responses to noradrenaline were not established under equilibrium conditions before the addition at the antagonist, since this might have resulted in desensitization. Fitted regression lines for noradrenaline dose-response curves established in the absence of phentolamine, using perfused mesentery preparations from the three treatment groups, are shown in Fig. 1. The slopes of these lines did not differ significantly (P >0·05) from one another. Potency ratios (with 95% confidence limits) compared to control, were 1·75 (0·96, 3·45) and 1·70 (1·07, 2·82) for tissues from hyperthyroid and hypothyroid rats, respectively. The mean maximum increases in perfusion pressure (± s.e.m.(n)) recorded in response to 6 s infusion of noradrenaline were 180 ± 18 (9), 205 ± 17 (7) and 225 ± 9 (9) mmHg respectively for preparations from control, hyperthyroid and hypothyroid animals. A one-way analysis of variance of these data did not reveal a significant difference (P >0·05) among group means.

Phentolamine produced parallel rightward shifts in the position of the log dose-response curves for noradrenaline in preparations from each of the treatment groups. The slopes of the Schild plots obtained were linear and in all cases differed significantly from -1. The corresponding x- intercepts determined from these plots were similar in each treatment group (Table 2).

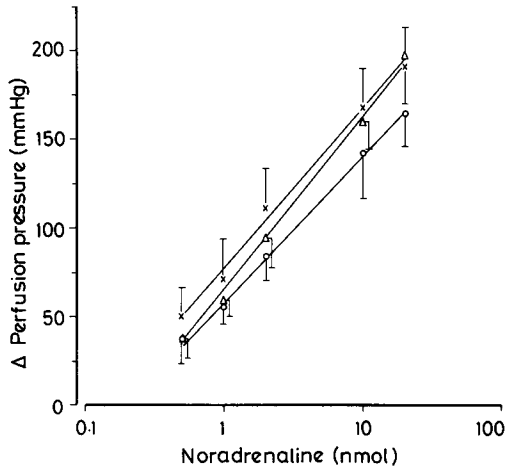

FIG. 1. Shows log dose-response curves for noradrenaline in perfused mesenteric arterioles from control (0), hyperthyroid (×) and hypothyroid (△) rats. Points on the graph are means ± s.e.m. from experiments from control (n = 9), hyperthyroid (n = 7) and from hypothyroid (n = 9) rats. Least squares regression lines were fitted to points on the linear part of each log dose-response curve.

Discussion

The results of our experiments using noradrenaline, suggest that long term alterations in thyroid state have little influence upon responses mediated by α-adrenoceptors within the mesenteric arteriolar bed of the rat. Thus no significant change in the potency of noradrenaline in preparations from hyperthyroid rats was revealed by regression analysis; and the increase in the potency in preparations from hypothyroid rats, though statistically significant, was small. The latter effect cannot be definitely attributed either to an enhanced responsives (i.e. an increased maximum response) or to a change in the affinity of α-adrenoceptors. It is possible that hypothyroidism, or methimazole treatment itself, leads to some alteration in the uptake of noradrenaline and/or to activation of β-adrenoceptors. However, our findings that the slopes and x-intercept values of Schild plots for phentolamine as an antagonist of noradrenaline do not support these possibilities, since in these experiments sites of catecholamine loss and β-adrenoceptors were not blocked, so that any influence of thyroid hormone could emerge.

The small increase in the potency of noradrenaline which we observed using preparations of mesenteric arterioles from rats made hypothyroid by treatment with methimazole is in apparent contrast with the findings of Brown & Pollock (1980). These authors studied the vasoconstrictor potency of the relatively selective α-adrenoceptor agonist, phenylephrine, using perfused hindlimb vessels of thyroidectomized rats, and found a small decrease in potency coupled with an increased maximum response. They suggested that their observations may in part be due to impairment of the regulation of calcium metabolism which accompanies surgical thyroidectomy and associated parathyroidectomy. Methimazole-treatment would not interfere in this way with calcium metabolism. In a parallel study we found that hypothyroidism induced by methimazole treatment of rats, did not influence the potencies of the constrictor agents, noradrenaline and angiotensin II upon preparations of portal vein (Yoong et al 1982).

Our studies using the α-adrenoceptor antagonist, phentolamine reinforce the finding that thyroid hormone has little influence upon the properties of α-adrenoceptors in the mesenteric vasculature. In this investigation the mean x-intercept estimate for phentolamine with noradrenaline in control preparations, exceeds those previously reported by Hepburn & Bentley (1982) and Coupar & McLennan (1978). The experimental technique used by us differed from that of Hepburn & Bentley who employed 'bolus' administration of the agonist, and included propranolol, cocaine and corticosterone in their perfusion medium. In the presence of agents which block sites of agonist loss, a one-to-one relationship between agonist and antagonist concentrations at the receptor biophase may result in a lower pA_2 value. Although Coupar & McLennan (1978) omitted such agents and perfused the agonist, the procedures they employed also differed from ours in that phentolamine was not included in their noradrenaline solutions during determinations of pA_2 values.

Whatever the reasons for these differences in absolute pA_2 values, it is clear from the present study (Table 2), that the potency of phentolamine as an antagonist of the vasoconstrictor action of noradrenaline upon rat mesenteric arterioles is little affected by thyroid status.

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