Br. J. Pharmac. (1970), **39**, 49–68.

Influence of thyroid hormones on the sensitivity of cardiac and smooth muscle to biogenic amines and other drugs

P. F. COVILLE* AND J. M. TELFORD*

Department of Pharmacology, School of Pharmacy, College of Technology, Brighton, Sussex

Summary

1. Rats and guinea-pigs were injected with l-thyroxine or thyroidectomized. Sensitivity of isolated tissues and of the intact cardiovascular system to drugs was investigated.

2. Thyroxine increased the sensitivity of the isolated perfused heart to noradrenaline, adrenaline, isoprenaline, acetylcholine and histamine.

3. Thyroxine increased the sensitivity of the rat isolated uterus to noradrenaline, adrenaline, isoprenaline, acetylcholine, histamine and 5-hydroxytryptamine.

4. In contrast, thyroxine decreased the sensitivity of the isolated ileum to acetylcholine, histamine and 5-hydroxytryptamine, but not to adrenaline.

5. Thyroxine also decreased the sensitivity of the isolated aorta to noradrenaline, adrenaline, histamine and 5-hydroxytryptamine.

6. In pithed rats, thyroxine potentiated pressor responses to (+)-amphetamine but not to noradrenaline or synephrine.

7. In anaesthetized thyroxine-treated rats, blood pressure responses to (+)-amphetamine were potentiated while those to noradrenaline were unaltered. Responses to acetylcholine were increased, and the response to isoprenaline became purely pressor.

8. Thyroxine-induced changes in the sensitivity of the isolated uterus and ileum to modification of the concentration of calcium in the physiological salt solution paralleled the changes in drug sensitivity of these tissues.

9. In general, thyroidectomy produced the opposite effects to those of thyroxine.

10. It is concluded that there is no specific interaction between thyroxine and sympathomimetic amines, the effect of thyroxine on drug sensitivity being non-specific.

Introduction

The similarity between the symptoms of hyperthyroidism and those of increased sympathetic activity has been known and studied for over a century. In 1865, many

* Present address: Biological Research Department, B.D.H. (Research) Ltd., Borough Road, Godalming, Surrey.

physiologists believed that hyperthyroidism was caused by over-activity of the cervical sympathetic nerves (Reith, 1865). Since then, a vast literature has accumulated on thyroid-sympathetic-adrenal medullary relationships, but until now no satisfactory explanation has emerged to explain why, in hyperthyroidism, sensitivity to many of the effects of adrenaline and noradrenaline is increased.

Thyroid hormones have been shown to increase the pressor and myocardial responses to adrenaline and noradrenaline, both experimentally and clinically (for references, see Harrison, 1964 ; Waldstein, 1966). Explanation of the phenomenon has been sought in terms of modification of catecholamine uptake, storage or metabolism, but many of the data are contradictory (Harrison, 1964), and some recent publications produce evidence that thyroid hormones may not, in fact, potentiate the cardiovascular effects of catecholamines after all (Margolius & Gaffney, 1965 ; van der Schoot & Moran, 1965 ; Aoki, Wilson, Theilen, Lukensmeyer & Leaverton, 1967). The picture is further complicated by the findings of others that thyroid hormones reduce sensitivity to sympathomimetic amines in some tissues, for example, the rabbit aorta (Macmillan & Rand, 1962), and have no effect on others, for example, the rabbit intestine (Aumann & Youmans, 1940). It would appear, therefore, that the sensitizing action of thyroxine, if indeed it has one, is confined to certain tissues.

Administration of thyroid hormones has been shown to increase toxicity to a variety of drugs. These include, besides sympathomimetic amines, histamine, alloxan, endotoxin, cocaine, morphine, chlorpromazine, chlordiazepoxide, monoamine oxidase inhibitors and barbiturates (for references, see Ashford & Ross, 1968). Thus, it seems that sensitivity changes following thyroxine are by no means confined to sympathomimetic amines.

The purpose of the present experiments was to elucidate more precisely the influence of thyroid hormones on sensitivity to catecholamines, and to establish whether or not any induced changes are accompanied by parallel changes in sensitivity to other biogenic amines. The results indicate that the effects of thyroxine on cardiac and smooth muscle are non-specific, and that, surprisingly, the hormone has opposite effects in different tissues. The possibility is discussed that the effects are due to modification of calcium metabolism.

Some of the experiments have been described briefly to the British Pharmacological Society (Coville & Telford, 1968, 1969).

Methods

Animals

Rats and guinea-pigs of either sex were used. The rats were of the Albino Wistar strain (weight 180–250 g) fed on 41B diet (Ranks) and the guinea-pigs were Porton albino (weight 450–600 g) fed on RGP diet (Dixons). The animals were given drinking water *ad lib.* and were housed at 21° ±0·5° C.

Hyperthyroidism was induced in both species by daily subcutaneous injection of 1 mg/kg l-thyroxine sodium in alkaline solution (0·001 N NaOH in 0·9% NaCl) for 10–14 consecutive days. This dose regimen was used by Myant & Witney (1967), who showed that the effect on metabolic rate reached a maximum after 5 days, remaining constant thereafter. Hypothyroidism was induced by surgical

removal of the thyroid gland, and the animals were maintained for 5 days post-operatively with 1% calcium gluconate in their drinking water, to avoid symptoms of calcium deficiency. Hyperthyroid animals were killed 10–14 days after the start of injections of thyroxine. Hypothyroid animals were killed not less than 14 days after the operation ; the effects of thyroidectomy on metabolic rate are maximal at or before this time (Neilson, Loizzi & Klitgaard, 1961).

For rats, changes in basal metabolic rate were determined on the eighth day (hyperthyroid animals) or fourteenth day (hypothyroid animals). Oxygen consumption was measured in individual animals at 25° C using a closed-circuit apparatus (Scientific Research Instruments). For guinea-pigs, effectiveness of treatment was determined by weight loss on the tenth day of treatment.

All animals were killed by cervical dislocation.

Isolated preparations

In all experiments on isolated tissues, weighting and magnification of the lever systems were kept constant, so that accurate comparisons could be made of the relative sensitivity of tissues from hyperthyroid, euthyroid and hypothyroid animals. Mean values for responses to doses of individual drugs were calculated together with their standard errors, and these were compared by Student's *t* test.

Heart

Detailed studies were carried out in the rat. The thorax was opened and the heart removed rapidly into cold Locke solution. Post-mortem examination of the neck region was performed to determine completeness of thyroidectomy. The heart was perfused with oxygenated Locke solution at 37° C, using the method of Langendorff (1895), at a pressure of approximately 30 mmHg (1 mmHg≡1·333 mbar). Heart rate and force of contraction were recorded with a heart spring lever writing on a smoked drum. Drugs were introduced into the perfusion fluid in a volume of 0·2 ml or less at intervals of 10 min. Responses to drugs were taken as a percentage change in the heart rate.

Uterus

Oestrus rats only were used, the stage of oestrus being determined by vaginal smear before killing. Loops were tied round the fallopian tubes and the two uterine horns dissected clear from the connective tissue. The whole uterine horn was suspended in a 10 ml organ bath containing aerated Locke solution at 37° C, and the movements recorded isotonically under 1 g tension. Drugs were added to the organ bath for 30 s every 10 min and responses were recorded either by an inhibitory effect on spontaneous movement or by direct spasmogenic effect. Inhibitory effects were measured by percentage decrease in the height of spontaneous movement, while contractile effects were measured as the height of the drug-induced contraction from the base-line, this being expressed as percentage increase over the height of the spontaneous contraction.

Ileum

Segments of rat or guinea-pig ileum 2·5 cm long in cold Tyrode solution were cut approximately 15 cm from the proximal end and suspended in a 20 ml bath con-

taining aerated Tyrode solution at 32° C. Responses were recorded isotonically using a tension of 1 g for rat and 2 g for guinea-pig ileum. Drug contact was 30 s at 90 s intervals. For transmural stimulation a 4 cm segment was taken from the distal ileum and mounted on an electrode as described by Paton (1955). The ileum was stimulated once every 10 s by a 10–40 V, 800 μs pulse, the voltage being varied until the maximal twitch was obtained.

Aorta

The whole of the thoracic aorta from rats or guinea-pigs was dissected from its connective tissue and a spiral cut approximately 2 mm wide. The strip was suspended in a 10 ml bath containing Krebs-bicarbonate solution bubbled with 95% oxygen and 5% carbon dioxide at 37° C. Isotonic contractions were recorded, using 1 g tension (rat) or 2 g tension (guinea-pig). The tissue was allowed to relax for one hour before commencing the experiment. Drugs were added at 15 min intervals, and left in contact with the tissue for 5 min.

Blood pressure measurements

Carotid blood pressure was recorded, using a mercury manometer and smoked drum. Male rats were used, and they were either pithed under ether anaesthesia (Shipley & Tilden, 1947) and maintained with artificial ventilation, or anaesthetized with urethane (150 mg/100 g intraperitoneally).

Drugs were given into the jugular vein and washed in with 0·3 ml of saline solution.

Experiments with calcium

Uterus and ileum

Calcium concentrations of the physiological salt solutions were varied between 0 and 4·4 mM in different experiments. The effect of this on spontaneous activity of the isolated uterus was measured by comparing the height of spontaneous contractions in 2·2 mM Ca^{2+} with that obtained in raised or lowered calcium concentration. This was done for uteri from thyroxine-treated, control and thyroidectomized rats.

The effect of variation of calcium concentration on the acetylcholine-induced response of either uterus or ileum taken from thyroxine-treated and control animals was also studied. The ED50 for acetylcholine was determined and the effect of altered calcium concentration was measured when three consecutive responses showed no consistent change in size.

The rate of decline of electrically-induced contractions was also determined in uterus and ileum taken from thyroxine-treated and control animals, because this may be related to the binding of calcium in the tissue (Knifton, 1966). The rat uterus was stimulated by paired ring electrodes ; for the guinea-pig ileum, coaxial electrodes (Paton, 1955) were used. For both tissues, 80 V pulses of 5 ms duration were applied at a frequency of 20 Hz for 5 s each min. Contractions were recorded on a Devices 4 channel pen recorder using a tension transducer attached to the free end of the tissue. Each tissue was allowed a rest period after dissection of 15–30 min ; resting tension was then adjusted, and stimulation commenced. After a short

interval, when the tension was steady for several responses, the Krebs-bicarbonate solution was substituted by one containing no calcium, and the responses allowed to decline until the tension developed fell below 50% of the maximum. The time taken for this to occur ($T50$) was measured from the time of removal of calcium, and these $T50$s compared for tissues from thyroxine-treated and control animals.

Drugs

(−)-Adrenaline acid tartrate, (−)-isoprenaline sulphate, acetylcholine bromide, histamine acid phosphate, (−)-thyroxine sodium (B.D.H.); (−)-noradrenaline bitartrate (Bayer); 5-hydroxytryptamine creatinine sulphate (May & Baker); (+)-amphetamine sulphate (Smith, Kline and French); synephrine tartrate (Koch-Light) and heparin (Evans Medical). All doses are expressed in terms of the free base.

Physiological salt solutions

The Locke solution contained (mM): NaCl 154, KCl 5·6, $CaCl_2$ 2·2, $NaHCO_3$ 6·0, dextrose 5·6. Tyrode solution: NaCl 136·9, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 0·5, Na_2HPO_4 139·6, $NaHCO_3$ 11·9, dextrose 5·6. Krebs-bicarbonate solution: NaCl 118·4, KCl 4·6, $CaCl_2$ 2·4, $MgSO_4$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25, dextrose 11·1.

All reagents used were " Analar " grade (B.D.H.).

Results

As a parameter of basal metabolic rate in rats and as a check on the effectiveness of respective pretreatments, oxygen consumption of individual rats was measured, and the mean values are shown in Table 1. At a dose of 1 mg thyroxine/kg daily, oxygen consumption in thyroxine-treated rats increased by about 60% on the eighth day, whilst that for thyroidectomized rats decreased by about 14% on the fourteenth day after the operation. Changes in oxygen consumption for thyroid hormone-treated rats (Myant & Witney, 1967) and thyroidectomized rats (Neilson *et al.*, 1961) are maximal before these times.

In guinea-pigs, thyroxine treatment decreased body weight by $78·0 \pm 4·9$ g after 10 days. Control and thyroidectomized guinea-pigs, on the other hand, gained $35·2 \pm 3·6$ g and $50·3 \pm 5·3$ g respectively. The difference between thyroxine-treated and control groups is significant ($P<0·001$). No weight loss followed thyroxine injection into rats.

Isolated tissue preparations

Heart

The mean rate of beat of isolated perfused hearts from six thyroxine-treated rats was $280 \pm 10·1$ beats/min. This was significantly faster ($P<0·001$) than hearts

TABLE 1. *Oxygen consumption of hyperthyroid, hypothyroid and control rats*

Treatment	Oxygen consumption ((ml/g)/h ± S.E.)	Significance
Thyroidectomy (14 days)	2·43 ± 0·178 (10)	$P<0·05$
Saline	2·82 ± 0·165 (10)	
Thyroxine (1 mg/kg, daily, 8 days)	4·64 ± 0·375 (10)	$P<0·01$

Results are expressed as a group mean ± S.E. Number of animals in parentheses.

from control rats, which beat at a mean rate of 167 ± 5·1 beats/min. Thyroidectomy, on the other hand, had no significant effect on heart rate ($P>0.05$). As shown in Table 2, similar results were obtained with guinea-pigs.

Detailed drug sensitivity studies were carried out in the rat. It was found that, without exception, the hearts from thyroxine-treated animals were more sensitive to noradrenaline, adrenaline, isoprenaline and acetylcholine. The results are given in detail in Table 3. Both the positive chronotropic effect of the catecholamines and the negative chronotropic effect of acetylcholine were potentiated. Thus, for noradrenaline, the dose ratio for hearts from thyroxine-treated and control rats was approximately 0·5 : 1, while that for acetylcholine was approximately 0·1 : 1. Thyroidectomy, on the other hand, had the opposite effect, depressing sensitivity to these agents very markedly (Table 3). As shown for noradrenaline in Fig. 1, the dose response curves were shifted to the left after thyroxine treatment, and to the right after thyroidectomy, and for each drug the changes in sensitivity were highly significant ($P<0.001$). Histamine, which had no action on the heart except in large doses (10–50 mg), was toxic to the hearts of thyroxine treated rats in doses of 500 ng–1 μg. These doses did not affect hearts of control or thyroidectomized animals.

TABLE 2. *Resting rate (beats/min) of hearts isolated from hyperthyroid, hypothyroid and control rats and guinea-pigs*

Treatment	Rat	Guinea-pig
Thyroidectomy (14 days)	152 ± 5·9 (n.s.)	164 ± 6·7 (n.s.)
Saline	167 ± 5·1	174 ± 5·2
Thyroxine (1 mg/kg, daily, 10 days)	280 ± 10·1*	268 ± 5·9*

Results are expressed as the mean ± S.E. of groups of six animals.
n.s., Not significantly different from control ($P>0.05$).
* Significantly different from control ($P<0.001$).

TABLE 3. *Mean responses (± S.E.) of rat isolated perfused heart to noradrenaline, adrenaline, isoprenaline and acetylcholine*

		Response (% effect on resting heart rate)		
Drug	Dose	Control	Thyroxine-treated	Thyroidectomized
Noradrenaline	5 ng	0·0 (7)	8·4 ± 1·8 (6) <0·001	0·0 (6)
	10 ng	5·2 ± 1·7 (7)	21·6 ± 2·2 (6) <0·001	0·0 (6) <0·02
	20 ng	21·3 ± 2·0 (7)	42·0 ± 2·8 (6) <0·001	0·0 (6) <0·001
	50 ng	30·0 ± 2·6 (7)	58·6 ± 4·2 (6) <0·001	10·2 ± 2·2 (6) <0·001
	100 ng	45·0 ± 2·9 (7)	68·0 ± 4·6 (6) <0·01	25·6 ± 2·5 (6) <0·001
Adrenaline	5 ng	0·0 (6)	5·8 ± 1·9 (6) <0·02	0·0 (6)
	10 ng	8·0 ± 1·9 (6)	27·3 ± 2·6 (6) <0·001	0·0 (6) <0·01
	20 ng	25·9 ± 2·6 (6)	50·0 ± 3·7 (6) <0·001	7·4 ± 1·7 (6) <0·001
	50 ng	29·6 ± 2·2 (6)	74·0 ± 4·6 (6) <0·001	11·1 ± 2·6 (6) <0·001
Isoprenaline	1 ng	0·0 (7)	0·0 (5)	0·0 (5)
	2 ng	0·0 (7)	7·5 ± 1·6 (5) <0·001	0·0 (5)
	5 ng	10·8 ± 2·0 (7)	20·4 ± 2·6 (5) <0·02	0·0 (5) <0·001
	10 ng	28·4 ± 2·5 (7)	43·0 ± 2·9 (5) <0·001	11·2 ± 2·4 (5) <0·001
	20 ng	50·0 ± 3·8 (7)	72·0 ± 4·6 (5) <0·01	19·6 ± 3·0 (5) <0·001
Acetylcholine	10 ng	0·0 (5)	32·0 ± 2·6 (5) <0·001	0·0 (5)
	20 ng	6·2 ± 1·5 (5)	58·5 ± 4·3 (5) <0·001	0·0 (5) <0·01
	50 ng	19·3 ± 2·4 (5)	100·0 (5) <0·001	0·0 (5) <0·001
	100 ng	40·0 ± 2·9 (5)	100·0 (5) <0·001	21·5 ± 2·1 (5) <0·001

Number of animals in parentheses, followed by significance of difference from control expressed as P value.

In some further experiments, the effects of adrenaline, noradrenaline, isoprenaline and acetylcholine were studied at single dose levels on guinea-pig hearts, and it was found that, as in the rat, thyroxine pretreatment increased sensitivity to each of these drugs, and that thyroidectomy had the opposite effect.

Uterus

Pretreatment of rats with thyroxine increased the sensitivity of the isolated oestrus uterus to isoprenaline, adrenaline, noradrenaline and histamine (which are inhibitory in this species) and to acetylcholine and 5-hydroxytryptamine (which cause contraction) by 2 to 5 times. These effects are highly significant ($P<0{\cdot}001$). The detailed results are given in Table 4. The dose response curves to all of these drugs were shifted to the left, as shown for isoprenaline by Fig. 2. The spontaneously active oestrus uterus is extremely sensitive to the inhibitory actions of catecholamines, yet (as shown in Table 4) pretreatment with thyroxine increased sensitivity even further. Isoprenaline produced the greatest inhibitory effect in control animals and responses to concentrations as low as 10^{-11} g/ml were recorded in tissues from hyperthyroid rats.

Acetylcholine and 5-hydroxytryptamine contract the rat uterus, and thyroxine potentiated the contractile effect of these drugs. In the rat uterus, therefore, as in the rat and guinea-pig heart, thyroxine potentiates the actions of both spasmogenic and inhibitory drugs.

In two experiments using tissues from thyroidectomized rats, the inhibitory response to histamine was studied. It was found that in control uteri, 100 ng/ml histamine produced approximately 50% inhibition of spontaneous contractions, whereas in uteri from thyroidectomized rats 500 ng/ml was required to produce the same effect.

Neither thyroxine-treatment nor thyroidectomy *per se* produced any significant change in the height of spontaneous contractions of the rat oestrus uterus.

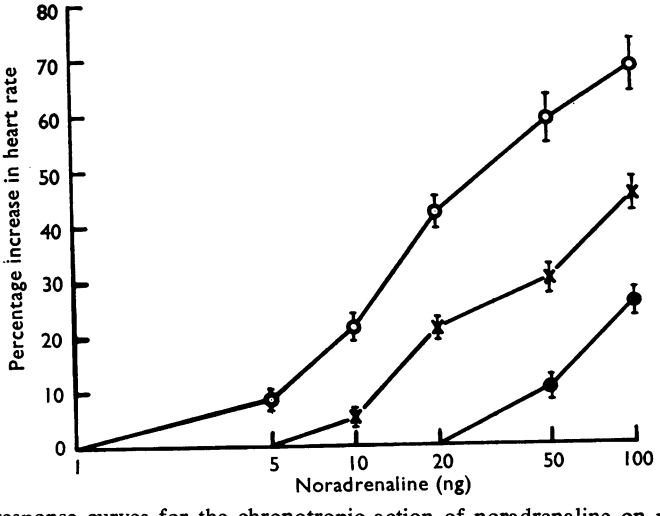

FIG. 1. Dose-response curves for the chronotropic action of noradrenaline on perfused hearts from thyroxine-treated (1 mg/kg subcutaneously daily (10–14 days) ○——○), saline treated (1 ml/kg s.c. daily (10–14 days) ×——×), or thyroidectomized (14 days after operation, ●——●) rats. Each point is the mean ± S.E. for six (○), seven (×) and six (●) experiments.

Hormonal domination of the guinea-pig uterus cannot be reliably determined by vaginal lavage as in the rat, and no experiments were done on this tissue.

Ileum

The effect of thyroxine pretreatment was studied on acetylcholine responses in the rat, and on acetylcholine, histamine and 5-hydroxytryptamine responses in the guinea-pig. The results are given in Table 5. In contrast to the results obtained in the heart and uterus, pretreatment of rats with thyroxine decreased sensitivity of the isolated ileum to acetylcholine. In the guinea-pig, pretreatment with thyroxine likewise decreased sensitivity to histamine and 5-hydroxytryptamine, as well as to acetylcholine. Sensitivity to histamine and 5-hydroxytryptamine was determined on the guinea-pig ileum only, since the rat ileum is relatively insensitive to these drugs. Thyroidectomy had the opposite effect to that of thyroxine treatment, and in the guinea-pig markedly increased the sensitivity of the ileum to all three

TABLE 4. *Mean responses* (±S.E.) *of rat isolated oestrus uterus to isoprenaline, adrenaline, noradrenaline, histamine, acetylcholine and 5-hydroxytryptamine*

Drug	Dose (ng/ml)	Response (%) Control	Thyroxine-treated
Isoprenaline*	0·005	0·0 (6)	0·0 (6)
	0·01	0·0 (6)	14·3 ±2·1 (6) <0·001
	0·025	10·0 ±2·2 (6)	64·6 ±4·8 (6) <0·001
	0·05	30·5 ±2·7 (6)	90·4 ±5·6 (6) <0·001
	0·1	58·6 ±4·4 (6)	100·0 (6) <0·001
	0·2	100·0 (6)	100·0 (6)
Adrenaline*	0·01	0·0 (6)	0·0 (6)
	0·05	10·0 ±2·4 (6)	22·5 ±2·7 (6) <0·01
	0·1	37·4 ±2·8 (6)	67·4 ±4·5 (6) <0·001
	0·2	63·2 ±4·5 (6)	95·6 ±5·8 (6) <0·01
	0·4	84·6 ±5·3 (6)	100·0 (6) <0·02
	1·0	100·0 (6)	100·0 (6)
Noradrenaline*	2·0	0·0 (7)	0·0 (5)
	5·0	0·0 (7)	24·1 ±2·6 (5) <0·001
	10·0	20·2 ±2·6 (7)	42·5 ±3·4 (5) <0·001
	20·0	56·8 ±4·3 (7)	83·4 ±5·6 (5) <0·01
Histamine*	10·0	0·0 (5)	10·2 ±2·0 (5) <0·001
	25·0	0·0 (5)	25·6 ±2·6 (5) <0·001
	50·0	15·6 ±2·5 (5)	86·9 ±5·3 (5) <0·001
	100·0	52·5 ±3·6 (5)	100·0 (5) <0·001
	500·0	80·1 ±5·4 (5)	100·0 (5) <0·01
	1,000·0	100·0 (5)	100·0 (5)
Acetylcholine†	10·0	18·0 ±2·2 (6)	35·3 ±2·8 (5) <0·001
	20·0	24·5 ±2·6 (6)	47·6 ±3·7 (5) <0·001
	40·0	32·8 ±2·6 (6)	59·4 ±4·8 (5) <0·01
	100·0	40·9 ±2·9 (6)	83·5 ±5·0 (5) <0·001
	200·0	65·5 ±4·6 (6)	98·2 ±5·8 (5) <0·01
	500·0	71·6 ±4·9 (6)	101·6 ±5·6 (5) <0·01
5-Hydroxytryptamine†	50·0	3·2 ±0·9 (5)	18·5 ±2·5 (5) <0·001
	100·0	12·1 ±2·0 (5)	26·1 ±2·5 (5) <0·01
	200·0	28·2 ±2·6 (5)	53·2 ±3·6 (5) <0·001
	500·0	62·3 ±4·5 (5)	90·1 ±4·9 (5) <0·01
	1,000·0	79·4 ±4·6 (5)	110·2 ±5·5 (5) <0·01

* Inhibitory effects, measured as % decrease in height of spontaneous movement.
† Spasmogenic effects, quoted as % increase of the height of the drug-induced contraction over the height of the spontaneous contraction.
Number of animals in parentheses, followed by significance of difference from control expressed as *P* value.

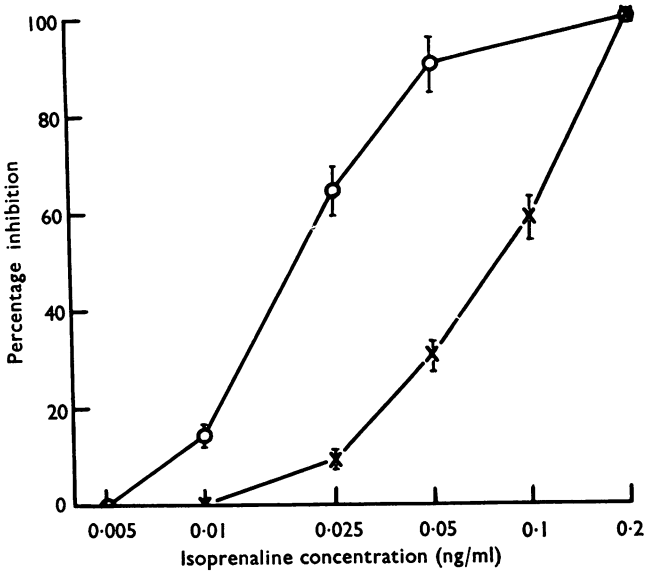

FIG. 2. Dose-response curves for the inhibitory action of isoprenaline on oestrus uteri from thyroxine-treated (○——○) and saline treated (×——×) rats. Each point is the mean ± S.E. for six experiments.

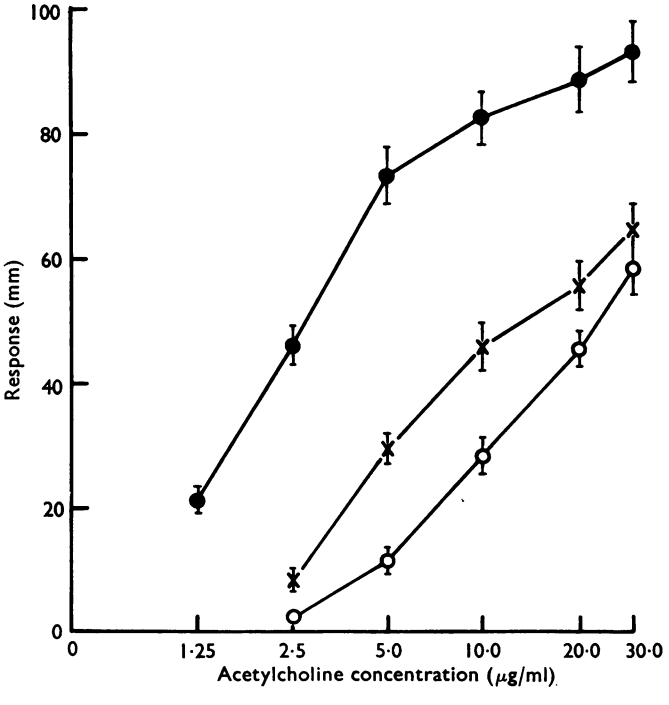

FIG. 3. Dose-response curves for acetylcholine on ileum isolated from thyroxine-treated (○——○), saline-treated (×——×) and thyroidectomized (●——●) rats. Each point is the mean ± S.E. for six (○), seven (×) and five (●) experiments. Note that alteration in thyroid hormone levels has had the opposite effect on drug sensitivity in this tissue to that obtained in the heart and uterus.

spasmogens, in some cases as much as five-fold. In the ileum, therefore, the effect of thyroidectomy was very marked, and directly opposite to that obtained in heart and uterus. This is shown for acetylcholine in Fig. 3.

Adrenaline potency was estimated by inhibition of contractile responses to acetylcholine and to transmural electrical stimulation. Approximately 50% maximal responses of the guinea-pig ileum to acetylcholine were obtained, and the inhibitory effects of graded doses of adrenaline, added to the bath 30 s before the acetylcholine, were estimated. Adrenaline-induced inhibition of transmural twitches was also determined. Both methods showed that the adrenaline sensitivity of the ileum was unaffected by thyroxine treatment (Table 6). In this respect, therefore, the ileum differs from heart and uterus.

Aorta

The guinea-pig isolated aortic strip preparation behaved like the ileum, in that pretreatment of the animals with thyroxine reduced the sensitivity to the spasmo-

TABLE 5. *Mean responses* (±S.E.) *of isolated ileum of rat and guinea-pig to acetylcholine, histamine and 5-hydroxytryptamine*

Drug	Dose (ng/ml)	Response (contraction, mm) Control	Thyroxine	Thyroidectomized
Acetylcholine (rat)	1·25			21·5 ±2·1 (5)
	2·5	8·3 ±1·8 (7)	2·4 ±0·8 (6) <0·02	46·2 ±3·1 (5) <0·001
	5·0	29·6 ±2·5 (7)	11·6 ±2·1 (6) <0·001	73·3 ±4·6 (5) <0·001
	10·0	46·0 ±3·7 (7)	28·5 ±2·8 (6) <0·001	82·5 ±4·2 (5) <0·001
	20·0	55·6 ±3·8 (7)	45·5 ±3·1 (6) n.s.	88·5 ±5·1 (5) <0·001
	30·0	64·6 ±4·0 (7)	58·6 ±4·3 (6) n.s.	93·0 ±4·8 (5) <0·01
Acetylcholine (guinea-pig)	5·0	72·5 ±4·5 (6)	37·7 ±3·2 (6) <0·001	104·3 ±6·3 (4) <0·01
	7·5	118·3 ±5·4 (6)	66·1 ±4·5 (6) <0·001	146·5 ±8·1 (4) <0·02
	10·0	151·2 ±9·1 (6)	93·3 ±5·8 (6) <0·001	183·2 ±9·2 (4) <0·05
	15·0	180·0 ±10·1 (6)	104·0 ±6·1 (6) <0·001	
	20·0	190·0 ±10·6 (6)	130·2 ±8·5 (6) <0·01	215·2 ±10·1 (4) n.s.
	40·0	195·2 ±11·2 (6)	133·1 ±8·2 (6) <0·001	225·0 ±9·9 (4) n.s.
Histamine (guinea-pig)	2·5	23·6 ±2·6 (6)	12·1 ±2·2 (6) <0·01	53·1 ±3·6 (4) <0·001
	5·0	45·4 ±3·2 (6)	21·6 ±2·4 (6) <0·001	70·0 ±5·2 (4) <0·01
	10·0	95·6 ±5·8 (6)	59·4 ±4·8 (6) <0·001	129·5 ±8·1 (4) <0·05
	20·0	134·0 ±8·2 (6)	110·2 ±6·3 (6) n.s.	154·5 ±9·3 (4) n.s.
	40·0	148·6 ±9·1 (6)	135·6 ±7·8 (6) n.s.	
5-hydroxy-tryptamine (guinea-pig)	50·0	9·6 ±2·0 (6)	2·4 ±0·6 (6) <0·01	24·2 ±2·8 (4) <0·01
	100·0	32·6 ±2·6 (6)	16·6 ±2·3 (6) <0·001	57·3 ±4·5 (4) <0·01
	250·0	62·0 ±4·2 (6)	48·5 ±3·5 (6) <0·05	120·0 ±6·6 (4) <0·001
	500·0	112·5 ±5·8 (6)	104·3 ±5·8 (6) n.s.	162·4 ±8·8 (4) <0·01

Number of animals in parentheses, followed by significance of difference from control expressed as *P* value. n.s., Not significant ($P>0·05$).

TABLE 6. *Mean responses* (±S.E.) *of guinea-pig isolated ileum to adrenaline*

Dose (ng/ml)	Response* Control	Thyroxine-treated
5·0	45·8 ±4·3 (5)	23·3 ±4·8 (5) <0·01
10·0	48·2 ±4·2 (5)	46·5 ±2·8 (5) n.s.
15·0	52·8 ±3·1 (5)	58·6 ±3·0 (5) n.s.
20·0	58·3 ±2·9 (5)	56·0 ±3·6 (5) n.s.
30·0	63·9 ±2·8 (5)	60·6 ±2·9 (5) n.s.

* Adrenaline response shown as percentage inhibition of reference acetylcholine contraction. Number of animals in parentheses, followed by significance of difference from control expressed as *P* value. n.s., Not significant ($P>0·05$).

genic drugs histamine and 5-hydroxytryptamine. The inhibitory action of thyroxine on the aorta was very striking ($P<0{\cdot}001$), the dose response curves being shifted to the right and the maximal responses being considerably reduced. This is shown for histamine in Fig. 4. The aorta differs from the ileum in that sympathomimetic amines have a spasmogenic effect and this action was likewise depressed, whereas in the ileum the inhibitory effect of adrenaline was unaffected by thyroxine treatment. Isoprenaline has little or no effect on the aorta. The effects of thyroxine on the responses of the guinea-pig aorta to noradrenaline, adrenaline, histamine and 5-hydroxytryptamine are summarized in Table 7.

In the rat, thyroxine pretreatment likewise decreased sensitivity of the isolated aorta to adrenaline. Thyroidectomy, however, did not modify sensitivity to adrenaline. These results are shown in Table 8.

Blood pressure measurements

Pithed rats

The low resting blood pressure of the pithed rat makes it an unsuitable preparation for testing drugs which have depressor actions. The influence of thyroxine was studied on responses to noradrenaline, synephrine and (+)-amphetamine. Noradrenaline and synephrine both act directly upon the adrenoceptors of the cardiovascular system, whereas (+)-amphetamine acts indirectly by release of endogenous noradrenaline from storage sites (Burn & Rand, 1958). Synephrine was chosen for this experiment because, unlike noradrenaline, it is not metabolized by catechol-*o*-methyl transferase (COMT) (Axelrod, 1959) and any effect of thyroxine upon COMT would be evident by a differential change in sensitivity of the cardiovascular

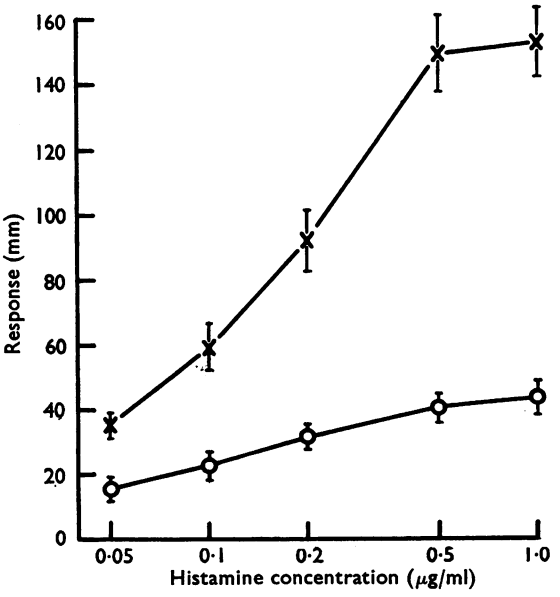

FIG. 4. Dose-response curves for histamine on aortic strips isolated from thyroxine-treated (○——○) and saline-treated (×——×) guinea-pigs. Each point is the mean ± S.E. for four (○) and fixe (×) experiments. Note that in the aorta, as in the ileum (Fig. 3), thyroxine reduces drug sensitivity, but that the shift of the dose-response curve is different.

TABLE 7. *Mean responses* (±S.E.) *of isolated aorta of guinea-pig to noradrenaline, adrenaline, histamine and 5-hydroxytryptamine*

Drug	Dose (μg/ml)	Response (contraction, mm) Control	Thyroxine-treated
Noradrenaline	0·01	19·2 ±2·6 (6)	4·5 ±1·4 (6)
	0·05	35·4 ±3·2 (6)	9·2 ±2·1 (6)
	0·1	65·2 ±4·1 (6)	15·6 ±2·3 (6)
	0·2	97·8 ±5·2 (6)	20·4 ±2·5 (6)
	0·5	129·3 ±7·4 (6)	27·6 ±3·0 (6)
	1·0	140·4 ±8·0 (6)	32·5 ±3·4 (6)
Adrenaline	0·05	36·1 ±3·2 (6)	8·2 ±2·1 (6)
	0·1	70·4 ±4·5 (6)	15·8 ±2·3 (6)
	0·2	100·2 ±5·5 (6)	22·6 ±2·9 (6)
	0·5	125·0± 6·8 (6)	26·5 ±2·8 (6)
Histamine	0·05	35·6 ±2·6 (5)	15·5 ±2·2 (4)
	0·1	59·2 ±4·5 (5)	22·6 ±2·7 (4)
	0·2	92·4 ±6·2 (5)	31·5 ±2·6 (4)
	0·5	148·8 ±7·8 (5)	40·6 ±2·8 (4)
	1·0	152·3 ±7·2 (5)	43·5 ±3·3 (4)
5-hydroxy-tryptamine	0·1	43·2 ±3·1 (5)	9·4 ±1·8 (4)
	0·2	71·6 ±4·2 (5)	15·6 ±2·2 (4)
	0·5	112·5 ±5·3 (5)	24·9 ±3·1 (4)
	1·0	135·6 ±7·5 (5)	34·6 ±3·7 (4)

Number of animals in parentheses. In every case, the response in the thyroxine-treated group was significantly less than that in the control group ($P<0·001$).

TABLE 8. *Mean responses* (±S.E.) *of isolated aorta of rat to adrenaline*

Dose (ng/ml)	Response (contraction, mm) Control	Thyroxine-treated	Thyroidectomized
0·01	10·0 ±1·5 (6)	3·5 ±0·9 (6) <0·01	10·0 ±1·8 (3) n.s.
0·02	12·2 ±1·4 (6)	5·6 ±1·6 (6) <0·02	12·0 ±2·1 (3) n.s.
0·04	16·4 ±2·2 (6)	6·2 ±1·8 (6) <0·001	17·0 ±2·6 (3) n.s.

Number of animals in parentheses, followed by significance of difference from control expressed as *P* value. n.s., Not significant ($P>0·05$).

TABLE 9. *Mean pressor responses* (±S.E.) *of pithed rats to noradrenaline, synephrine and* (+)*-amphetamine*

Drug	Dose (ng/kg)	Response (mmHg) Control	Thyroxine-treated
Noradrenaline	25	3·0 ±0·36 (6)	2·3 ±0·28 (5) n.s.
	50	6·0 ±0·26 (6)	5·3 ±0·28 (5) n.s.
	100	10·8 ±1·86 (6)	9·6 ±1·11 (5) n.s.
	250	15·0 ±1·29 (6)	16·5 ±1·35 (5) n.s.
	500	20·6 ±2·35 (6)	22·0 ±2·12 (5) n.s.
Synephrine	250	5·0 ±0·56 (6)	4·0 ±0·36 (5) n.s.
	500	8·6 ±0·44 (6)	8·0 ±0·28 (5) n.s.
	750	9·5 ±0·53 (6)	10·5 ±0·51 (5) n.s.
	1,000	15·0 ±0·96 (6)	13·5 ±0·48 (5) n.s.
(+)-amphetamine (μg/kg)	25	3·5 ±0·28 (6)	3·6 ±0·31 (5) n.s.
	50	6·3 ±0·84 (6)	6·5 ±0·52 (5) n.s.
	100	8·6 ±0·65 (6)	11·0 ±0·56 (5) <0·05

Number of animals in parentheses, followed by significance of difference from control. n.s., Not significant ($P>0·05$).

system to these two amines. The results presented in Table 9 show that thyroxine did not alter sensitivity to either noradrenaline or synephrine. The pressor response to the highest dose (100 mg/kg) of (+)-amphetamine was slightly potentiated, but the effect of the low doses was unaltered (Table 9).

Anaesthetized rats

The resting blood pressure of anaesthetized thyroxine-treated rats was $80 \pm 3{\cdot}99$ mmHg, that of control rats $70 \pm 3{\cdot}42$ mmHg, and thyroidectomized rats $65 \pm 6{\cdot}13$ mmHg. The difference between thyroxine-treated and control rats is significant ($P<0{\cdot}02$).

As in pithed hyperthyroid rats, the sensitivity of the cardiovascular system in anaesthetized hyperthyroid rats to noradrenaline was not significantly different from controls. On the other hand, in hypothyroid rats, the sensitivity to noradrenaline was significantly reduced (Table 10). The effect of isoprenaline on the cardiovascular system of untreated animals was usually biphasic, a small pressor response preceding a longer fall. In hyperthyroid animals, only the pressor effect was seen, except when larger doses (250–500 ng/kg) were tested. In hypothyroid rats, only the depressor effect of isoprenaline was observed and it was no greater than in control rats. It appears, therefore, that the heart in hyperthyroid animals plays a larger part than normal in the determination of the cardiovascular response. The depressor response to low doses of acetylcholine was slightly greater in thyroxine-treated rats (Table 10).

Since the action of (+)-amphetamine rapidly declines due to tachyphylaxis, only the initial responses to this drug could be compared in hyper- and hypothyroid rats. As shown in Fig. 5, it was found that the response to (+)-amphetamine was increased by about 40% in hyperthyroid rats ($P<0{\cdot}001$), but was not significantly changed in hypothyroid rats. The increase in sensitivity to (+)-amphetamine in hyperthyroid rats may reflect an effect of thyroxine upon the release of endogenous noradrenaline rather than upon sensitivity to the latter, since, as found in these experiments, exogenous noradrenaline was not potentiated in hyperthyroid rats.

TABLE 10. *Mean responses* (±S.E.) *of cardiovascular system of anaesthetized rats to noradrenaline and acetylcholine*

Drug	Dose (ng/kg)	Response (mmHg) Control	Thyroxine-treated	Thyroidectomized
Noradrenaline	25	8·0 (3)	8·0 (2)	
	50	11·2 ±1·06 (6)	9·0 ±0·40 (6) n.s.	7·3 ±0·42 (5) <0·02
	100	16·5 ±1·18 (6)	12·2 ±1·20 (6) n.s.	12·0 ±0·82 (5) <0·05
	250	17·2 ±1·65 (6)	16·0 ±1·19 (6) n.s.	13·5 ±1·04 (5) <0·02
	500	23·2 ±2·22 (6)	16·5 ±2·06 (6) n.s.	17·5 ±1·65 (5) <0·05
	1,000	23·0 (2)	23·0 (2)	
Acetylcholine	25	8·0 (3)	12·5 (2)	
	50	12·0 ±0·41 (6)	15·3 ±1·06 (6) <0·05	14·0 ±0·98 (5) n.s.
	100	13·5 ±1·04 (6)	17·0 ±1·68 (6) <0·05	16·5 ±1·10 (5) n.s.
	250	14·5 ±1·89 (6)	18·9 ±1·29 (6) n.s.	15·5 ±1·65 (5) n.s.
	500	17·8 ±1·96 (6)	20·0 ±1·58 (6) n.s.	19·0 ±1·41 (5) n.s.

Number of animals in parentheses, followed by significance of difference from control expressed as P value. n.s., Not significant ($P>0{\cdot}05$).

Experiments with calcium

Calcium is an essential factor in the drug-induced responses of smooth muscle (Daniel, 1964). Thyroxine is known to modify calcium metabolism (Krane, Brownell, Stanbury & Corrigan, 1956; Adams, Jowsey, Kelly, Riggs, Kinney & Jones, 1967) and it is therefore possible that thyroxine-induced modifications in sensitivity to drugs are due to modification in the availability of calcium ions. For this reason, some experiments were carried out to determine the influence of thyroxine on the sensitivity of the uterus and ileum to calcium ions.

Uterus

The relationship between thyroxine and calcium in the uterus was investigated using three parameters: spontaneous movement, drug-induced contraction, and electrically induced tension. In the first place, it was found that variation in the calcium concentration of the Locke solution bathing the uterus produced a much more marked change in the height of spontaneous contractions of uteri from thyroxine-treated rats than in uteri from control or thyroidectomized rats. This is shown in Fig. 6. Second, it was found that the variation in size of acetylcholine responses induced by alteration of the concentration of calcium in the Locke solution

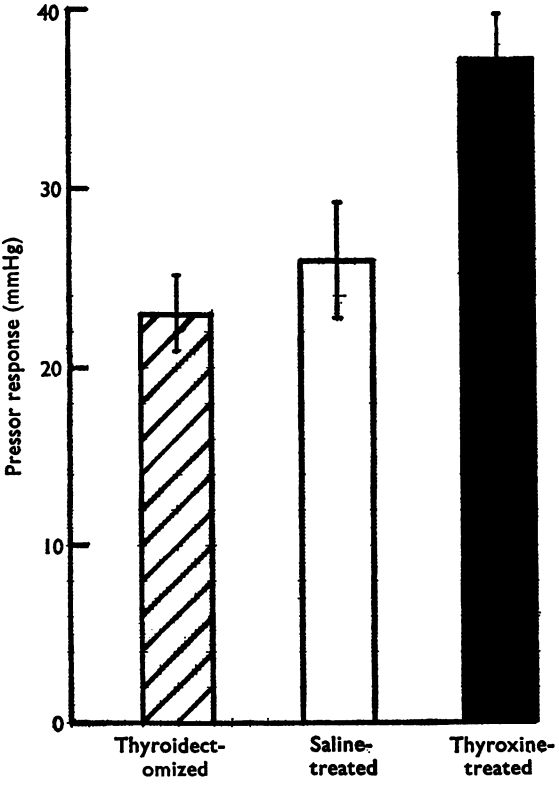

FIG. 5. Pressor responses of anaesthetized rats to 50 μg/kg (+)-amphetamine intravenously. Each column represents the mean response ± S.E. for five thyroidectomized (▨), six saline-treated (□) and six thyroxine-treated (■) rats. The difference between the response from thyroxine-treated and saline-treated rats is highly significant ($P<0.001$).

TABLE 11. *Effect of variation in the concentration of calcium on the acetylcholine-induced response of rat uterus and guinea-pig ileum*

Response as a percentage of acetylcholine response in Locke solution containing 2·2 mM Ca^{2+}

	Uterus			Ileum		
Ca^{2+} (mM)	Control	Thyroxine-treated	*P*	Control	Thyroxine-treated	*P*
1·1	70·1 ±1·3	36·5 ±1·8	<0·001	68·3 ±0·6	77·1 ±1·2	<0·001
2·2	100	100		100	100	
3·3	124·7 ±4·3	168·9 ±3·3	<0·001	176·8 ±8·7	123·3 ±7·7	<0·001

Each result is the mean ±S.E. of six determinations.

TABLE 12. *Effect of thyroxine treatment on the rate of decrease of electrically-induced contraction of the rat uterus and guinea-pig ileum in calcium-free physiological salt solution*

	Time (min) taken for tension to fall by 50% (*T*50)		
	Control	Thyroxine-treated	*P*
Dioestrus uterus	5·63 ±0·34	3·12 ±0·28	<0·001
Oestrus uterus	2·66 ±0·17	1·86 ±0·29	<0·01
Ileum	1·48 ±0·22	1·62 ±0·32	>0·05

Each result is the mean ±S.E. of six (uterus) and nine (ileum) determinations.

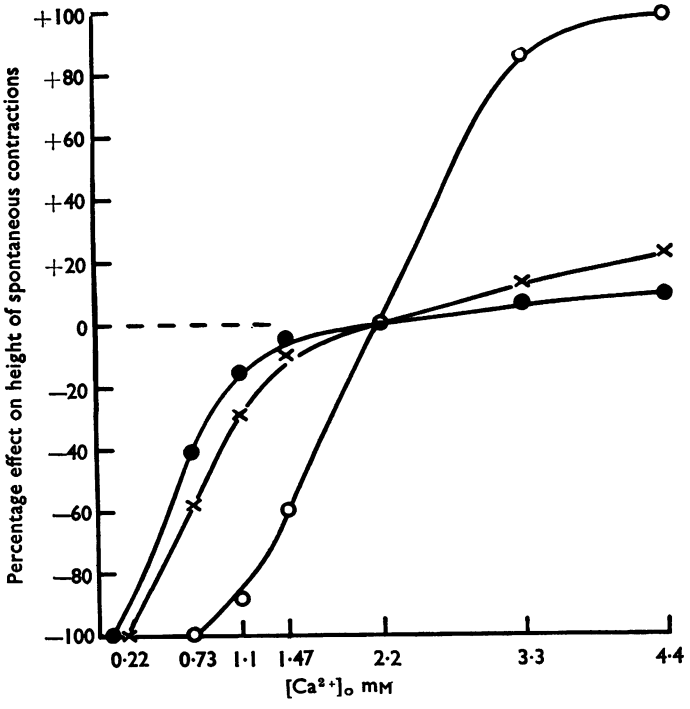

FIG. 6. Effect of variation of calcium concentration in the Locke solution on the height of spontaneous contractions of the isolated oestrus uterus from saline-treated (×), thyroxine-treated (○) and thyroidectomized (●) rats. The dotted line indicates the height of spontaneous contractions in Locke solution (2·2 mM Ca^{2+}). Each point is the mean of six determinations.

were much greater in uteri from thyroxine-treated rats than in those from control rats. This is shown in Table 11. Third, it was found that the decline in electrically-induced contractions following exposure to calcium-free bathing solution was significantly more rapid in uteri from thyroxine-treated oestrus or dioestrus rats than in control oestrus or dioestrus rats ($P<0{\cdot}01$ and $<0{\cdot}001$ respectively). These results are shown in Table 12.

Ileum

In the ileum, in contrast to the uterus, thyroxine reduced the changes in size of the acetylcholine response induced by variation in calcium concentration and also produced a slight reduction in the rate of decline of electrically-induced tension, following exposure to calcium-free medium. These findings are shown in Tables 11 and 12.

Discussion

The present experiments show that thyroxine increases sensitivity to biogenic amines non-specifically in some tissues (heart and uterus), but depresses sensitivity non-specifically in others (ileum and aorta). These parallel changes in sensitivity to biogenic amines occur irrespective of whether the agonist is excitatory or inhibitory in its effect. Barker (1964) has shown that thyroxine has different effects on metabolic rate in different tissues, but no parallelism exists between the changes in tissue metabolic rate reported by him and the changes in tissue sensitivity reported here. For example, the metabolic rate of the heart is greatly increased in hyperthyroidism, whereas the uterus exhibits hardly any change, yet both of these tissues are made much more sensitive to drugs by thyroxine pretreatment. It would seem, therefore, that the differing effect of thyroxine on drug sensitivity in different tissues is not directly related to the metabolic effect of the hormone, as gauged by oxygen consumption. The same lack of correlation is also true for tissues taken from thyroidectomized animals, and since changes in drug sensitivity are, in general, opposite to those induced after thyroxine treatment, it would appear that changes induced after thyroidectomy in the present experiments are due to lack of thyroid hormone, rather than loss of parathyroid hormone or thyrocalcitonin.

That thyroxine increases the negative chronotropic effect of acetylcholine as well as increasing the positive chronotropic effects of catecholamines implies that the hormone acts on a pathway which in the heart is common to the action of both excitatory and inhibitory drugs. In the uterus, thyroxine likewise increases sensitivity to both excitatory and inhibitory drugs, increasing sensitivity to the spasmogenic actions of acetylcholine and 5-hydroxytryptamine on the one hand, and to the inhibitory actions of histamine and catecholamines on the other. As pointed out by Diamond & Brody (1966a, b) both spasmogenic and inhibitory drugs raise phosphorylase *a* levels in the uterus. Thyroxine is also known to raise phosphorylase *a* levels, at least in the heart (Aronson & Hess, 1967) and the increase in sensitivity to both excitatory and inhibitory drugs in the heart and uterus may be related to this effect.

Thyroxine has been shown to increase tissue levels of 3′,5′ cyclic adenosine monophosphate (cyclic AMP) by inducing adenyl cyclase (Brodie, Davies, Hynie, Krishna & Weiss, 1966) and by inhibiting phosphodiesterase (Mandel & Kuehl,

1967). Cyclic AMP has been implicated in both α and β effects of catecholamines (Bartelstone, Nasmyth & Telford, 1967 ; Robison, Butcher & Sutherland, 1966). In the present experiments some actions of sympathomimetic amines (on the heart and uterus) were enhanced by thyroxine, whereas the action on aortic strips was inhibited. Pressor effects and the inhibitory action of catecholamines on guinea-pig ileum were unaltered. Thus, no simple explanation of the effects of thyroxine in terms of altered levels of cyclic AMP is possible.

Hyperthyroidism induces a marked tachycardia which is not neurogenic in origin. The possibility that the tachycardia is due to increased sensitivity to endogenous catecholamines has been extensively studied, but with equivocal results. Various workers (for references, see Harrison, 1964 ; Waldstein, 1966) have found that thyroid hormones increase the sensitivity of the heart to catecholamines, but others have failed to demonstrate any such effect. In the present experiments in the rat, thyroxine invariably caused both tachycardia and heightened sensitivity to catecholamines and to acetylcholine Since the heart is predominantly under vagal control in the intact animal, it seems unlikely that the tachycardia of hyperthyroidism is due to altered sensitivity to local humoral agents. This conclusion is supported by the failure of adrenoceptor blocking agents, adrenergic neurone blocking agents, or reserpine completely to ameliorate either experimental or clinical tachycardia due to hyperthyroidism (Sawyer & Lipner, 1961 ; Lee, Bronsky & Waldstein, 1962 ; Barker & Makiuchi, 1965 ; Cairoli & Crout, 1967 ; Goodkind, 1968).

The depressant effect of thyroxine on the sensitivity of the rat and guinea-pig aorta to drugs agrees with the results of Macmillan & Rand (1962) in the rabbit, and also with the work of Zsotér, Tom & Chappel (1964), who showed that the vasoconstrictor responses of the dog hind limb to catecholamines are depressed by thyroxine. The ability of thyroxine to induce marked (but opposing) changes in the sensitivity of the isolated aorta and heart to sympathomimetic amines contrasts with its failure to modify pressor responses. Since in the intact animal peripheral circulation pays a large part in the overall cardiovascular response to a drug, it is possible that decrease in peripheral response may balance any increase in cardiac output. As an alternative hypothesis to account for the failure of thyroxine to modify pressor responses, it may be argued that an effect of the hormone on catecholamine metabolism masks any potentiation of pressor action which would otherwise be seen. Thus, Zile (1959, 1960) found that thyroid feeding elevates the plasma levels of adrenaline and noradrenaline, and it is known that thyroxine depresses hepatic monoamine oxidase activity (Spinks & Burn, 1952). Thyroxine is reported to depress monoamine oxidase and catechol-*o*-methyl transferase activity in certain conditions, but as pointed out by Harrison (1964) these findings have not been confirmed by other workers and it is doubtful if they are physiologically significant. In the present work, thyroxine did not alter the sensitivity of the cardovascular system of the pithed rat to either noradrenaline or synephrine, and since the former but not the latter is inactivated by catechol-*o*-methyl transferase, it is doubtful whether any inhibition of this enzyme which may have been caused by thyroxine is relevant.

The pressor response to (+)-amphetamine is exceptional in that it is greatly potentiated in hyperthyroid rats. Moore (1965) showed that (+)-amphetamine produces marked depletion of endogenous noradrenaline stores. It is known that the cardiovascular action of the drug is due to local release of noradrenaline (Burn

& Rand, 1958). Although evidence for increased storage of endogenous noradrenaline is somewhat equivocal, release of this amine may be facilitated in hyperthyroidism, thereby potentiating the action of (+)-amphetamine. This may well be the case, since the response to exogenous noradrenaline is unaffected by hyperthyroidism.

The ability of thyroxine to increase sensitivity to drugs in the heart and uterus but to inhibit sensitivity to drugs in the intestine and aorta is difficult to explain. In the uterus, sensitivity to drugs is related to the oestrus state, being greatest in the oestrogen-dominated uterus and least in dioestrus. Nicol, Vernon-Roberts & Quantock (1965) have reported that thyroxine potentiates the stimulant effect of oestrogen on the reticuloendothelial system, thyroxine having no effect on its own. It is possible that thyroxine likewise potentiates the action of endogenous oestrogen on the uterus and that the increases in sensitivity to drugs are mediated in this way. Oestrogens are thought to increase the sensitivity of the myometrium to drugs by decreasing the amounts of membrane bound calcium (Coutinho & Csapo, 1958; Knifton, 1966). Thyroxine, like oestrogens, is known to modify calcium metabolism (Adams *et al.*, 1967) and an alternative possibility is that the induced sensitivity changes are mediated by this effect. The results of the experiments described in this paper where the effect of thyroxine on the sensitivity of the uterus and ileum to changes in calcium concentration were determined are consistent with this view. Thus, the results in the two tissues are in marked contrast. In the uterus, thyroxine not only increases the effect of variation in calcium level on the size of acetylcholine-induced contractions, but also increases the rate of loss of electrically-induced tension, following exposure to calcium-free solution. On the ileum, thyroxine has the opposite effects. These changes run parallel to the drug-sensitivity changes induced by thyroxine, and it is possible that they are related. Substantiation of this hypothesis will require a more detailed study, but the main conclusion we wish to draw from the present work is that since the effects of thyroxine are quite non-specific, there does not appear to be, contrary to the widely held view, a specific interaction between thyroxine and sympathomimetic amines.

We thank Dr. T. J. Sullivan for critical review of the manuscript. We also thank Smith, Kline and French for their generous gift of (+)-amphetamine.

REFERENCES

ADAMS, P. H., JOWSEY, J., KELLY, P. J., RIGGS, B. L., KINNEY, V. R. & JONES, J. D. (1967). Effects of hyperthyroidism on bone and mineral metabolism in man. *Q. Jl Med.*, **141**, 1–15.

AOKI, V. S., WILSON, W. R., THEILEN, E. O., LUKENSMEYER, W. W. & LEAVERTON, P. E. (1967). The effects of triiodothyronine on hemodynamic responses to epinephrine and norepinephrine in man. *J. Pharmac. exp. Ther.*, **157**, 62–68.

ARONSON, C. E. & HESS, M. E. (1967). Metabolic effects of bretylium and phentolamine in euthyroid and thyrotoxic rats. *Eur. J. Pharmac.*, **2**, 113–118.

ASHFORD, A. & ROSS, J. W. (1968). Toxicity of depressant and antidepressant drugs in hyperthyroid mice. *Br. med. J.*, **2**, 217–218.

AUMANN, K. W. & YOUMANS, W. B. (1940). Differential sensitization of adrenergic neuroeffector systems by thyroid hormones. *Am. J. Physiol.*, **131**, 394–401.

AXELROD, J. (1959). Metabolism of epinephrine and other sympathomimetic amines. *Physiol. Rev.*, **39**, 751–776.

BARKER, S. B. (1964). Physiological activity of thyroid hormones and analogues. In *The Thyroid Gland*, ed. Pitt-Rivers, R. & Trotter, W. R., vol. 1, pp. 199–236. London: Butterworth and Co. Ltd.

BARKER, S. B. & MAKIUCHI, M. (1965). Effect of guanethidine and bretylium on heart rate responses to thyroxine. *J. Pharmac. exp. Ther.*, **148**, 71–74.

BARTELSTONE, H. J., NASMYTH, P. A. & TELFORD, J. M. (1967). The significance of adenosine cyclic 3',5'-monophosphate for the contraction of smooth muscle. *J. Physiol., Lond.*, **188**, 159–176.

BRODIE, B. B., DAVIES, J. I., HYNIE, S., KRISHNA, G. & WEISS, B. (1966). Interrelationships of catecholamines with other endocrine systems. *Pharmac. Rev.*, **18**, 273–289.

BURN, J. H. & RAND, M. J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol., Lond.*, **144**, 314–336.

CAIROLI, V. J. & CROUT, J. R. (1967). Role of the autonomic nervous system in the resting tachycardia of experimental hyperthyroidism. *J. Pharmac. exp. Ther.*, **158**, 55–65.

COUTINHO, E. M. & CSAPO, A. I. (1958). Calcium, oxytocin and the regulation of the myometrium. *Biol. Bull.*, **115**, 334.

COVILLE, P. F. & TELFORD, J. M. (1968). Influence of thyroid hormones on the responses of smooth muscles to drugs. *Naunyn-Schmeidebergs Arch. exp. Path. Pharmak.*, **259**, 161–162.

COVILLE, P. F. & TELFORD, J. M. (1969). Changes in drug sensitivity in hyperthyroidism. *Br. J. Pharmac.*, **36**, 189*P*.

DANIEL, E. E. (1964). Effect of drugs on contractions of vertebrate smooth muscle. *Ann. Rev. Pharmac.*, **4**, 189–222.

DIAMOND, J. & BRODY, T. M. (1966a). Effect of catecholamines on smooth muscle motility and phosphorylase activity. *J. Pharmac. exp. Ther.*, **152**, 202–211.

DIAMOND, J. & BRODY, T. M. (1966b). Relationship between smooth muscle contraction and phosphorylase activation. *J. Pharmac. exp. Ther.*, **152**, 212–220.

GOODKIND, M. J. (1968). Left ventricular myocardial contractile response to aortic constriction in the hyperthyroid guinea-pig. *Circulation Res.*, **22**, 605–614.

HARRISON, T. S. (1964). Adrenal medullary and thyroid relationships. *Physiol. Rev.*, **44**, 161–185.

KNIFTON, A. (1966). The influence of the female sex hormones on the response of the pig uterus to electrical stimulation. *J. Pharm. Pharmac.*, **18**, Suppl. 151S–159S.

KRANE, S. M., BROWNELL, G. L., STANBURY, J. B. & CORRIGAN, H. (1956). Effect of thyroid disease on calcium metabolism in man. *J. clin. Invest.*, **35**, 874–887.

LANGENDORFF, O. (1895). Untersuchungen am überlebenden Sägetierherzen. *Pflügers Arch. ges. Physiol.*, **61**, 291–332.

LEE, W. Y., BRONSKY, D. & WALDSTEIN, S. S. (1962). Studies of thyroid and sympathetic nervous system interrelationships. II. Effects of guanethidine on manifestations of hyperthyroidism. *J. clin. Endocr. Metab.*, **22**, 879–885.

MACMILLAN, W. H. & RAND, M. J. (1962). The effects in rabbits of thyroidectomy and treatment with triiodothyronine on the sensitivity to noradrenaline, and the content of noradrenaline in the aorta and spleen. *J. Pharm. Pharmac.*, **14**, 257–267.

MANDEL, L. R. & KUEHL, F. A. Jr. (1967). Lipolytic action of l-triodothyronine—a cyclic 3'5'-AMP phosphodiesterase inhibitor. *Fedn Proc.*, **26**, 810.

MARGOLIUS, H. S. & GAFFNEY, T. E. (1965). The effects of injected norepinephrine and sympathetic nerve stimulation in hypothyroid and hyperthyroid dogs. *J. Pharmac. exp. Ther.*, **149**, 329–335.

MOORE, K. E. (1965). Amphetamine toxicity in hyperthyroid mice: effects on endogenous catecholamines. *Biochem. Pharmac.*, **14**, 1831–1837.

MYANT, N. B. & WITNEY, S. (1967). The time course of the effect of thyroid hormones upon basal oxygen consumption and plasma concentration of free fatty acid in rats. *J. Physiol., Lond.*, **190**, 221–228.

NEILSON, R. R., LOIZZI, R. F. & KLITGAARD, H. M. (1961). Metabolic changes in the intact rat and excised tissues after thyroidectomy. *Am. J. Physiol.*, **200**, 55–57.

NICOL, T., VERNON-ROBERTS, B. & QUANTOCK, D. C. (1965). The influence of various hormones on the reticulo-endothelial system; endocrine control of body defence. *J. Endocr.*, **33**, 365–383.

PATON, W. D. M. (1955). The response of the guinea-pig ileum to electrical stimulation by coaxial electrodes. *J. Physiol., Lond.*, **127**, 40–41*P*.

REITH, A. (1865). Exophthalmus—enlargement of thyroid gland—affection of cervical sympathetic. *Medical Times and Gazette*, **2**, 521.

ROBISON, G. A., BUTCHER, R. W. & SUTHERLAND, E. W. (1966). Adenyl cyclase as an adrenergic receptor. *Ann. N.Y. Acad. Sci.*, **139**, 703–723.

SAWYER, H. K. Jnr. & LIPNER, H. J. (1961). Effects of dihydroergotamine in athyroid and thyroxinized rats. *Am. J. Physiol.*, **201**, 264–266.

VAN DER SCHOOT, J. B. & MORAN, N. C. (1965). An experimental evaluation of the reputed influence of thyroxine on the cardiovascular effects of catecholamines. *J. Pharmac. exp. Ther.*, **149**, 336–345.

SHIPLEY, R. E. & TILDEN, J. H. (1947). A pithed rat preparation suitable for assaying pressor substances. *Proc. Soc. exp. Biol. Med.*, **64**, 453–455.

SPINKS, A. & BURN, J. H. (1952). Thyroid activity and the amine oxidase activity of the liver. *Br. J. Pharmac. Chemother.*, **7**, 93–98.

WALDSTEIN, S. S. (1966). Thyroid-catecholamine interrelationships. *Ann. Rev. Med.*, **17**, 123–132.

ZILE, M. H. (1959). Monoamine oxidase activity in liver of thyroid fed rats. *Fedn Proc.*, **18**, 359.

ZILE, M. H. (1960). Effect of thyroxine and related compounds on monoamine oxidase activity. *Endocrinology*, **66**, 311–312.

ZSOTÉR, T., TOM, H. & CHAPPEL, C. (1964). Effect of thyroid hormones on vascular response. *J. Lab. clin. Med.*, **64**, 433–441.

(*Received January* 12, 1970)