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The effects of thyroxine treatment of rats on neuronal and extraneuronal uptake and metabolism of catecholamines in the heart

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1 The effects of thyroxine (T_4) treatment of rats on the neuronal and extraneuronal uptake and subsequent metabolism of catecholamines in the heart were examined, to determine whether changes in the local dissipation of catecholamines might contribute to the enhanced sympathetic cardiac responses that occur when thyroid hormone levels are elevated.

2 T_4-treated rats were injected subcutaneously with L-thyroxine sodium 1 mg kg^{-1} on days 1, 3 and 5, and controls were injected with the normal saline vehicle on the same days. The experiments on isolated, perfused hearts were carried out on day 8. The T_4-treated rats had only 50% of the growth rate of the controls and their heart weights were 18% greater than the controls. The experimental data were adjusted to allow for the increase in heart weight caused by the T_4 treatment.

3 The initial rates of neuronal uptake of noradrenaline and of extraneuronal uptake of noradrenaline and isoprenaline in the hearts from T_4-treated rats were not significantly different from those in hearts from control rats.

4 The steady-state rates of extraneuronal *O*-methylation of isoprenaline and of extraneuronal deamination of noradrenaline in hearts from T_4-treated rats were not significantly different from those in hearts from control rats.

5 The steady-state rate of neuronal deamination of noradrenaline was significantly lower and the accumulation of unmetabolized noradrenaline in the hearts was significantly greater in T_4-treated rats than in the controls. These findings could be explained by a decrease in neuronal monoamine oxidase activity or by an increase in intraneuronal binding of noradrenaline in hearts from T_4-treated rats. If the first of these two speculations should be correct, the changes in neuronal dissipation could contribute to cardiac supersensitivity to noradrenaline or adrenaline, since these amines are substrates for neuronal uptake. However, it could not explain cardiac supersensitivity to isoprenaline in T_4-treated rats.

6 The study has shown that it is unlikely that increased plasma thyroid hormone levels cause cardiac supersensitivity to catecholamines by affecting the local dissipation processes for those amines in the heart.

Introduction

Various attempts have been made to explain the enhanced cardiac responses to catecholamines in hyperthyroid patients and in animals treated with thyroid hormones. In hearts of rats treated with thyroid hormones, there are reports of an increase in the release of neuronal noradrenaline (Nagel-Hiemke *et al.*, 1981), an increase in the number of cardiac β-adrenoceptors (Williams *et al.*, 1977; Stiles & Lefkowitz, 1981), an increase in the affinity of catecholamines for β-adrenoceptors (Stiles & Lefkowitz, 1981), and enhanced coupling of cardiac β-adrenoceptors to adenylate cyclase (Stiles & Lefkowitz, 1981), although the rate of synthesis of noradrenaline is decreased, and not increased (Prange *et al.*, 1970), and the *in vitro* activity of adenylate cyclase is not changed (McNeill *et al.*, 1969). However, the majority of studies have invoked an increase in the number of cardiac β-adrenoceptors as the main reason for the increased inotropic and chronotropic responses to

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catecholamines in hyperthyroidism. Nevertheless, one possibility that has not previously been examined directly is that increased concentrations of catecholamines in the adrenoceptor biophase, and hence the increased cardiac responses, might be due to attenuation by elevated thyroid hormone levels of the normal processes for the local dissipation of catecholamines in the heart. In the present study, the effects of thyroxine (T_4) treatment of rats on the neuronal uptake and subsequent intraneuronal metabolism of noradrenaline by monoamine oxidase (MAO) and on the extraneuronal uptake and subsequent intracellular metabolism of catecholamines by MAO and catechol-*O*-methyltransferase (COMT) have been systematically studied.

Some of the results of this work were presented to the December 1984 meeting of the Australasian Society of Clinical and Experimental Pharmacologists (Williams *et al.*, 1985).

Methods

Male, albino, Wistar rats (170–212 g, 184 ± 1.5 g, $n = 38$) were injected subcutaneously with L-thyroxine sodium (T_4) 1 mg kg^{-1} on days 1, 3 and 5, and the experiments were carried out on day 8. Age- and weight-matched controls (170–212 g, 184 ± 1.7 g, $n = 33$) were injected with 1 ml kg^{-1} of the normal saline vehicle (154 mM NaCl in water) on the same days. This T_4 treatment regime has been shown to cause an increase in serum levels of the thyroid hormones, T_4 and triiodothyronine (T_3) by 4.1 fold and 3.7 fold, respectively (Mustafa *et al.*, 1985). The growth rate of the T_4-treated rats (2.3 g day^{-1}, $n = 38$) during the one week treatment regime was 50% of that of controls (4.6 g day^{-1}, $n = 33$). Despite this decrease in growth rate, the heart wet weights on the day of the experiment were 18% greater in the T_4-treated rats (0.93 ± 0.014 g, $n = 38$) than in the controls (0.79 ± 0.013 g, $n = 33$).

In some series of experiments, the rats were also injected with reserpine or pargyline (see Table 1). In all experiments, the rats were injected intraperitoneally with 5000 u kg^{-1} heparin, 20 min before they were killed by cervical dislocation and bleeding. The hearts were perfused by the Langendorff technique with Tyrode solution at 36.5°C. The Tyrode solution contained (in mM): Na^+ 149.2, K^+ 2.7, Ca^{2+} 1.3, Mg^{2+} 1.05, Cl^- 144.3, $H_2PO_4^-$ 0.4, HCO_3^- 11.9, glucose 5.0, (−)-ascorbic acid 0.3 and Na_2EDTA 0.04, and was aerated with 95% O_2 and 5% CO_2. The perfusion pressure ranged from 35–55 mmHg. A constant flow rate of about 10 ml g^{-1} min^{-1} (range: 9–11 ml g^{-1} min^{-1}) was used for the controls, and the same absolute rate (in ml min^{-1}) was used for each control and the matched T_4-treated rat. Hence, the flow rate expressed in ml g^{-1} min^{-1} was lower for hearts from T_4-treated rats (range 7.6–9.6 ml g^{-1} min^{-1}) than for hearts from controls, due to the increase in heart weight in the T_4-treated rats. The hearts were initially perfused for 20 min with Tyrode solution with other drugs present where appropriate to achieve optimal conditions for the particular series of experiments (Table 1). The hearts were then perfused with [^{3}H]-noradrenaline or [^{3}H]-isoprenaline at the concentration and for the time shown in Table 1, and with other drugs present as shown in Table 1. In all experiments except those in which extraneuronal uptake was measured, samples of venous effluent (collected from a hole cut in the pulmonary artery close to the right ventricle) were collected at appropriate intervals (see Results) during the perfusion with the amine.

At the end of each experiment, the heart was removed from the cannula, blotted, weighed and placed in 0.4 M perchloric acid containing antioxidants (2.7 mM Na_2EDTA and 10 mM Na_2SO_3 in all experiments, plus 5.7 mM ascorbic acid in experiments with isoprenaline) at 4–6°C. After at least 1 h, the hearts were homogenized and then centrifuged at 10000 *g* for 10 min. Also, a sample of perfusion solution was collected immediately after removal of the heart from the cannula (arterial sample).

Samples of venous effluent, arterial solution and the supernatant from the heart homogenate were taken for liquid scintillation counting (^{3}H and, where appropriate, ^{14}C) and, except in uptake experiments, for column chromatographic separation of noradrenaline and its metabolites (Fiebig & Trendelenburg, 1978; Trendelenburg *et al.*, 1983) or of isoprenaline and its metabolite, 3-*O*-methylisoprenaline (OMI) (Bönisch, 1978; Bryan *et al.*, 1983). In experiments on neuronal and extraneuronal deamination, preliminary results showed that no detectable amounts of *O*-methylated metabolites of noradrenaline appeared in the venous effluent or in the heart, so the fractions containing *O*-methylated metabolites in the separation procedure were not collected in subsequent experiments. In some experiments on neuronal uptake of noradrenaline, the hearts were perfused with [^{3}H]-noradrenaline for an additional 15 s, during which a sample of venous effluent was collected. There were no detectable amounts of metabolites of noradrenaline in these venous effluent samples.

Drugs and solutions

The drugs used were: cocaine hydrochloride (Drug Houses of Australia, Sydney, Australia); corticosterone (Sigma Chemical Company, St Louis, MO, U.S.A.); 3′,4′-dihydroxy-2-methylpropiophenone (U-0521; Upjohn, Kalamazoo, MI, U.S.A.); heparin sodium (as ampoules of 5000 u ml^{-1}; Weddel Phar-

Table 1 Experimental design for each series of experiments in the study

	Process studied in each series of experiments		
A *Uptake experiments*	*Neuronal uptake*	*Extraneuronal uptake*	*Extraneuronal uptake*
Amine	Noradrenaline	Isoprenaline	Noradrenaline
Amine conc (μM)	0.01	1.0	1.0
Perfusion time with amine (min)	2.0	2.0	2.0
Reserpine[a]	*		*
Pargyline[b]	*		*
[^{14}C]-sorbitol[c]	*	*	*
Corticosterone[d]	*		
Cocaine[e]			*
U-0521[f]	*		
Tropolone[g]		*	*
B *Metabolism experiments*	*Neuronal deamination*	*Extraneuronal O-methylation*	*Extraneuronal deamination*
Amine	Noradrenaline	Isoprenaline	Noradrenaline
Amine conc (μM)	0.01	0.01	0.01
Perfusion time with amine (min)	30.5	15.5	30.5
Reserpine[a]	*		*
Corticosterone[d]	*		
Cocaine[e]			*
U-0521[f]	*		*

*Drug included in the series of experiments.
[a]Rats were injected intraperitoneally with reserpine 1 mg kg^{-1}, 24 h prior to the experiment, to deplete endogenous noradrenaline and to prevent uptake of exogenous noradrenaline into synaptic vesicles in adrenergic neurones.
[b]Rats were injected intraperitoneally with pargyline 75 mg kg^{-1}, 18 h and 2 h prior to the experiment, to inhibit MAO.
[c][^{14}C]-sorbitol (100 μM) was present during the perfusion with amine so that the extracellular space of the heart could be determined.
[d]Corticosterone (100 μM) was present in the perfusion solution throughout the experiment to inhibit extraneuronal uptake.
[e]Cocaine (30 μM) was present from the 10th min of the initial perfusion without amine and throughout the perfusion with amine, to inhibit neuronal uptake.
[f]U-0521 (10 μM) was present in the perfusion solution throughout the experiment to inhibit COMT.
[g]Tropolone (100 μM) was present in the perfusion solution throughout the experiment to inhibit COMT.

maceuticals Ltd, London, England); (±)-isoprenaline sulphate (Sigma); [^{3}H]-(±)-isoprenaline hydrochloride (Amersham International, Amersham, U.K.; approximately 500 Bq pmol^{-1}, purified over alumina before use and diluted with unlabelled isoprenaline to the desired specific activity; the final concentration of labelled isoprenaline was approximately 5 nM in uptake experiments and approximately 2 nM in *O*-methylation experiments); (−)-noradrenaline bitartrate (Sigma); (−)-[7-^{3}H]-noradrenaline (New England Nuclear, Boston, MA, U.S.A.; approximately 650 Bq pmol^{-1}, purified over alumina before use and diluted with unlabelled noradrenaline to the desired specific activity; the final concentration of labelled noradrenaline was approximately 3 nM in extraneuronal deamination experiments and approximately 0.8 nM in other experiments); pargyline hydrochloride (Sigma; administered as a 92.5 mg ml^{-1} solution in normal saline); reserpine (as Serpasil ampoules; 1 mg ml^{-1}; Ciba-Geigy Australia Ltd, Sydney, Australia); D-sorbitol (Sigma); D-[^{14}C]-sorbitol (Amersham; 12 Bq pmol^{-1}, diluted with unlabelled sorbitol to the desired specific activity; the final concentration of labelled sorbitol was 30–60 nM); L-thyroxine sodium (Sigma; administered as a suspension of 1 mg ml^{-1} in normal saline); tropolone (Aldrich Chemical Company, Milwaukee, WI, U.S.A.).

Stock solutions of isoprenaline and noradrenaline (10 mM) were prepared in 10 mM HCl. Corticosterone was dissolved in dimethylsulphoxide (DMSO) and diluted with Tyrode solution such that the final concentration of DMSO was 4.6 mM. Other drugs and

all dilutions were prepared in Tyrode solution.

Calculation of results

Initial rates of neuronal uptake of noradrenaline were determined as the slopes of plots of the cumulative removal of noradrenaline from the perfusion fluid versus perfusion time (Graefe *et al.*, 1978). The cumulative removal of noradrenaline was corrected for the amine in the extracellular space (calculated from the [^{14}C]-sorbitol concentrations in the venous effluent and arterial solutions).

Initial rates of extraneuronal uptake were calculated as the amine content of the heart, corrected for the amine in the extracellular space (calculated from the [^{14}C]-sorbitol content of the heart), divided by the perfusion time (2 min). The perfusion time was not corrected for the time to clear the dead volume of the apparatus (270 µl; cleared in approximately 1.5 s).

In experiments on deamination of noradrenaline, rates of dihydroxyphenylglycol (DOPEG) and dihydroxymandelic acid (DOMA) appearance in the venous effluent and the DOPEG, DOMA and noradrenaline contents of the heart were calculated as described by Fiebig & Trendelenburg (1978). In experiments on *O*-methylation of isoprenaline, rates of OMI appearance in the venous effluent and the OMI and isoprenaline contents of the heart were calculated as described by Bönisch (1978). The rate constants for efflux of DOPEG and OMI were calculated by dividing the rate of DOPEG or OMI appearance in the venous effluent just prior to the end of the experiment by the DOPEG or OMI content of the heart.

For each heart, the initial rate of uptake, the rate of metabolite appearance in the venous effluent or the amine or metabolite content of the heart, each expressed per g of heart weight, was divided by a factor comprising the ratio of the mean heart weight of T_4-treated rats to the mean heart weight of corresponding controls (using mean heart weights from the particular group of rats used for that series of experiments). This was necessary to allow for the increase in heart weight in the T_4-treated rats, compared with the controls (see Discussion).

Rate constants for efflux for DOPEG and OMI are expressed as geometric means with 95% confidence limits. All other results are expressed as arithmetic means ± s.e. The significance of differences between means was assessed by Student's *t* test.

Results

Effects of T_4 treatment of rats on neuronal uptake and metabolism in the heart

The effects of T_4 treatment of rats on neuronal uptake were examined in hearts perfused with noradrenaline. The initial rate of neuronal uptake of noradrenaline was significantly lower in T_4-treated rats than in the controls, before, but not after, the data were adjusted for the T_4-induced increase in heart weight (Table 2A). Hence, the neuronal uptake of noradrenaline in the

Table 2 Effects of T_4 treatment of rats on (A) neuronal uptake of noradrenaline and (B) neuronal deamination of noradrenaline in rat perfused hearts

	(A) *Initial rate of neuronal uptake of noradrenaline* (pmol g^{-1} min^{-1})	(B) *Steady-state rate of neuronal DOPEG formation*[a] (pmol g^{-1} min^{-1})	*Noradrenaline content of heart* (pmol g^{-1})
Controls	17.40 ± 0.69 (n = 4)	12.31 ± 0.91 (n = 4)	116.8 ± 9.7 (n = 4)
T_4-treated	13.61 ± 0.32** (n = 4)	7.81 ± 0.82** (n = 5)	186.4 ± 7.6*** (n = 5)
T_4-treated data after adjustment for heart weight increase[b]	15.89 ± 0.37 (n = 4)	8.65 ± 0.91* (n = 5)	206.6 ± 8.5*** (n = 5)

Hearts were perfused with [^{3}H]-noradrenaline 0.01 µM for 2 min in (A) and for 30.5 min in (B). In all experiments, rats were pretreated with reserpine, and COMT and extraneuronal uptake were inhibited; in (A), MAO was also inhibited (see Methods for details). Data are mean results from n hearts for each group of rats.

[a]Steady-state rates of DOPEG formation were calculated as the mean of the rates of DOPEG appearance in the venous effluent at the 28th and 30th min of perfusion.

[b]Details of the method of adjustment of data to allow for the heart weight increase due to the T_4 treatment are in Methods.

Significantly different from corresponding values for controls: *$0.05 > P > 0.01$; **$0.01 > P > 0.001$; ***$P < 0.001$.

whole heart appeared to be unaffected by the T_4 treatment.

The effects of T_4 treatment of rats on the neuronal deamination of noradrenaline were determined by obtaining the rate of appearance in the venous effluent of DOPEG only, since there were no detectable amounts of DOMA (the other deaminated metabolite of noradrenaline) in the effluent and only very small amounts of DOMA in the heart at the end of the experiment. The rates of DOPEG appearance in the venous effluent were lower in T_4-treated rats than in controls, even after adjustment for the T_4-induced increase in heart weight (Figure 1). Hence, the steady-state rate of neuronal DOPEG formation was significantly lower in the T_4-treated rats, than in the controls (Table 2B). Also, the rate constant for efflux of DOPEG was lower in T_4-treated rats ($1.02\,min^{-1}$, 95% confidence limits: 1.00 and $1.04\,min^{-1}$; $n = 5$) than in the controls ($1.29\,min^{-1}$, 95% confidence limits: 1.22 and $1.36\,min^{-1}$; $n = 4$; $t = 12.8$, d.f. 7, $P < 0.001$). This reduction in the rate constant for DOPEG efflux may explain the observation that the rates of DOPEG appearance in the venous effluent appeared to approach steady state more slowly for the T_4-treated rats than for the controls (Figure 1). The noradrenaline content of the heart at the end of the experiment was significantly greater for T_4-treated rats than for controls (Table 2B).

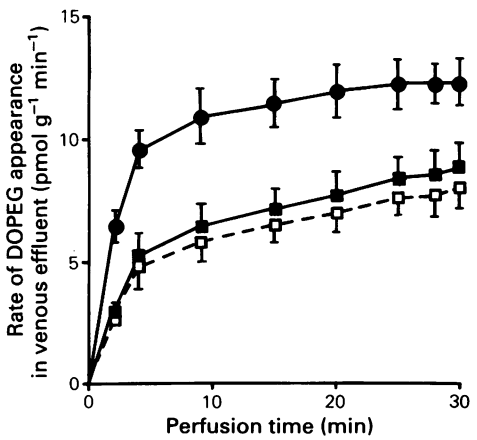

Figure 1 Effects of T_4 treatment of rats on neuronal deamination of noradrenaline in rat perfused hearts. The hearts were perfused with [^{3}H]-noradrenaline 0.01 μM. Means of rates of DOPEG appearance in the venous effluent (pmol g^{-1} min^{-1}) are plotted against perfusion time (min) for controls (●, $n = 4$) and for T_4-treated rats ($n = 5$), before (□) and after (■) adjustment of the results to allow for heart weight increase due to the T_4 treatment (see Methods for details): s.e. means shown by vertical lines.

Effects of T_4 treatment of rats on extraneuronal uptake and metabolism in the heart

The effects of T_4 treatment of rats on extraneuronal uptake were examined in hearts perfused with isoprenaline or noradrenaline. Initial rates of extraneuronal uptake of both amines were significantly lower in T_4-treated rats than in the corresponding controls before, but not after, adjustment for the T_4-induced increase in heart weight (Table 3). In other words, the apparent decrease in rates of extraneuronal uptake (expressed per g heart weight) in the T_4-treated rats was due to the increase in heart weight, and the extraneuronal uptake in the whole heart appeared to be unaffected by the T_4 treatment.

The effects of T_4 treatment on extraneuronal *O*-methylation by COMT were examined in hearts perfused with isoprenaline. The steady-state rate of OMI formation was lower in hearts from T_4-treated rats than from controls, but again there was no difference after adjustment for the T_4-induced increase in heart weight (Table 4A). There was also no significant difference in the rate constants for efflux of OMI between the hearts from the T_4-treated rats ($0.661\,min^{-1}$; 95% confidence limits: 0.618 and

Table 3 Effects of T_4 treatment of rats on extraneuronal uptake of isoprenaline and noradrenaline in rat perfused hearts

	Initial rate of extraneuronal uptake (pmol g^{-1} min^{-1})	
	Isoprenaline[a]	*Noradrenaline*[a]
Controls	412 ± 16 ($n = 5$)	253 ± 17 ($n = 8$)
T_4-treated	326 ± 29** ($n = 9$)	199 ± 13* ($n = 8$)
T_4-treated data after adjustment for heart weight increase[b]	409 ± 37 ($n = 9$)	242 ± 16 ($n = 8$)

Hearts were perfused with [^{3}H]-isoprenaline 1 μM or [^{3}H]-noradrenaline 1 μM for 2 min. COMT was inhibited in all experiments. In experiments with noradrenaline, rats were pretreated with reserpine, and MAO and neuronal uptake were inhibited (see Methods for details). Data are mean results from *n* hearts for each group of rats.

[a]It should be noted that the isoprenaline or noradrenaline concentration (1 μM) used in these experiments was greater than that used (0.01 μM) in all other experiments in the study.

[b]Details of the method of adjustment of data to allow for the heart weight increase due to the T_4 treatment are in Methods.

Significantly different from value for corresponding controls: *$0.05 > P > 0.01$; **$0.01 > P > 0.001$.

$0.707\,min^{-1}$; $n = 6$) and the controls ($0.614\,min^{-1}$; 95% confidence limits: 0.563 and $0.671\,min^{-1}$; $n = 6$).

The effects of T_4 treatment on extraneuronal deamination by MAO were examined in hearts perfused with noradrenaline. The steady-state rate of DOPEG formation in hearts from T_4-treated rats was not significantly different from that in hearts from controls, whether or not the results were adjusted for the T_4-induced increase in heart weight (Table 4B). There was also no significant difference in the rate constants for efflux of DOPEG between the hearts from T_4-treated rats ($0.235\,min^{-1}$; 95% confidence limits: 0.180 and $0.307\,min^{-1}$; $n = 6$) and controls ($0.210\,min^{-1}$; 95% confidence limits: 0.181 and $0.245\,min^{-1}$; $n = 6$). The steady-state rate of DOPEG formation slightly underestimates the total rate of extraneuronal deamination of noradrenaline in the hearts, since a small amount of DOMA was also formed. The DOMA did not appear in the venous effluent samples in detectable quantities, but there were detectable amounts in the hearts at the end of the experiment. The DOMA contents of the hearts from T_4-treated rats, whether corrected for the T_4-induced increase in heart weight ($8.09 \pm 1.33\,pmol\,g^{-1}$, $n = 6$) or not ($7.19 \pm 1.18\,pmol\,g^{-1}$, $n = 6$) were not significantly different from that of hearts from controls ($9.14 \pm 1.73\,pmol\,g^{-1}$, $n = 6$). Hence, the extraneuronal deamination of noradrenaline in the hearts appeared to be unaffected by the T_4 treatment of the rats.

Table 4 Effects of T_4 treatment of rats on (A) extraneuronal *O*-methylation of isoprenaline, and (B) extraneuronal deamination of noradrenaline in rat perfused hearts

	(A) *Steady-state rate of OMI formation*[a] ($pmol\,g^{-1}\,min^{-1}$)	(B) *Steady-state rate of extraneuronal DOPEG formation*[b] ($pmol\,g^{-1}\,min^{-1}$)
Controls	6.34 ± 0.34	1.42 ± 0.09
T_4-treated	4.82 ± 0.34*	1.33 ± 0.12
T_4-treated data after adjustment for heart weight increase[c]	5.65 ± 0.39	1.50 ± 0.14

Hearts were perfused with [^{3}H]-isoprenaline 0.01 μM for 15.5 min (A) or with [^{3}H]-noradrenaline 0.01 μM for 30.5 min (B). In experiments in (B), rats were pretreated with reserpine, and COMT and neuronal uptake were inhibited (see Methods for details). Data are mean results from 6 hearts for each group of rats.

[a]Steady-state rates of OMI formation were calculated as the mean of the rates of OMI appearance in the venous effluent at the 11th, 13th and 15th min of perfusion.

[b]Steady-state rates of DOPEG formation were calculated as the mean of the rates of DOPEG appearance in the venous effluent at the 28th and 30th min of perfusion.

[c]Details of the method of adjustment of data to allow for the heart weight increase due to the T_4 treatment are in Methods.

*Significantly different from value for corresponding controls, $0.05 > P > 0.01$.

Discussion

An increase in the concentration of catecholamines in the adrenoceptor biophase of the heart could occur in animals treated with thyroid hormones if the normal processes for the local dissipation of catecholamines were inhibited. This could occur (a) if there were inhibition of neuronal uptake and/or extraneuronal uptake, since there would be a decrease in the removal of catecholamines from the coronary circulation, or (b) if there were inhibition of the subsequent metabolism of the amine, provided that this inhibition was accompanied by an increase in the efflux of unchanged amine into the adrenoceptor biophase from either the adrenergic neurones (Graefe *et al.*, 1971) or the extraneuronal cells (Trendelenburg, 1980). In the present study, we have examined the above possibilities by measuring the initial rates of neuronal and extraneuronal uptake of catecholamines and, for metabolism, the steady-state rates of appearance of the relevant metabolite in the venous effluent in isolated perfused hearts from T_4-treated and control rats.

There was no difference in either neuronal uptake (of noradrenaline) or extraneuronal uptake (of noradrenaline or isoprenaline) between hearts from T_4-treated and control rats. Thus, increased thyroid hormone levels do not seem to affect the uptake of catecholamines in the heart. This confirms the conclusions of McNeill & Brody (1968) and Tu & Nash (1975), which were based on very indirect evidence.

In the hearts from T_4-treated rats, there was a reduction in the neuronal deamination of noradrenaline, accompanied by an increase in the accumulation of unchanged noradrenaline. There was also a decrease in the rate constant for the neuronal efflux of the catechol metabolite, DOPEG. These results could indicate either that the activity of MAO had decreased or that the intraneuronal binding of noradrenaline, and possibly DOPEG, had increased. Since the rats in these experiments had been treated with reserpine, any increase in binding is unlikely to have been in synaptic vesicles. Also, even if increased intraneuronal binding of noradrenaline did occur, it would not result in an increase in the amine concentration in the adrenoceptor biophase, since there would be no increase in the

efflux of noradrenaline. The first possibility, i.e. decreased neuronal MAO activity, could explain supersensitivity of the cardiac responses to noradrenaline and adrenaline, but not to isoprenaline, since isoprenaline is not a substrate for neuronal uptake. There are no reports in the literature on the effects of elevated thyroid hormone levels on MAO activity in adrenergic neurones, although thyroid hormone treatment of rabbits has been shown to reduce the activity of MAO in the liver (Spinks & Burn, 1952).

There was no difference in either extraneuronal *O*-methylation or extraneuronal deamination between hearts from T_4-treated and control rats. For the necessary increase in the efflux of catecholamines into the adrenoceptor biophase to have occurred, inhibition of COMT would have been more important than inhibition of MAO. This is because inhibition of COMT has been shown to result in an increase in the accumulation of noradrenaline in extraneuronal cells in rat perfused hearts, whereas inhibition of MAO results in a compensatory increase in the metabolism of noradrenaline by COMT, and this prevents any increase in the efflux of noradrenaline from the cells (Trendelenburg, 1984). The lack of effect of elevated thyroid hormone levels on extraneuronal *O*-methylation supports the results reported for COMT activity in heart homogenates (Wurtman *et al.*, 1963). In contrast, the lack of effect of elevated thyroid hormone levels on extraneuronal deamination is not in agreement with reports of increased MAO activity in heart homogenates from T_4-treated rats (Lyles & Callingham, 1974; 1979).

The T_4 treatment regime used for the rats in the present study was selected because other experiments in our laboratory have shown that (a) it caused an increase in serum levels of both T_4 and T_3 (Mustafa *et al.*, 1985), and (b) it increased the β_1-adrenoceptor-mediated relaxation of isolated pulmonary artery preparations, as did a three-day treatment regime with T_3 (O'Donnell *et al.*, 1985 and personal communication). It has also been shown that the responses of isolated atria to catecholamines were enhanced in rats treated with T_3 for either three days (Rub *et al.*, 1981) or eight days (Gross & Lues, 1985). Thus, it is likely that the T_4 treatment regime used in the present study would also have increased the cardiac responses of the rats, although experiments were not carried out to examine this aspect specifically. Another reason for selecting a relatively short T_4 treatment regime was to minimize cardiac hypertrophy. When hypertrophy occurs, the myocardium is more susceptible to hypoxia (Taylor, 1970), and myocardial contractility and relaxation are impaired (Palacios *et al.*, 1979), so that isolated perfused heart preparations would be less viable. However, some cardiac hypertrophy has been shown to occur as early as three days after starting T_4 treatment (Sanford *et al.*, 1978). Thus, the 18% increase in heart weight observed in the T_4-treated rats in the present study was not unexpected.

The increase in heart weight in the T_4-treated rats was due to an increase in the size of existing myocardial cells (i.e. cardiac hypertrophy), and not to an increase in the number of myocardial cells (i.e. cardiac hyperplasia), in that there was no change in the DNA content of the heart (Rabinowitz & Zak, 1972). The increase in heart weight was taken into account in expressing the data, by including a factor of the ratio of heart weights of the T_4-treated and control rats (see Methods). This was necessary to avoid artefactual effects of the T_4 treatment on the rates of uptake and metabolism, since these rates are conventionally expressed per g of heart weight (Fiebig & Trendelenburg, 1978; Bönisch, 1978; Bryan *et al.*, 1983).

In conclusion, the thyroid hormone regime used in the present study had no effect on the neuronal uptake of catecholamines in the rat heart. It also had no effect on either extraneuronal uptake or the subsequent extraneuronal metabolism of catecholamines by MAO or COMT. The only apparent effect of the T_4 treatment was to reduce the deamination of noradrenaline, subsequent to neuronal uptake. This could contribute to enhanced cardiac responses to noradrenaline and adrenaline, if it should reflect a decrease in the activity of neuronal MAO, rather than an increase in binding of the amine within the neurone. However, this effect of T_4 treatment could not contribute to enhanced cardiac responses to isoprenaline, since this amine is not a substrate for neuronal uptake. Thus, it is unlikely that increased plasma thyroid hormone levels cause cardiac supersensitivity to catecholamines by an effect on the local dissipation processes for these amines in the heart.

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