Biochemical Pharmacology, Vol. 32, No. 20, pp. 3115–3117, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

SHORT COMMUNICATIONS

Sex- and substrate-dependent effects of the thyroid gland on drug metabolism in the rat*

(*Received* 10 *October* 1982; *accepted* 27 *January* 1983)

The existence of a thyroid effect on hepatic drug metabolism has been known for some time. Kato and co-workers in a series of publications between 1965 and 1970 [1—5] showed apparent sex- and substrate-differences in the effect of L-thyroxine. Enzyme activities that were androgen-dependent were decreased by L-thyroxine treatment whereas other enzyme activities were increased in the male. All enzyme activities were increased in the female [1]. The significance of these results, however, is open to discussion as the investigators used supraphysiological doses of L-thyroxine (1–1.5 mg/kg per day) and these can lead to a thyrotoxic state in the animals. Rumbaugh *et al.* [6] repeated the procedures of Kato *et al.* using smaller doses of L-thyroxine (50–500 μg/kg per day) and found decreases in all enzyme acitivies following thyroidectomy in both the male and female animals (although smaller decreases in the female). All these effects were reversed by low dose (50 μg/kg per day) L-thyroxine treatment. In some cases higher doses of L-thyroxine had less effect than the lower doses indicating that, indeed, the thyrotoxic state may have different effects on drug metabolism from the euthyroid state.

The existence of sex differences in the effect of thyroidectomy is also substantiated by the findings of Lax *et al.* [7] using steroid substrates. 3β- and 17β-hydroxysteroid dehydrogenase activities were decreased in the male and 5α-reductase activity was decreased and 5β-reductase activity increased in the female. For the 11β-hydroxysteroid dehydrogenase the activity in the male was decreased while that in the female was increased.

Evidence, thus, points to a sex-related difference in the effect of thyroid hormones on hepatic drug metabolism but some data indicate that this may be related to the thyrotoxic state rather than the euthyroid state. It was decided, therefore, to test the euthyroid/thyrotoxic hypothesis using different substrates known to exhibit sex differences in their hepatic metabolism.

Materials and methods

Animals. Control, sham-operated and thyroidectomized animals (~200 g) were obtained from Charles River Ltd. The animals were allowed 3 days to settle in the animal house before beginning any treatment. The animals were allowed free access to food and water, and were kept (5 animals/cage) in a light- and temperature-controlled environment (lights on 07.00 to 20.00; temperature 19 ± 1°. L-thyroxine was administered once a day i.p. at a dose of 50 μg/kg (low dose) or 500 μg/kg (high dose) as a solution in 0.9% sodium chloride solution. The low dose was administered to thyroidectomized animals to make them euthyroid—this was checked by measuring serum L-thyroxine levels by radioimmunoassay using an Amerlex T_4-kit—and the high dose was given to intact animals to render them thyrotoxic.

* This work was presented in part at the joint Meeting of the Scandinavian and British Pharmacological Societies, Stockholm, Sweden, 5–6 July 1982.

Materials. Cofactors and L-thyroxine sodium salt were obtained from Sigma Chemical Co. Ltd., U.K. and the L-thyroxine test kit, [^{14}C]imipramine and [^{14}C]diazepam were purchased from Amersham International Ltd., U.K. [^{14}C]Lidocaine was obtained from New England Nuclear Ltd., U.K. Authentic standards for the assays were generously given by Ciba–Geigy AG, Basel, Switzerland (imipramine); Astra Läkemedel AB, Sodertälje, Sweden (lidocaine); Roche Products Ltd., Welwyn Garden City and Wyeth Products Ltd., Maidenhead, U.K. (diazepam).

Enzyme analyses. Animals were killed by CO_2-asphyxiation and blood was collected by heart puncture. The liver was rapidly removed and put in ice-cold 0.25 M sucrose solution. A 20% homogenate was made using a Potter–Elvehjem homogeniser and microsomes prepared by the method of Cinti *et al.* [8]. The microsomal drug-metabolizing capacity was tested using lidocaine, imipramine and diazepam as described previously [9, 10]. Protein content was assayed by the method of Lowry [11] using bovine serum albumin as standard.

Statistics. Four to eight animals were used in each group and results expressed as % of the relevant control. Means and standard deviations were calculated and statistical analysis was performed by means of Student's *t*-test or, where appropriate, Duncan's multiple range test. The level of significance was set at $P < 0.05$ in all cases.

Results and discussion

Measurement of the metabolites formed allow the calculation of the following enzyme activities: 3-hydroxylase and *N*-deethylase (for lidocaine); *N*-oxidase, 2-hydroxylase and *N*-demethylase (for imipramine); 3-hydroxylase and *N*-demethylase (for diazepam).

Effect of thyroidectomy and replacement therapy. The results are shown in Table 1. Thyroidectomy in the male caused an increase in lidocaine 3-hydroxylase and *N*-deethylase without affecting any other parameter significantly. Treatment of male thyroidectomized animals with physiological doses of L-thyroxine [L-thyroxine levels in serum were (μg/100 ml): control 5.35 ± 0.95; thyroidectomised 1.40 ± 0.08; L-thyroxine-treated 5.26 ± 0.82] reversed the effects of thyroidectomy on lidocaine metabolism but potentiated the effect of thyroidectomy on cytochrome P-450 content (giving a significant increase) and imipramine N-oxidase (giving a significant decrease). L-Thyroxine treatment also increased diazepam metabolism significantly.

In the female, thyroidectomy significantly decreased cytochrome P-450 content and increased lidocaine 3-hydroxylase, imipramine *N*-oxidase and *N*-demethylase activities. Replacement therapy with L-thyroxine to physiological levels [serum levels (μg/100 ml): control 5.72 ± 0.88; thyroidectomized 1.40 ± 0.33; L-thyroxine-treated 6.12 ± 2.19] reversed the effect of thyroidectomy on lidocaine metabolism but did not significantly affect any other parameter.

These results indicate that, even at physiological levels, L-thyroxine can have both a sex- and substrate-dependent effect. This contrasts with the results of Rumbaugh *et al.*

Table 1. Effect of thyroidectomy and thyroxine-replacement therapy on hepatic drug metabolism

Group	Cytochrome P-450	Lidocaine 3-OHase	Lidocaine *N*-Demethylase	Lidocaine *N*-Oxidase	Imipramine *N*-Demethylase	Imipramine 2-OHase	Diazepam 3-OHase	Diazepam *N*-Demethylase
Control ♂ (A,8)*	100 ± 64†‡	100 ± 29	100 ± 34	100 ± 62	100 ± 18	100 ± 14	100 ± 51	100 ± 46
TX ♂ (B,4)	147 ± 46	202 ± 80	257 ± 71	60 ± 26	88 ± 15	143 ± 15	95 ± 41	94 ± 36
TX + T4 ♂ (C,4)	280 ± 108	138 ± 44	161 ± 20	50 ± 14	84 ± 20	142 ± 29	175 ± 28	162 ± 17
Sign.§	CBA	BCA	BCA	CBA	CBA	ACB	BAC	BAC
Control ♀ (D,8)	100 ± 20‡	100 ± 33	100 ± 43	100 ± 31	100 ± 26	100 ± 33	100 ± 37	100 ± 33
TX ♀ (E,4)	78 ± 13	150 ± 4	171 ± 50	246 ± 23	153 ± 28	133 ± 33	135 ± 43	137 ± 41
TX + T4 ♀ (F,4)	57 ± 16	100 ± 25	131 ± 12	418 ± 309	173 ± 40	140 ± 33	130 ± 14	146 ± 12
Sign.§	FED	DFE	DFE	DEF	DEF	DEF	DFE	DEF

* Group letter, No. of animals in group,

† % of control ± 1 S.D.

‡ Control levels in male are: 0.31 nmole/mg protein (cytochrome P-450), 0.04 (3OH), 0.29 (*N*-deethylase), 0.09 (*N*-oxidase), 0.20 (*N*-demethylase), 0.05 (2OH) all nmole/min per mg protein, 17.3 (3OH), 19.6 (*N*-demethylase) both pmole/min per mg protein. Control levels in female are: 0.22 nmole/mg protein (cytochrome P-450), 0.03 (3OH), 0.06 (*N*-deethylase), 0.05 (*N*-oxidase), 0.08 (*N*-demethylase), 0.05 (2OH) all nmole/min per mg protein, 3.7 (3OH), 3.5 (*N*-demethylase) both pmole/min per mg protein.

§ Means arranged in rank order, those underlined are not significantly different ($P > 0.05$) according to Duncan's multiple range test.

Table 2. Effect of high dose (500 μg/kg) L-thyroxine on hepatic drug metabolism

	Cytochrome P-450	Lidocaine		Imipramine		
		N-Deethylase	3-Hydroxylase	*N*-Oxidase	*N*-Demethylase	2-Hydroxylase
♂ Control (5)†	100 ± ‡	100 ± 21	100 ± 33	100 ± 22	100 ± 34	100 ± 24
♂ T4-Treated (5)	45 ± 18**§	63 ± 7*§	68 ± 22	84 ± 22	95 ± 22	97 ± 20
♀ Control (5)	100 ± 28	100 ± 24	100 ± 30	100 ± 21	100 ± 7	100 ± 5
♀ T4-Treated (5)	72 ± 23	75 ± 7	66 ± 16	170 ± 123	113 ± 28	105 ± 25

† No. of animals in each group.

‡ Values expressed as % of control. Control values are: ♂ 0.25 (*N*-deethylase), 0.06 (3OH), 0.11 (*N*-oxidase), 0.09 (*N*-demethylase), 0.08 (2OH) all nmole/min per mg protein. ♀:0.06 (*N*-deethylase), 0.06 (3OH), 0.04 (*N*-oxidase), 0.10 (*N*-demethylase), 0.08 (2OH) all nmole/min per mg protein.

§ Value significantly different from control values; * = $P < 0.05$, ** = $P < 0.01$.

[6] who only found sex-dependent effects at supraphysiological levels of L-thyroxine which could be due to the substrates used in that study not being appropriate to the problem under investigation due to the substrate-dependence of the effects.

Effect of thyrotoxic doses of L-*thyroxine.* The results are shown in Table 2. There are very few effects of hyperthyroidism and the effects are, with one exception, those that would be expected from the previous experiment: decrease in metabolism of lidocaine in both sexes and increase in imipramine *N*-oxidase activity in the female. The unexpected finding is the sharp decrease in cytochrome P-450 content seen in the male. This is the only effect which could possibly be explained in terms of a thyrotoxic action of excess L-thyroxine but in this case the sex-dependent effect of physiological doses of L-thyroxine is lost in the hypothyroid state.

In conclusion, these results substantiate the findings of Kato *et al.* [1] that the effects of the thyroid gland on drug metabolism are both sex- and substrate-dependent even at physiological levels of L-thyroxine and that, contrary to the results of Rumbaugh *et al.* [6], excess L-thyroxine leads to a disappearance of the sex-dependent effects with the substrates used in this study. The hypothesis that the sex-dependent effects of L-thyroxine are due to the induction of a hyperthyroid state can thus be discounted.

* To whom correspondence should be addressed.

† Present address: Department of Veterinary Pharmacology, Veterinary School, Bearsden Road, Bearsden, Glasgow G21 1QH, U.K.

Acknowledgement—This work was supported by the University of Glasgow Medical Research Funds.

Department of Pharmacology PAUL SKETT*
The University ALLAN WEIR†
Glasgow G12 8QQ, U.K.

REFERENCES

1. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150**, 285 (1965).
2. R. Kato and A. Takahashi, *Molec. Pharmac.* **4**, 109 (1968).
3. R. Kato, A. Takahashi and Y. Omori, *J. Biochem., Tokyo* **68**, 603 (1970).
4. R. Kato, A. Takanaka and A. Takahashi, *J. Biochem., Tokyo* **68**, 613 (1970).
5. R. Kato, A. Takahashi and Y. Omori, *Biochim. biophys. Acta* **208**, 116 (1970).
6. R. C. Rumbaugh, R. E. Kramer and H. D. Colby, *Biochem. Pharmac.* **27**, 2027 (1978).
7. E. R. Lax, R. Ghraf, H. Schreifers and K.-H. Voight, *Hoppe-Seyler's Z. Physiol. Chem.* **360**, 137 (1979).
8. D. L. Cinti, P. Moldéus and J. B. Schenkman, *Biochem. Pharmac.* **21**, 3249 (1972).
9. P. Skett, A. Mode, J. Rafter, L. Sahlin and J-Å. Gustafsson, *Biochem. Pharmac.* **29**, 2759 (1980).
10. P. Skett and C. Young, *Acta Endo. KbH* **100**, 421 (1982).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).

Biochemical Pharmacology, Vol. 32, No. 20, pp. 3117–3120, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

α_2-Adrenergic inhibition of lipolysis and respiration in rat brown adipocytes

(*Received* 25 *January* 1983; *accepted* 19 *April* 1983)

Brown adipose tissue is a highly specialized organ with two known functions: (1) to produce regulative heat in order to keep body temperature constant in a cold environment (non-shivering thermogenesis, NST); (2) the combustion of excess calories in cases of prolonged intake of an unbalanced diet, i.e. rich in carbohydrate relative to protein (diet-induced thermogenesis, DIT) [1–4]. The major product under these two conditions is heat derived from a high rate of uncoupled mitochondrial respiration. The mechanisms by which the brown adipocytes are triggered for oxidation of fatty acids during NST and during DIT seem to be identical. Norepinephrine (NE) which is released from the dense sympathetic nerve supply binds to the adrenergic receptors of the adipocytes [5]. Lipolysis is then initiated through the 'cascade effect' of cyclic AMP production, activation of protein kinase and hormone-sensitive lipase. The fatty acids (or their derivatives) uncouple the mitochondria and are oxidized at a high rate within these organelles [3, 6, 7–9].

The brown adipocytes possess different kinds of adre-