EFFECT OF THYROXINE ON ACID LIPASE ACTIVITY OF ADULT RAT LIVER

Paul M. COATES, Spencer A. BROWN, Herbert LAU, L. KRULICH* and Otakar KOLDOVSKY

The Joseph Stokes Jr Research Institute, Children's Hospital of Philadelphia and *The Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104* and **The Department of Physiology, University of Texas Southwestern Medical School, Dallas, TX 75235, USA*

Received 11 November 1977

1. Introduction

Lysosomal enzymes are important in many aspects of normal and pathological events in mammalian tissues [1–4]. The regulation of their activity appears to be under the control of a number of influences; genetic, temporal, and hormonal. Whereas the effect of glucocorticoid hormones on lysosomes and lysosomal enzymes of rat liver has been extensively studied [5–10], little attention has been paid to the effect of thyroid hormones on these enzymes. Hyperthyroidism (induced by feeding iodine) or hypothyroidism (induced by feeding thiourea) were found to have no effect on the activity of liver acid phosphatase or arylsulphatase; however, both conditions increased the fragility of liver lysosomes [11]. We have shown that administration of thyroxine for several days evokes an increase in the activity of three acid β-glycosidases (acid β-galactosidase, *N*-acetyl-β-glucosaminidase and β-glucuronidase) in the liver of adult rats [12] and thyroidectomy causes a decrease in their activity [13].

We questioned whether this effect could be demonstrated for other lysosomal enzymes and in the present study, we chose to examine the effect of thyroxine and thyroidectomy on acid lipase activity of the adult rat liver. This is a lysosomal enzyme with quite a different function, namely the hydrolysis of cholesteryl esters and triglycerides.

Abbreviations: LT_4, L-thyroxine; DT_4, D-thyroxine; LT_3, L-triiodothyronine; b.w., body weight; TSH, thyroid-stimulating hormone; THX, thyroidectomy

Address correspondence to: Paul M. Coates, The Joseph Stokes Jr Research Institute, Children's Hospital of Philadelphia, 34th and Civic Center Blvd., Philadelphia, PA 19104, USA

2. Materials and methods

Male rats of the Charles River CD strain (Wilmington, MA) were air-shipped at age 1 month and housed in our animal facility for 1–3 months prior to inclusion in any experiments. They were fed Purina Laboratory Chow (#5001) and water ad libitum. Animals for the thyroidectomy experiments underwent the operation at age 6 weeks by the producer (Charles River) and were air-shipped to our animal facility 4 days later, where they were maintained until age 10 weeks before inclusion in the experiments. In place of water, they were offered 0.85% (w/v) calcium chloride. Age-matched controls were used for these experiments.

Animals were randomly assigned, 3 per cage for most experiments, and differentiated according to treatment by clipping rear toes. Animals were sacrificed by decapitation; the livers removed, weighed and immediately frozen at −20°C to −40°C. Tissue homogenates, usually 1:4 (w/v), were prepared in ice-cold deionized water using a Potter-Elvehjem homogenizer with a Teflon pestle.

4-Methylumbelliferyl oleate and 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide were obtained from Research Products International (Elk Grove Village, IL). The free acids of L-thyroxine (LT_4), D-thyroxine (DT_4) and 3,3′,5-triiodo-L-thyronine

Table 1
Effects of thyroid hormone administration and thyroidectomy on body and liver weight, liver protein content and acid lipase activity in adult rats

Experiment	Treatment	n	Body wt (g) Start	Body wt (g) End	Liver wet wt (g)	Liver protein content (% liver wet wt)	Acid lipase activity (nmol/min/mg protein)
I	Control	11	289 ± 3[a]	318 ± 4	14.20 ± 0.30	16.5 ± 0.2	10.33 ± 0.80
	LT_4, 200 μg[b], 4 days	11	288 ± 3	292 ± 4[c3]	11.38 ± 0.21[c3]	19.7 ± 0.2[c3]	15.90 ± 0.89[c3]
II[d]	Control	7	148 ± 2	332 ± 8	13.45 ± 0.53	17.2 ± 0.8	9.13 ± 0.73
	Thyroidectomy	11	157 ± 3	198 ± 4[c3]	6.05 ± 0.21[c3]	17.9 ± 0.3	5.16 ± 0.14[c3]
III	Control	6	388 ± 10	406 ± 11	14.33 ± 0.80	23.7 ± 1.5	10.20 ± 0.82
	LT_4, 20 μg, 4 days	6	379 ± 9	373 ± 7[c1]	11.50 ± 0.37[c1]	24.3 ± 1.3	12.92 ± 0.88[c1]
	LT_4, 200 μg, 4 days	7	381 ± 10	364 ± 9[c1]	13.14 ± 0.83	27.7 ± 1.5	13.84 ± 0.84[c2]
	DT_4, 200 μg, 4 days	8	381 ± 11	385 ± 11	12.84 ± 0.89	29.8 ± 1.8	13.72 ± 0.65[c2]
IV	Control	3	257 ± 9	266 ± 8	n.d.[e]	n.d.	9.32 ± 0.72
	LT_3, 1000 μg, 1 day	3	265 ± 8	258 ± 7	n.d.	n.d.	13.32 ± 0.72[c1]

[a] Results expressed as mean ± SEM
[b] Doses are per 100 g body wt/day for the number of days indicated
[c] Significant difference from control: [1] $p<0.05$; [2] $p<0.01$; [3] $p<0.001$
[d] Experimental detail given in section 2
[e] n.d., not determined

(LT_3) and L-α-phosphatidyl choline were from Sigma Chemical Co. (St Louis, MO). Taurodeoxycholic acid sodium salt was from Calbiochem (La Jolla, CA). All other chemicals were reagent grade. Deionized water was used throughout.

Stock solutions of LT_4, DT_4 and LT_3 were made in 0.005 N sodium hydroxide and were administered to the rats subcutaneously for 1, 2, 3, 4 or 10 days. Control animals received injection of solvent alone.

Acid lipase activity was measured in a fluorometric assay described [14,15], but modified by replacing sodium taurocholate with taurodeoxycholic acid, sodium salt. *N*-Acetyl-β-glucosaminidase activity was measured as in [15]. Protein concentration was determined by the method in [16]. Enzyme activities were expressed as nmol substrate hydrolyzed/min/mg protein (specific activity) or μmol substrate hydrolyzed/min/total liver (total activity).

The success of thyroidectomy (THX) was verified by monitoring T_4 and TSH in the serum of THX and control rats using a radioimmunoassay kit for T_4 generously provided by Nuclear Medical Laboratories (Dallax, TX) and the NIAMDD radioimmunoassay kit for rat TSH.

Data were presented as mean ± SEM. Statistical significance of the difference of means was evaluated using the Student's *t*-test.

3. Results

The first experiments were designed to explore whether treatment with LT_4 influences liver acid lipase activity. As in [12], animals were treated with 200 μg LT_4/100 g b.w./day for 4 days, then sacrificed on the 5th day. The results, summarized in exp. I of table 1, show that this treatment evoked a substantial increase (54%) in the specific activity of acid lipase. The same results were obtained if the activity of acid lipase was expressed per total liver (36.3 ± 1.5 μmol/min/liver compared to control levels of 28.1 ± 1.9 μmol/min/liver). *N*-Acetyl-β-glucosaminidase activity was also measured and in agreement with [12], the specific activity of this enzyme was significantly increased by LT_4 treatment (LT_4-treated, 11.5 ± 0.8 nmol/min/mg protein versus control, 8.1 ± 0.6; $p<0.01$).

The next experiments examined the effect of thyroidectomy on liver acid lipase activity. Thyroidectomy was judged to be successful because the thyroidectomized rats had a mean TSH concentration of 5190 ± 420 ng/ml (control: 636 ± 49 ng/ml); thyroxine was undetectable in the serum of thyroidectomized rats (control: 51.0 ± 3.0 ng/ml). The data in exp. II (table 1) show that thryoidectomy evoked a 43% decrease in acid lipase activity.

In order to exlcude the possibility that the observed changes in acid lipase activity of hormone-treated or thyroidectomized animals were due to the presence or absence of an activator or inhibitor, we performed mixing experiments. Equal amounts of liver homogenates from hormone-treated (or thyroidectomized) and control animals were assayed together; the mixed samples showed activities which did not differ from the expected arithmetic mean by more than ±10%.

Further experiments were designed to characterize the time dependency of the response to LT_4. Figure 1 shows that treatment with LT_4 at a dose of 200 μg/100 g b.w./day evoked a significant increase in acid lipase activity within the first 24 h. Maximal increase in enzyme activity was seen between days 3 and 4. By 10 days, the activity was slightly lower than at 3–4

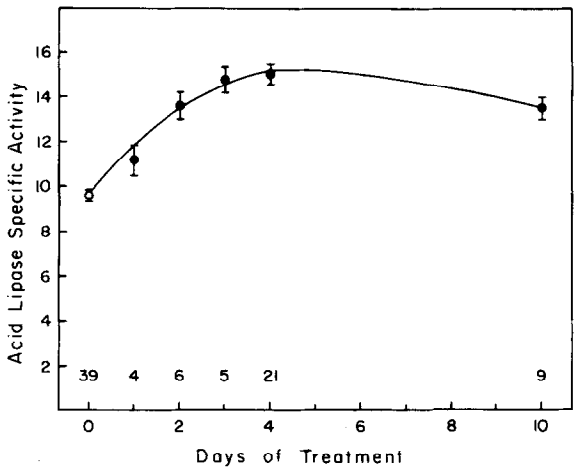

Fig.1. Change in specific activity of liver acid lipase in adult rats treated with L-thyroxine at a dose of 200 μg/100 g b.w./day for 0, 1, 2, 3, 4 and 10 days. Closed symbols denote a significant difference ($p<0.01$) from controls (day 0). Vertical lines denote 2 SEM. The number of animals per treatment group is shown below the curve. Because the acid lipase activities of control animals at each time were not significantly different, their values were pooled (day 0).

days, but this difference was not significant ($p>0.20$).

Experiment III (table 1) shows the effect of a 10-fold lower dose of LT_4 (20 μg/100 g b.w./day for 4 days) on the activity of acid lipase. This dose evoked a 27% increase in enzyme activity, lower than that seen with the 200 μg/100 g b.w. dose, but still significantly different from control levels.

In expt III (table 1), we also examined the effect of treating rats with DT_4 (the stereoisomer of LT_4) at a dose of 200 μg/100 g b.w./day for 4 days. This analogue also evoked a substantial increase in acid lipase activity, comparable to that observed after treatment with the same dose of LT_4.

Finally, expt IV (table 1) shows a preliminary experiment in which an extremely high dose (1000 μg/100 g b.w.) of LT_3 was administered for 1 day. This hormone, in a single dose, evoked a 43% increase in acid lipase activity within 1 day.

4. Discussion

Acid lipase activity of the adult rat liver is shown here to be increased by treatment with thyroxine and decreased after thyroidectomy. This response differs from that seen using cortisone acetate (5 mg/100 g b.w./day for 4 days) which caused a significant decrease in acid lipase activity (unpublished data).

In general, acid lipase activity shows responses to thyroid hormone treatment and thyroidectomy similar to those observed for acid β-glycosidases [12,13]; however, there were differences in the time-dependency of response to L-thyroxine treatment. Whereas the glycosidase activities were increased only after 2–3 days of thyroxine treatment, acid lipase activity was increased within 1 day; furthermore, the glycosidase activities increased essentially linearly with treatment time up to 10 days, while acid lipase activity appeared to level off after a peak response at 3–4 days. The finding that D-thyroxine had a similar effect to its naturally-occurring L-isomer deserves further attention in light of our data and several studies on the action of this analogue on various biological functions [17].

Thus, thyroid hormones appear to have effects not only on the acid β-glycosidases of rat liver, but also on another lysosomal enzyme. The effect of thyroxine administration and thyroidectomy on acid lipase activity is especially intriguing in view of the effect of thyroid status on serum cholesterol levels and cholesteryl ester deposition in arteries during atherogenesis [18].

Acknowledgements

This work was supported in part by Grants HL 18723, HD 08536 and HD 09988 from the National Institutes of Health, by Grant-in-Aid 6-80 from the National Foundation-March of Dimes, and Grant 76-768 from the American Heart Association.

References

[1] Dingle, J. T. and Fell, H. G. eds (1969) Lysosomes in Biology and Pathology, vol. 1, 2, North-Holland, Amsterdam.
[2] Dingle, J. T. ed (1973) Lysosomes in Biology and Pathology, vol. 3, North-Holland, Amsterdam.
[3] Dingle, J. T. and Dean, R. T. eds (1975–1976) Lysosomes in Biology and Pathology, vol. 4, 5, North-Holland, Amsterdam.
[4] Hers, H. G. and Van Hoof, F. eds (1973) Lysosomes and Storage Diseases, Academic Press, New York.
[5] Weissman, G. and Dingle, J. T. (1961) Exp. Cell Res. 25, 207–210.
[6] Weissman, G. and Thomas, L. (1962) J. Exp. Med. 116, 433–450.
[7] DeDuve, C., Wattiaux, R. and Wibo, M. (1962) Biochem. Pharmacol. 9, 97–116.
[8] Berg, T. and Bird, J. W. C. (1970) Acta Physiol. Scand. 79, 335–350.
[9] Ignarro, L. J. (1972) J. Pharmacol. Exp. Therap. 182, 179–188.
[10] Smith, R. J., Crawford, S., Gilchrest, H. and Williams, S. (1976) Biochem. Pharmacol. 25, 2172–2177.
[11] Kishore, G. S., Perumal, A. S. and Cama, H. R. (1971) Int. J. Vit. Nutr. Res. 41, 171–179.
[12] Horowitz, C., Comer, S., Lau, H. and Koldovsky, O. (1977) submitted.
[13] Lau, H., Krulich, L., Jumawan, J. and Koldovsky, O. (1977) submitted.
[14] Cortner, J. A., Coates, P. M., Swoboda, E. and Schnatz, J. D. (1976) Pediat. Res. 10, 927–932.
[15] Coates, P. M., Brown, S. A., Jumawan, J. and Koldovsky, O. (1977) Biochem. J. 166, 331–336.
[16] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[17] Pittman, C. S. and Pittman, J. A. (1974) in: Handbook of Physiology, sect. 7, Endocrinology, vol. III, Thyroid, pp. 233–253, Waverly Press, Baltimore.
[18] Kritchevsky, D., Tepper, S. A. and Story, J. A. (1976) Atherosclerosis 23, 249–252.