site de liaison supérieure à celle de E_2[12] et le déplace compétitivement du récepteur hypophysaire[13].
Une molécule de synthèse dont l'affinité vis-à-vis du site de liaison est supérieure à celle de l'E_2 déplace l'E_2. Par contre, s'il existe une spécificité hormonale, les autres hormones stéroïdes ne doivent pas entrainer de modification de la fixation de la molécule d'E_2. Ceci est montré par la figure 2. La MDA n'est pas modifiée par l'injection de doses croissantes d'autres stéroïdes qui possèdent leur propre site de liaison[14-16]. De même la MDA du sérum anti-moxestrol est de 10^{-5}, celle-ci n'est pas modifiée par l'administration de P, T ou DXM, confirmant ainsi la spécificité hormonale et la fixation du moxestrol sur le site de liaison de l'E_2.
Cependant l'utilisation de la MDA ne doit pas être assimilée à une quantification des résultats. Les valeurs obtenues n'ont pas de signification absolue. Cependant la détermination de cette valeur est intéressante car elle permet d'apprécier les variations relatives d'un stéroïde dans une cellule cible.
En conclusion, l'immunocytochimie après cryo-ultramicrotomie permet de localiser des stéroïdes endogène, exogène ou de synthèse dans un tissu hétérogène tel que l'hypophyse. La MDA permet d'apprécier des affinités relatives et les caractéristiques des sites de liaison des hormones tel que la spécificité hormonale.

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Thyroid hormones and cathepsin D activity in the rat liver, kidney and brain

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Summary. The effect of thyroidectomy and subsequent treatment with tri-iodothyronine (T_3), as well as that of thyrotoxicosis, was examined on cathepsin D activity in the rat liver, kidney and brain. Thyrotoxicosis resulted in a generalized increase in the enzyme activity in the 3 tissues; the effect of other thyroidal states was diverse and tissue-specific.

The role of thyroid hormones in regulating microsomal protein synthetic activity and mitochondrial energy metabolism has been extensively studied[1,2]. In recent years, however, it has become increasingly apparent that thyroid hormones may also influence lysosomal enzyme activities[3-6].
Earlier studies from our laboratory indicated a tissue-specific regulatory role for thyroid hormones with respect to energy metabolism in mitochondria[7] and turnover of their protein components[8]. It was therefore of interest to see whether a similar effect could also be seen in the case of a typical lysosomal enzyme, viz. cathepsin D. Results of these studies are summarized below.
Materials and methods. Male albino rats of Wistar strain were used throughout these experiments. Thyroidectomy was performed on weanling male rats (20–22 days old, 30–35 g b.wt) and the animals were allowed to grow for 8–10 weeks; a periodic record of their body weights and basal metabolic rates was kept. Only those animals showing considerable decrease in body weight (30–35% decrease) and basal metabolic rate (35–40% decrease) were used for further studies. Treatment of the thyroidectomized animals with tri-iodothyronine (T_3) (20 µg/100 g b.wt) was as described previously[9]. Thyroidectomized animals treated with T_3 were sacrificed 48 h after hormone administration. Thyrotoxicosis was induced experimentally by giving repeated doses of 200 µg T_3 per day s.c. for 5 days to rats weighing between 150–160 g[10]; the animals were sacrificed on the 6th day.
Liver and kidney homogenates (10% w/v) were made in 0.25 M sucrose containing 10 mM Tris-HCl, pH 7.4 and 1 mM EDTA[7]. Of the brain a 20% homogenate (w/v) was made in 0.3 M mannitol containing 0.1 mM EDTA, pH 7.4[7].
Free and total cathepsin D activities were determined according to the method of Gianetto and De Duve[11] at pH 3.8, using denatured hemoglobin as the substrate. The Folin-positive material released in the supernatant was estimated according to the method of Spies[12].
For estimation of the free pool size, separate aliquots of homogenates were deproteinized with trichloroacetic acid and the Folin-positive material released in the supernatant was determined as described above[12]. The values are expressed in terms of µg tyrosine/g tissue. Protein was estimated by the method of Lowry et al[13]. Student's t-test was used to determine statistical significance of differences between means.
Results and discussion. Results on the cathepsin D activities in the liver, kidney and brain, as influenced by the thyroid status of the rat, are shown in table 1. It can be noted that the free cathepsin D activity in the liver decreased significantly (43% decrease) after thyroidectomy, in agreement with the report of De Martino et al.[4]; and subsequent treatment with T_3 resulted in restoring the free activity to the level of normal euthyroid animals. In fact, a slight increase over the normal value was evident, which, however, was not statistically significant. Thyrotoxicosis caused a significant elevation (200% increase) of the free cathepsin D

activity. A similar trend was also observed for total activity, where thyroidectomy resulted in about a 20% decrease; about 35% stimulation over the normal value was noted after treatment of thyroidectomized rats with T_3. Most significantly, however, in thyrotoxic animals a 120% increase was evident. The ratio of total/free activity for normal, thyroidectomized and T_3-treated thyroidectomized rats ranged from 6.8 to 8.7, whereas it was lowered to 4.9 in the thyrotoxic condition. This is attributable to a greater increase in the free activity than in the total activity indicating probable lysosomal damage. Such an increase in the free cathepsin D activity in lysosomal damage has also been reported by other investigators[14-16]. The per cent bound activity, however, remained practically unaltered under these conditions.

In the kidney, on the other hand, the free as well as the total cathepsin D activity was not affected by a deficiency of thyroid hormones and administration of T_3 resulted in a further decrease in free activity (35% decrease), which may possibly relate to a tissue-specific effect of thyroid hormones[7,8].

The total activity, however, once again remained practically unaltered and was comparable to that of the normal controls. In the thyrotoxic condition, both free as well as total activities increased significantly by 67–96%, with the result that the ratio of total/free activity was slightly elevated. The percent bound activity increased significantly in animals receiving single or repeated doses of T_3, indicating that the thyroid hormones may perhaps regulate the percent binding of cathepsin D in this tissue, although the same does not seem to be the case for the liver or brain.

In the brain, as in the case of the liver, thyroidectomy resulted in a decrease (41%) in the free activity as well so in the total activity (30% decrease). Injection of a physiological dose of T_3 to hypothyroid animals brought about a further decrease in the free activity (60% decrease compared to normal; 27% decrease compared to the hypothyroid rats). A practically similar trend was also observed in the case of total activity. In thyrotoxicosis, the free activity was not affected significantly, in fact it was comparable to the value for euthyroid control, but the total activity increased by about 55%. The ratio of total/free activity ranged from 2.3–3.5 and no significant changes were noticeable in the percent bound activity.

Other workers have also shown hormonal influence on lysosomal enzyme activities. Thus it has been reported that treatment of thyroidectomized rats with T_3 evokes acid phosphatase activity in the liver[3]. Treatment of pregnant rats with T_3 during the last week of gestation results in an increased specific activity of 5 acid glycosidases in the fetal forebrain and cerebellum of rats during the first month of postnatal development[5]. Administration of L-thyroxine (T_4) or cortisone similarly affected several lysosomal enzymes in the forebrain and cerebellum of rats during the first month of postnatal development[6]. The results of our present studies have clearly shown the thyroidal influence on lysosomal cathepsin D activity and support the idea of regulation of this enzyme activity and protein turnover by thyroid hormones[4,17]. In this context it is of particular interest to note that a feature common to all tissues was a generalized elevation in the cathepsin D activity as a result of experimentally induced thyrotoxicosis - a condition which essentially represents a catabolic state.

Since tissue specific changes were noted with respect to free and total cathepsin D activities as influenced by the thyroid status of the rat, we tried to correlate the observed changes

Table 1. Effect of thyroid status on cathepsin D activity in the rat liver, kidney and brain

Tissue	Animals[a]	Cathepsin D activity (μg tyrosine/10 min/mg protein) Free	Total	Bound activity (%)[b]	Ratio of total/free activity
Liver	Normal (16)	0.572 ± 0.056	3.86 ± 0.208	85.58 ± 2.89	6.75
	Thyroidectomized (8)	0.325 ± 0.060[e]	3.11 ± 0.240[c]	87.49 ± 2.15 (NS)	8.65
	Thyroidectomized + T_3 (7)	0.641 ± 0.120 (NS)[h]	5.16 ± 0.360[e,k]	88.09 ± 2.90 (NS)	8.06
	Thyrotoxic (8)	1.760 ± 0.210[g]	8.56 ± 0.410[g]	78.95 ± 2.74 (NS)	4.86
Kidney	Normal (24)	1.652 ± 0.088	6.346 ± 0.370	69.52 ± 2.04	3.84
	Thyroidectomized (8)	1.921 ± 0.180 (NS)	6.709 ± 0.340 (NS)	66.24 ± 2.42 (NS)	3.49
	Thyroidectomized + T_3 (8)	1.260 ± 0.070[f,j]	6.400 ± 0.330 (NS)	74.81 ± 1.91 (NS)[i]	5.08
	Thyrotoxic (8)	2.760 ± 0.170[g]	12.440 ± 0.620[g]	77.22 ± 2.16[d]	4.51
Brain	Normal (12)	1.173 ± 0.095	2.734 ± 0.150	59.09 ± 4.42	2.33
	Thyroidectomized (8)	0.694 ± 0.075[g]	1.934 ± 0.130[g]	63.98 ± 5.27 (NS)	2.79
	Thyroidectomized + T_3 (12)	0.506 ± 0.028[g,h]	1.735 ± 0.130[g]	69.29 ± 2.88 (NS)	3.46
	Thyrotoxic (8)	1.200 ± 0.110 (NS)	4.210 ± 0.260[g]	69.88 ± 4.81 (NS)	3.51

The values are given as mean ± SEM. [a] Figures in parentheses represent the number of animals, [b] the percent bound activity is (total − free)/total × 100, [c] $p < 0.05$ compared with normal, [d] $p < 0.02$ compared with normal, [e] $p < 0.01$ compared with normal, [f] $p < 0.002$ compared with normal, [g] $p < 0.001$ compared with normal, [h] $p < 0.05$ compared with thyroidectomized, [i] $p < 0.02$ compared with thyroidectomized, [j] $p < 0.01$ compared with thyroidectomized, [k] $p < 0.001$ compared with thyroidectomized; NS, not significant compared with normal.

Table 2. Effect of thyroid status on the amino acid pool of the rat liver, kidney and brain

Animals[a]	Amino acid pool (μg tyrosine/g tissue) Liver	Kidney	Brain
Normal (24)	750.18 ± 35.66	773.72 ± 36.57	183.06 ± 7.06
Thyroidectomized (8)	762.04 ± 23.70 (NS)	634.40 ± 14.50[b]	197.10 ± 9.47 (NS)
Thyroidectomized + T_3 (12)	645.57 ± 49.63 (NS)[d]	354.52 ± 22.11[c,e]	112.42 ± 11.09[c,e]
Thyrotoxic (8)	575.90 ± 35.30[b]	674.40 ± 39.90 (NS)	161.44 ± 18.52 (NS)

The values are given as mean ± SEM. [a] Figures in parentheses represent the number of animals, [b] $p < 0.002$ compared with normal, [c] $p < 0.001$ compared with normal, [d] $p < 0.05$ compared with thyroidectomized, [e] $p < 0.001$ compared with thyroidectomized; NS, not significant compared with normal.

with the amino acid pool size in the tissues. These results are given in table 2. It is clear that tissue specific changes are also evident in the pool size. Thus the pool size in the liver and brain was not affected by thyroidectomy but decreased in the kidney by 18%. Treatment of the thyroidectomized rats with T_3 resulted in an overall decrease in the pool size ranging from 14–54% in the 3 tissues, the maximum effect being seen in the kidney in comparison with the brain and liver. The decrease in liver (14% decrease), however, was not statistically significant when compared with the controls. The observed decrease in the pool size following T_3 treatment of the thyroidectomized rats can probably be correlated with the onset of synthetic processes[1,2]. In thyrotoxic animals, the pool size did not change significantly for the kidney and brain and was in fact reduced in the liver (23% decrease), inspite of the fact that total cathepsin D activities were increased in all the tissues (table 1). Apparently, more complex mechanisms, such as a balance between retention in the tissues and excretion as well as re-utilization, may be involved under these conditions. Nevertheless, the results of the present studies have clearly shown that the thyroid status of the organism does influence the lysosomal cathepsin D activity and that the effect is tissue-specific.

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