Thyroid-hormone effects on ornithine decarboxylase

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We examined thyroidectomized, normal and hyperthyroid rats and found that ornithine decarboxylase activity was directly correlated with thyroid functional state in heart and liver and unaffected in brain, testes and spleen, phenomena that correlate with the known effect of thyroid hormone on protein synthesis.

Ornithine decarboxylase (EC 4.1.1.17) is a rate-limiting enzyme in the conversion of ornithine into the polyamines. These substances, intimately involved in the protein-synthetic machinery of the cell, have been shown to be increased in a number of endocrine target tissues when those tissues were exposed to tropic hormones. For example, lutropin administration stimulates ovarian ODC activity (Kobayashi *et al.*, 1971), insulin stimulates hepatic ODC (Mallette & Exton, 1973), and oestradiol stimulates uterine ODC (Kaye *et al.*, 1971).

The effect of hyperthyroidism or hypothyroidism on ODC in thyroid-hormone target tissues has not yet been examined. Previous work has shown that T_3 added to the isolated perfused rat heart prevented loss of ODC activity that otherwise occurred, but somatotropin or dibutyl cyclic AMP were equally effective (Antony *et al.*, 1976). T_3 administration has been shown to stimulate ODC in the immediately post-natal rat brain (Anderson & Schonberg, 1975). Large doses of T_3 have been reported to increase hepatic ODC activity 4 h after administration to the rat *in vivo* (Combest & Russell, 1978). Finally, thyroid hormone has been demonstrated to inhibit the thyroïdal increase in ODC induced by thyrotropin (Yu *et al.*, 1976).

As judged by effects on protein synthesis, oxygen consumption and the activity of a number of enzymes involved in intermediary metabolism, thyroid hormone exerts a profound effect on heart and liver (Michels *et al.*, 1963; Barker & Kitgaard, 1952). Effects of thyroid hormone on a number of other tissues, including testes, brain (adult) and spleen, are nowhere near as large, and in fact have

been difficult to document. We have decided to look at ornithine decarboxylase activity in each of these tissues in hypothyroid, euthyroid and hyperthyroid rats in an effort to ascertain the effect of thyroid hormones.

Materials and methods

The studies were performed on male Sprague-Dawley rats. All rats weighed between 280 and 320 g at the time of death. After thyroidectomized rats were obtained from Zivic Miller (Attison Park, PA, U.S.A.), they were weighed weekly and judged to be adequately hypothyroid when they no longer increased in weight. At that time, T_4 concentrations were measured in the laboratory of Dr. P. Reed Larsen (Boston, MA, U.S.A.) and were essentially undetectable. Hyperthyroidism was achieved by daily subcutaneous injections of T_3 (free acid, from Sigma Chemical Co.) for 3 days, at a dose of 25 µg/100 g body wt. Experiments were performed in the morning and all rats were allowed free access to food until the morning of experimentation.

The animals were killed by decapitation; tissues were immediately removed and homogenized for determinations of ODC activity. The apex of the heart, the distal right lobe of the liver, the decapsulated testes, the entire spleen and the neocortex of the brain were the tissues studied.

Ornithine decarboxylase was assayed by the method of Russell & Snyder (1968) with modifications (Rozovski *et al.*, 1979). The tissue was homogenized in 2½ vol. of ice-cold 0.25 M-sucrose (pH 7.2) containing 1 mM-2-mercaptoethanol and 0.3 mM-disodium EDTA, with a Polytron homogenizer (Brinkman Instruments). The homogenate was centrifuged at 40 000 g for 1 h at 3°C and the supernatant used for determination of enzyme activity. ODC was assayed by measuring the

Abbreviations used: ODC, ornithine decarboxylase; T_3 , tri-iodothyronine; T_4 , thyroxine.

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evolution of $^{14}\text{CO}_2$ from [*carboxy*- ^{14}C]ornithine (New England Nuclear). Incubations were carried out in Pyrex test tubes in which was suspended a polyethylene centre well; these contained 0.3 ml of Protosol (New England Nuclear) as a CO_2 -trapping reagent. The incubation mixture contained 0.05 mM-pyridoxal 5'-phosphate, 2.5 mM-dithiothreitol, 0.1 ml of supernatant, 0.5 μCi of DL- ^{14}C ornithine (sp. radioactivity 52.4 Ci/mol), 50 μM -L-ornithine (unlabelled), 50 μM -Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (pH 7.2), sufficient to make a final volume of 1 ml. Tubes were incubated in a shaking water bath at 37°C for 60 min (during which the reaction proceeds linearly), after which 0.5 ml of 40% (w/v) trichloroacetic acid was injected to stop the reaction. They were allowed to shake for an additional 30 min. The $^{14}\text{CO}_2$ trapped in the Protosol was counted for radioactivity as described by Russell & Snyder (1968). Protein was determined as previously described (Rozovski *et al.*, 1979), and the ODC activity was expressed as pmol of CO_2 released/h of incubation per mg of protein determined in the supernatant.

Results and discussion

In the heart and liver, activity increased in a stepwise fashion from the hypothyroid to the euthyroid to the hyperthyroid state (Fig. 1*a*). In the testes, spleen and brain (Fig. 1*b*), there was no relationship between ODC activity and thyroid status.

Because we chose to express the ODC activity in terms of mg of soluble protein, we needed to know

that the concentration of soluble protein/weight of tissue was not a variable in our experiments. We therefore made this determination under various conditions (Table 1) and found it to be constant. Were we to express ODC activity in terms of μg of DNA, the relationships in Fig. 1 would still be maintained in that (a) concentrations of DNA/weight of tissue are either unchanged or slightly increased in the liver of hypothyroid or hyperthyroid animals (Aranda *et al.*, 1972), and (b) the weights of the heart decreased in our hypothyroid animals and increased by 12% in our hyperthyroid animals.

ODC is an ubiquitous enzyme, found in all living tissues. Its function is closely related to the protein-synthetic machinery of the cell. Under many differing conditions, changes in ODC activity are correlated with changes in protein-synthetic activity (Stevens, 1970). Thyroid hormone has a potent effect on protein synthesis in heart and liver (Michels *et al.*, 1963). We have demonstrated that thyroid hormone regulates heart and liver ODC. Thyroid hormone has minimal effects on protein

Table 1. Concentration of soluble protein (mg/g wet wt. of tissue) under various experimental conditions

By analysis of variance (Student's *t* test) there was no significant difference between any of these values for the heart or the liver.

	Hypothyroid	Euthyroid	Hyperthyroid
Heart	40.7 $\pm$ 1.9	39.6 $\pm$ 3.4	42.2 $\pm$ 1.2
Liver	108.5 $\pm$ 2.5	113.9 $\pm$ 3.6	108.2 $\pm$ 3.8

Fig. 1. Effect of thyroid functional state on ODC activity in various organs of the rat

Each group consists of six to eight animals; results are means $\pm$ s.e.m. Student's two-tailed *t* test was used to compare hypothyroid (▨) and hyperthyroid (■) with control (□) animals: NS, non-significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. In the heart and liver (a), ODC activity varies according to thyroid state, whereas in those tissues (b) thought to be relatively resistant to thyroid-hormone action, ODC activity is unaffected.

synthesis in testes, spleen and adult brain (Michels *et al.*, 1963). In the experiments reported here, the hormone was without effect on ODC activity in these latter tissues. The effect of the hormone on ODC thus appears to be specific, related to protein-synthetic response in the hormone's target tissue.

We do not believe that an increase in ODC is a necessary step for all the actions of thyroid hormone. Previously we have demonstrated that, in skeletal muscle, thyroid hormone does not exert a stimulatory effect on ODC (Chideckel & Belur, 1980). Nevertheless, in skeletal muscle thyroid hormone induces an increase in oxygen consumption and an increase in the activity of a number of enzymes of intermediary metabolism, but generalized protein synthesis is not stimulated (DeMartino & Goldberg, 1978). It thus appears that the action of thyroid hormone to increase protein synthesis is accompanied and perhaps mediated by an increase in ODC, but other actions of thyroid hormone may occur in the absence of increased ODC.

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References

- Anderson, T. R. & Schonberg, S. M. (1975) *Biochem. Pharmacol.* **24**, 495–501
- Antony, P., Gibson, K. & Harris, P. (1976) *J. Mol. Cell. Cardiol.* **8**, 589–597
- Aranda, A., Montoya, E. & Herrera, E. (1972) *Biochem. J.* **128**, 597–604
- Barker, S. B. & Kitgaard, H. M. (1952) *Am. J. Physiol.* **170**, 81–86
- Chideckel E. W. & Belur, E. B. (1980) *Endocrinology* in the press
- Combest, W. L. & Russell, D. H. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 797
- DeMartino, G. N. & Goldberg, A. L. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1369–1373
- Kaye, A. N., Ickson, I. & Lindner, H. R. (1971) *Biochim. Biophys. Acta* **252**, 150–159
- Kobayashi, Y., Kupelian, J. & Maudsley, P. V. (1971) *Science* **172**, 379–380
- Mallette, L. E. & Exton, J. H. (1973) *Endocrinology* **93**, 640–644
- Michels, R., Cason, J. & Sokoloff, L. (1963) *Science* **140**, 1417–1418
- Rozovski, S. J., Conover, C. A. & Ruderman, N. B. (1979) *Life Sci.* **25**, 533–560
- Russell, D. H. & Snyder, S. H. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **60**, 1420–1427
- Stevens, L. (1970) *Biol. Rev. Cambridge Philos. Soc.* **45**, 1–27
- Yu, S., Friedman, Y., Richman, R. & Burke, G. (1976) *J. Clin. Invest.* **57**, 745–755