SHORT COMMUNICATIONS

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Malate dehydrogenase (decarboxylating) (NADP) and α-glycerophosphate oxidase in the developing rat

In a previous study, the evolution of α-glycerophosphate oxidase (L-glycerol-3-phosphate:(acceptor) oxidoreductase, EC 1.1.99.5) activity of rat liver mitochondria and its relation to thyroid status have been described[1]. It is well known that in adult rat liver, thyroxin increases the activity of malate dehydrogenase (decarboxylating) (NADP) (L-malate:NADP oxidoreductase (decarboxylating), EC 1.1.1.40)[2,3], a cytoplasmic enzyme involved in the regulation of lipogenesis[3-5]. This communication reports results of studies of these two enzyme activities in the liver and brain of developing rats with different thyroid conditions. The brain was studied because of the importance of thyroxin in its differentiation, even though it has been proven that thyroxin does not affect α-glycerophosphate oxidase activity in the adult rat brain[6].

Wistar albino rats of both sexes (no systematic differences were observed), fed with laboratory chow, were sacrificed and the brain and liver were homogenized (10%, w/v) in a cold medium (0.3 M sucrose, 10 mM Tris–HCl buffer (pH 7.2), 2 mM Na_2EDTA). These homogenates were centrifuged for 5 min at 700 × *g*. The supernatants were then centrifuted at 7500 × *g* for 10 min. The pellets were washed and centrifuged again under the same conditions. This mitochondrial fraction was used for α-glycerophosphate oxidase assay by a manometric method previously described[1]. Supernatants from the first 7500 × *g* centrifugation were centrifuged 60 min at 105 000 × *g*. The cytoplasmic extracts thus obtained were used for malate dehydrogenase (decarboxylating) (NADP) assay by a method derived from the one described by OCHOA[7]. Proteins were determined by the biuret method after trichloroacetic acid

TABLE I

MALATE DEHYDROGENASE (DECARBOXYLATING) (NADP) IN LIVERS OF NORMAL AND HYPERTHYROID RATS

mμmoles $NADPH_2$ per min per mg cytoplasmic proteins at 30°; means ± confidence intervals for $P = 0.05$; number of determinations in parentheses.

A. Normal rats			*B. Hyperthyroid rats*		
Post-natal age (days)	*Malate dehydrogenase (decarboxylating) (NADP) activity*		*Post-natal age (days)*	DL-*Thyroxine injected (μg)*	*Malate dehydrogenase (decarboxylating) (NADP) activity*
0	0 ± 0	(6)	2	10	12.7 ± 2.5 (9)
10	0.5 ± 0.3	(8)	12	20	13.0 ± 3.6 (8)
21	1.2 ± 0.5	(9)	23	40	37.6 ± 10.3 (7)
28	24.1 ± 10.0	(8)	30	40	113.8 ± 38.9 (5)
35	52.9 ± 9.2	(4)	37	40	125.6 ± 34.0 (4)
42	33.9 ± 7.8	(16)	44	80	94.1 ± 29.1 (7)
Adults (2–3 months)	19.1 ± 4.3	(22)	Adults (2–3 months)	200	62.1 ± 9.2 (8)

TABLE II

α-GLYCEROPHOSPHATE OXIDASE ACTIVITY IN BRAINS OF NORMAL RATS

μl O_2 per h per mg mitochondrial proteins at 37°; means ± confidence intervals for $P = 0.05$; number of determinations in parentheses.

Post-natal age (days)	*α-Glycerophosphate oxidase activity*
0	23.6 ± 5.7 (5)
10	29.7 ± 5.2 (7)
21	24.3 ± 4.8 (6)
28	22.9 ± 5.4 (6)
42	25.5 ± 4.2 (6)
Adults (2–3 months)	22.0 ± 1.6 (10)

precipitation. In all cases DL-thyroxine was administered subcutaneously, 48 and 24 h before measurements.

Malate dehydrogenase (decarboxylating) (NADP) activity is almost undetectable in the liver during the suckling period and increases sharply during weaning. After a maximum during late post-natal development, it decreases to the lower adult values (Table 1A). This evolution is similar to the one already described[8]. Thyroxine increases malate dehydrogenase (decarboxylating) (NADP) activity at any stage, but the amplitude of this response is much greater from the weaning period onward (Table IB).

α-Glycerophosphate oxidase activity is high in the brain mitochondria of control animals, with little variation during development (Table II).

In hypothyroid rats both α-glycerophosphate oxidase and malate dehydrogenase (decarboxylating) (NADP) activities are depressed in liver, but not in brain (Table IIIA). Thyroxin increases the activity of liver enzymes, without affecting those of the

TABLE III

ENZYME ACTIVITIES IN HYPOTHYROID ANIMALS

The mother is force-fed daily with 50 mg of propyl thiouracil from 19 days *post-coïtum*. Units as in Tables I and II; means of 4–5 experiments; range in parentheses.

	Age (days)	*A. Without thyroxine*		*Age (days)*	*B. With thyroxine**	
		Malate dehydrogenase (decarboxylating) (NADP)	*α-Glycerophosphate oxidase*		*Malate dehydrogenase (decarboxylating) (NADP)*	*α-Glycerophosphate oxidase*
Liver	10	2.1 (1.0–3.8)	4.7 (2.7–5.8)	12	15.8 (10.4–24.8)	20.7 (14.3–25.8)
	28	0.9 (0–3.4)	1.8 (1.2–2.2)	30	6.1 (4.8–7.5)	16.6 (13.9–23.2)
Brain	10	8.2 (7.9–9.1)	27.0 (19.2–31.8)	12	10.9 (9.7–11.3)	32.1 (27.6–34.4)
	28	15.2 (14.6–15.4)	29.0 (24.0–34.1)	30	14.0 (12.1–15.0)	24.1 (19.3–32.4)

* 20 μg DL-thyroxine at day 12; 40 μg DL-thyroxine at day 30.

brain (Table IIIB). But the increase of malate dehydrogenase (decarboxylating) (NADP) activity remains limited, even at the age of 1 month, as in euthyroid suckling rats. It must be pointed out that 1-month-old hypothyroid rats are not yet weaned.

Our results confirm the fact that thyroxin does not increase α-glycerophosphate oxidase activity in the developing rat brain, a result which has been shown in hyperthyroid animals[9]. This conclusion can be extended to malate dehydrogenase (decarboxylating) (NADP) as for other adult rat tissues[10]. It seems, therefore, impossible to correlate the morphogenetic action of thyroxine with an action at the level of these enzymes.

The results concerning liver malate dehydrogenase (decarboxylating) (NADP) can be interpreted by considering the change of diet during development. Suckling rats received a high-fat diet. Their malate dehydrogenase response to thyroxine was low. From weaning onward, the fat content of their diet was decreased and lipogenesis was stimulated[8]. Correlatively, the sensibility of malate dehydrogenase (decarboxylating) (NADP) response to thyroxine increased. This agrees with observations in adult rats fed different diets[11]. Moreover, a similar interpretation has been given for the evolution of several other enzymes connected with lipogenesis[8,12].

In the brain, α-glycerophosphate oxidase and malate dehydrogenase (decarboxylating) (NADP) activities are not under thyroid control, even during post-natal brain differentiation. Liver malate dehydrogenase (decarboxylating) (NADP) activity is very small during the suckling period. The sharp increase of this activity during the weaning period seems to be due to an increase of its responsiveness to thyroxine correlated with the change of diet.

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