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α-Glycerophosphate Dehydrogenase Activities in Liver and Brain Mitochondria from Neonatal Rats Treated with L-Thyroxine

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Liver α-GPD undergoes a dramatic increase in newborn rats treated with large doses of L-thyroxine, indicating the elevated exposure of peripheral tissues to the hormone. In contrast, brain enzyme remains unchanged in the two mitochondria populations, free and synaptic.

The activity of many enzymes is increased by thyroid hormone administration. Mitochondrial α-glycerophosphate dehydrogenase (α-GPD), specially the liver enzyme, is one of the enzymes that responds more markedly (15). This response has been considered an excellent index of the thyroid status in peripheral tissues, particularly in the liver (19). In contrast, adult brain α-GPD activity is not increased by exogenous T_4. SCHAPIRO and PERCIN (21) showed that there was no increase in rat brain α-GPD from T_4 treated animals between 0 and 48 days of life. Nevertheless, these authors measured mitochondrial α-GPD in brain without distinguishing between free and synaptic mitochondrial populations, while it is well known that there is a different enzyme distribution in these two populations, and that these enzymes can be differently affected by T_4 (5).

On the other hand it is well established that large doses of T_4 administered to rats during the first days of life produce a sequence of perturbances of the CNS which lead to a permanent derangement in hystological parameters of cortical brain and, consequently, to an impaired behaviour of the adult animal (6, 7).

The effect of thyroxine also depends on the age of the animal. FAZEKAS *et al.* (9) showed an increase of oxygen consumption in the cerebral cortex of developing

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hyperthyroid rats which did not occur in the adult animals. Protein biosynthesis, a well-known response to thyroxine, is not stimulated in the adult brain by T_4 treatment (4, 17) whereas it is decreased in cerebral cortex by thyroidectomy at birth (10). Thyroid treatment increases amino-acid incorporation into infant brain (13). SCHAPIRO (20) has also shown that thyroxine increases brain cholesterol — an index of myelinization — during the first two weeks of life, but not later.

Keeping in mind that the T_4 doses used to produce the neo-T_4 syndrome are much larger than those administered to newborn rats by SCHAPIRO and PERCIN (21), it seemed interesting to study α-GPD activity in neo-T_4 rats both in liver mitochondria and in free and synaptic brain mitochondria.

Materials and Methods

Wistar rats were used throughout. Mothers were fed the available commercial diet containing 1-2 μg I/g. Spontaneous delivery was carefully observed and the first day of life was considered 24 hours after birth. The number of pups in every litter was matched to eight per dam. In all cases, litters were divided into two groups: four animals received the T_4 treatment while the rest served as controls.

Thyroxine (L-thyroxine sodium pentahydrate, purchased from Sigma) was dissolved (0.3 mg/ml) in physiological saline slightly alkalinized with OHNa 0.01 N. The hormone was injected subcutaneously; the treatment began at different ages, and it consisted of a maximum of five consecutive doses of 30 μg T_4/rat in a volume of 0.1 ml. Control animals received an equal volume of the solvent.

The animals were killed by decapitation without anaesthesia at differents ages as indicated. Liver samples and whole brains were removed for analysis and rapidly homogenized at 4° C. Hepatic mitochondria were obtained by the method of SCHNEIDER (22). Crude brain mitochondria were separated into free and synaptic fractions by the method of GRAY and WHITTAKER (11). The mitochondrial pellets were dispersed in phosphate buffer and their protein concentration was adjusted to about 9 mg/ml.

α-GPD activity was measured according to the method of LEE *et al.* (15). The protein content in each assay was about 90 μg; the reaction is proportional to protein in the range of 50-115 μg per assay (18). The values are expressed as optical density change per minute per milligram of protein at 500 nm. Protein was determined by the method of LOWRY *et al.* (16).

Results

Table I shows the body and brain weights of control and L-T_4-treated animals at different ages. These weights, as well as those of other organs, are significantly decreased after five doses of L-T_4, showing the delay of growth produced by the early excess of hormone. However, the T_4-injected animals are significantly smaller at 22 days of age only when the treatment starts on the 3rd day of life; when treatment begins later than that, animals recover normal body and brain weights some days after the end of treatment.

Figure 1 presents liver α-GPD activity in rats whose L-T_4 treatment starts on the 3rd day of life. The hormone produces a dramatic increase in enzyme activity after the second dose — at 5 days of age —; this effect is maintained with subsequent injections. After treatment, α-GPD activity is still very elevated during at least four days — at 11 days of life — and later gradually decreases. At 22 days (13 days after the last injection), there is no difference between neo-T_4 and control animals.

Liver α-GPD activity undergoes a similar increase in animals that receive the

Table I. *Body and brain weights ($\bar{x} \pm SD$) after T_4 treatment.*
Each value corresponds to 8-10 animals.

Onset of trt.	Days of life	Body (g)			Brain (g)		
		Control	Thyroxine	P	Control	Thyroxine	P
3rd day	6	16.5 ± 0.9	14.9 ± 0.9	xx	0.51 ± 0.03	0.50 ± 0.03	N.S.
	8	18.6 ± 0.9	15.0 ± 0.5	xx	0.64 ± 0.03	0.53 ± 0.03	xx
	13	27.8 ± 1.9	19.2 ± 1.3	xxx	0.87 ± 0.02	0.69 ± 0.03	xxx
	22	49.4 ± 1.4	33.6 ± 3.8	xxx	1.01 ± 0.01	0.87 ± 0.04	xxx
6th day	11	22.1 ± 1.9	19.6 ± 0.8	x			
	22	56.5 ± 5.1	51.1 ± 4.9	N.S.	0.97 ± 0.05	0.91 ± 0.02	N.S.
11th day	16	33.4 ± 2.3	27.0 ± 3.5	xx	0.93 ± 0.04	0.89 ± 0.04	xx
	22	37.5 ± 4.6	31.2 ± 4.2	N.S.	0.91 ± 0.03	0.92 ± 0.03	N.S.

x = $p < 0.05$, xx = $p < 0.01$, xxx = $p < 0.001$. N.S. = non significant.

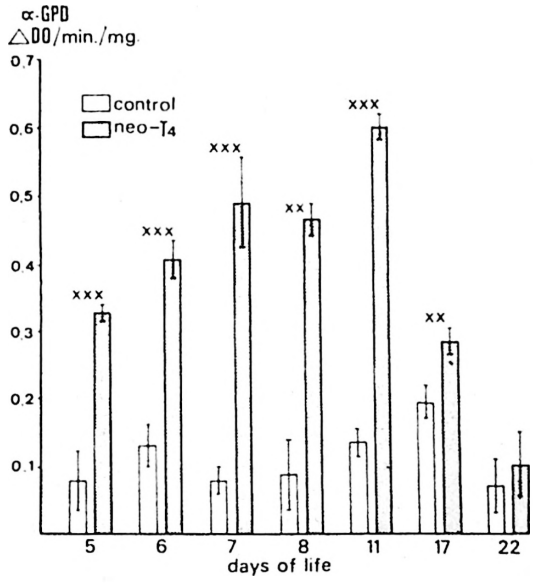

Fig. 1. *Liver α-glycerophosphate dehydrogenase (α-GPD) activity in mitochondria from control and neo-T_4 rats at several stages of life*
Onset of treatment: 3rd day of life. α-GPD activity is much higher in neo-T_4 rats than in controls at 5, 6, 7, 8, 11 and 17 days of life, i.e., when they have received 2, 3, 4 or 5 doses of T_4 or 4 and 10 days after the last injection. Each value corresponds to 10 animals.

first L-T_4 dose on the 6th day of life. Enzyme activity starts to increase after the first injection (unpresented data). Figure 2 illustrates the enzyme activity during the phase of recovery of normal

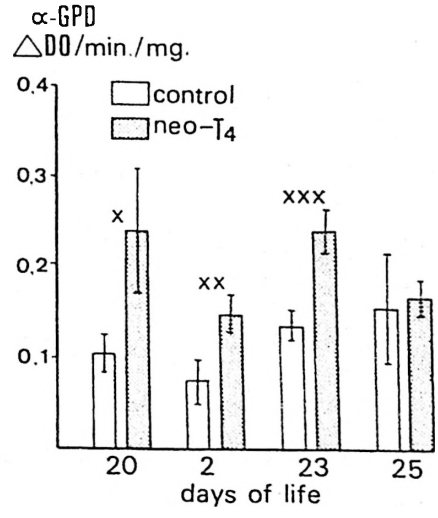

Fig. 2. *Liver α-GPD activity in mitochondria from control and neo-T_4 rats at several stages of life.*
Onset of treatment: 6th day of life. α-GPD activity is much higher in neo-T_4 rats than in controls at 20, 22 and 23 days of life, i.e., 10, 12, 13 days after the last injection. Each value corresponds to 10 animals.

values, which are reached at 25 days of life, 14 days after the end of treatment.

The results on brain α-GPD are presented in figures 3 and 4. In contrast with the liver enzyme, neither of the two models of treatment produces a significant change in brain α-GPD activity. Thus, immediatly after five doses of L-T_4 (150 μg of hormone in all) administered between the 3rd and 7th day of life, the level of α-GPD activity in free and synaptic brain mitochondria remains the same in control and hormone-injected rats. Similar results are obtained with equally treated animals killed at 13 days of age (fig. 3). When treatment is started on the 11th day of life (fig. 4) there are no differences in α-GPD activity between neo-T_4 and control animals either at 16 days of life (that is, the day following the last T_4 injection) or at 22 days of life.

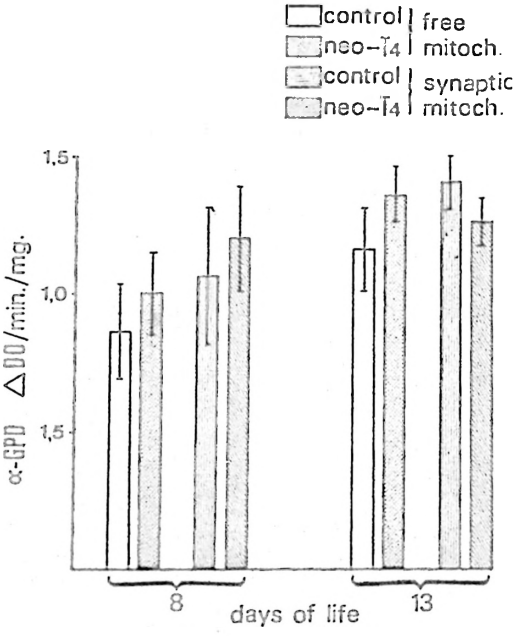

Fig. 3. *Brain α-GPD activity in free and synaptic mitochondria from control and neo-T_4 rats at several stages of life.*
Onset of treatment: 3rd day of life. α-GPD activity was the same in free and synaptic mitochondria from control and neo-T_4 rats killed at 8 or 13 days of life. Number of animals: 10.

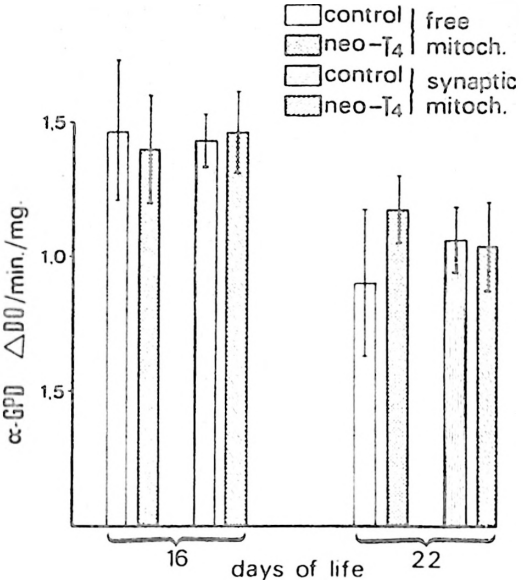

Fig. 4. *Brain α-GPD activity in free and synaptic mitochondria from control and neo-T_4 rats at several stages of life.*
Onset of treatment: 11th day of life. α-GPD activity was the same in free and synaptic mitochondria from control and neo-T_4 rats killed at 16 or 22 of life. Each value corresponds to 10 animals.

Discussion

At 22 days of age, only rats injected with T_4 from day 3 present a decrease in body and brain weight with respect to controls (table I). This agrees with the observation that the irreversibility of the neo-T_4 syndrome is so much the greater the earlier T_4 injections are started (1-3).

The results obtained for liver α-GPD in neo-T_4 rats agree with those of several authors (14, 15), who showed the high responsiveness of liver enzyme to thyroid

hormone. In this way, after a total of 150 μg of L-T_4, liver enzyme activity exhibits a sevenfold increase (fig. 1), showing a response roughly proportional to the L-T_4 doses administered (19). Moreover, these results agree with those found in newborn rats with smaller T_4 doses, and seem to indicate a specific action of thyroxine on the liver enzyme (20), independent of other T_4 effects.

The adult brain is only minimally affected by changes in endocrine activity (9) but its growth is essentially influenced by thyroid hormones, which play a major role in several aspects of neural maturation. Thus, thyroid hormones affect the synthesis of certain brain enzymes only while the organ is developing (5), so that it seems to be an age-dependent effect (24). Our preliminary studies dealt with α-GPD activity in crude brain mitochondrial fraction of neo-T_4 rats. We chose several stages characterized by their increased oxygen consumption as a consequence of T_4-treatment (9) in the hope of finding some possible response, but the enzyme remained unaffected in all cases (unpublished data). These results are in keeping with those of SCHAPIRO and PERCIN (21).

Nevertheless, it is known that brain mitochondria can be distributed into two populations, namely free and synaptic, and that the latter can roughly be assimilated to the high myelin content fraction. The distribution of several enzyme activitites is different in both populations. There is evidence that a distinct hormonal control of metabolism can be exerted on free and synaptic mitochondria (3). Thyroidectomy at birth has been seen to influence the enzymes in each population differently: glutamate dehydrogenase — whose activity is mainly found in free mitochondria — remains unaffected, while succinate dehydrogenase — essentially located in mitochondria from synaptosomes — exhibits an irreversible decrease in activity.

In this work, we show that brain α-GPD activity from synaptic and non-synaptic mitochondria, does not change in response to a series of high T_4 doses, either when the injections are started on the 3rd day of life (fig. 3) when the brain is not yet mature, or when they are administered during a later period of development, that is, on the 11th day of life (fig. 4).

It is not yet clear whether or not thyroid hormones exert their effects on brain through a direct action, or through the metabolic alterations that they produce (8). Moreover, assumption of a direct effect does not exclude the possibility that brain α-GPD is not sensitive to thyroid hormones. LEE and LARDY (14) early pointed out several possible reasons to explain the lack of response. Nevertheless, it has been well established that T_3 reaches the brain and binds to specific nuclear receptors. However, DE GROOT *et al.* (12) found a discrepancy between intensity of T_3 binding and α-GPD stimulation. SCHWARTZ *et al.* (23) concluded that α-GPD response to thyroid hormones is modulated at a postreceptor level and, consequently, it is not directly dependent on concentration of nuclear receptors. However, the sort of biochemical changes at postreceptor levels are still unknown.

Acknowledgements

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Resumen

En ratas recién nacidas tratadas con grandes dosis de L-tiroxina la actividad de α-GPD hepática se incrementa significativamente, lo cual indica una elevada exposición de los tejidos periféricos a esta hormona. Contrariamente, la actividad de esta enzima en cerebro no cambia en las dos poblaciones de mitocondrias analizadas, libres y sinápticas.

References

1. BAKKE, J. L. and LAWRENCE, N.: *J. Lab. Clin. Med.*, **67**, 477-482. 1966.
2. BAKKE, J. L., LAWRENCE, N. and ROBINSON, S.: *Neuroendocrinol.*, **10**, 183-195, 1972.
3. BALÀZS, R., COCKS, W. A., EAYRS, J. T. and KOVÀCS, S.: In «Hormones in Development» (M. Hamburg and E. J. Barrington, ed.). Pub. Appleton-Century-Crofts, New York, 1971, pp. 357-379.
4. BALÀZS, R., KOVÀCS, S. and PATEL, A. J.: I.S.N. Tokyo Meeting, 51-60, S5-4, 1973.
5. BALÀZS, R., LEWIS, P. D. and PATEL, A. J.: In «Growth and development of the brain. Nutritional, Genetic and Environmental Factors» (M. A. B. Brazier, ed.). Raven Press, New York, 1975, pp. 83-115.
6. EAYRS, J. T. and HOLMES, R. L.: *J. Endocr.*, **29**, 71-81, 1964.
7. EAYRS, J. T.: In «Hormones in Development» (M. Hamburg and E. J. Barrington, ed.). Pub. Appleton-Century-Crofts, New York, 1971, pp. 345-356.
8. ESCRIVÁ, F. and PASCUAL-LEONE, A. M.: *Endocrinol.*, **108**, 336-340, 1981.
9. FAZEKAS, J. F., BURTON, F. and ALMAN, R. W.: *Endocrinol.*, **48**, 169-174, 1951.
10. GEEL, S. E. T., VALKANA and TIMIRAS, P. S.: *Brain Res.*, **4**, 143-150, 1967.
11. GRAY. E. G. and WHITTAKER, V. P.: *J. Anat.* (London), **96**, 79-96, 1962.
12. GROOT, L. J. DE, ROBERTSON, M. and RUE, P. A.: *Endocrinol.*, **100**, 1511-1515, 1977.
13. KLEE, C. B. and SOKOLOFF, L.: *J. Neurochem.*, **11**, 709-800, 1964.
14. LEE, Y. P. and LARDY, H.: *J. Biol. Chem.*, **241**, 1427-1436, 1965.
15. LEE, Y. P., TAKEMORY, A. E. and LARDY, H.: *J. Biol. Chem.*, **234**, 3051-3054, 1959.
16. LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. and RANDALL, R. J.: *J. Biol. Chem.*, **193**, 265-275, 1951.
17. MICHELS, R., CASON, J. and SOKOLOFF. L.: *Science*, **140**, 1417-1420, 1963.
18. OPPENHEIMER, J. H., SILVA, E., SCHWARTZ, H. L. and SURKS, M. I.: *J. Clin. Invest.*, **59**, 517-527, 1977.
19. RUEGAMER, W. R., WESTERFIELD, W. W. and RICHTER. D. A.: *Endocrinol.*, **75**, 908-916, 1964.
20. SCHAPIRO, S.: *Endocrinol.*, **78**, 527-532, 1966.
21. SCHAPIRO, S. and PERCIN, C. J.: *Endocrinol.*, **79**, 1075-1078, 1966.
22. SCHNEIDER, W. C.: *J. Biol. Chem.*, **176**, 259-265, 1948.
23. SCHWARTZ, H. L., FORCIEA, M. A., MARIASH, C. N. and OPPENHEIMER, J. H.: *Endocrinol.*, **105**, 41-46, 1979.
24. VELLIS, J. and INGLISH, D.: *J. Neurochem.*, **15**, 1061-1070, 1968.