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Effects of Thyroid Hormones upon Flavin Adenine Dinucleotide Pyrophosphorylase Activity in Novikoff Hepatoma in Rats (40946)

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Abstract. This study was performed to explore basic mechanisms underlying the striking difference between the 5- to 10-fold increase in activity of mitochondrial α-glycerophosphate dehydrogenase produced by thyroid hormones in normal rat liver, and the complete lack of increase in enzyme activity in Novikoff hepatoma under similar conditions. The activity of flavin adenine dinucleotide (FAD) pyrophosphorylase, the enzyme which synthesizes the cofactor (FAD) of mitochondrial α-glycerophosphate dehydrogenase, was determined in liver and in Novikoff hepatoma grown in the rat peritoneal cavity after treatment with thyroid hormones. Thyroxine (T_4) in large doses increased FAD pyrophosphorylase activity 40% in normal livers and in livers of hepatoma-bearing animals, but not in Novikoff hepatomas. Similarly, triiodothyronine increased FAD pyrophosphorylase activity in normal livers and in livers of hepatoma-bearing animals, but not in Novikoff hepatomas. These findings suggest that one mechanism which relates to the unresponsiveness of enzymes of Novikoff hepatoma to regulation by thyroid hormones may be lack of hormonal increase of FAD biosynthesis, which is required to stabilize newly formed apoenzymes.

In a previous investigation of the biochemical regulation of neoplasms by thyroid hormones, we demonstrated that the activity of mitochondrial α-glycerophosphate dehydrogenase in Novikoff hepatoma is not induced by thyroid hormones (1). By contrast, in livers of normal animals, and of animals bearing intraperitoneal transplants of Novikoff hepatoma, 5- to 10-fold increases in activity occur after administration of thyroid hormones (1–4).

The present studies were undertaken to elucidate possible mechanisms underlying the unresponsiveness of mitochondrial α-glycerophosphate dehydrogenase activity from Novikoff Hepatoma to regulation by thyroid hormones. One possibility considered was a disturbance in the control of synthesis of flavin adenine dinucleotide (FAD), the cofactor required by mitochondrial α-glycerophosphate dehydrogenase. To explore this hypothesis, measurements were made in liver and in Novikoff hepatoma of the activity of FAD pyrophosphorylase, which converts flavin mononucleotide (FMN) to FAD (5) and which is probably the rate-limiting step in the biosynthesis of FAD from riboflavin (6). Changes of the activity of this enzyme have been shown to correlate closely with determinations of the actual rates of FAD formation *in vivo* under a variety of experimental conditions (7). Studies were performed in rats after treatment with either thyroxine or triiodothyronine.

Materials and methods. Adult male Holtzman rats, obtained from the Holtzman Rat Company, Madison, Wisconsin, were used in all experiments. Animals were fed a commercial diet of Purina rat chow *ad libitum*.

For studies of FAD pyrophosphorylase activity, normal and tumor-bearing rats were treated with daily intraperitoneal injections of either thyroxine in pharmacological doses (300–500 μg/100 g/body wt) for 4–5 days or triiodothyronine (25 μg/100 g body wt) for 4 days. These extremely large doses were chosen to show that the tumor is truly unresponsive to thyroid hormones. Rats of the same age, sex, strain, and where possible, weight, were used as controls for the studies, and received injections of 0.9% NaCl solution of the same volume and pH as the thyroxine or triiodothyronine solutions. All rats were sacrificed 24 hr after the last injection.

To prepare tumor for transplantation,

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animals bearing the Novikoff hepatoma (9) were sacrificed and viable tumors were removed promptly. The tumor was minced and passed through a sieve, 0.914 mm in diameter. Tumor tissue was suspended in 3 vol of 0.9% NaCl, and a suspension of approximately 0.4–0.5 ml injected through a 20-gauge needle into the peritoneal cavity of the recipient animal. In studies of enzyme activities, animals were sacrificed 5–6 days after transplantation of the tumor. Novikoff hepatomas were obtained from animals in which the tumor line has been maintained by serial passage on a weekly basis for several years.

Animals were sacrificed by a blow to the head followed by decapitation and immediate exsanguination. Animals were generally sacrificed between 8 and 10 AM. Samples of normal liver and hepatoma were promptly removed. All studies were performed on fresh tissues.

Samples of tissues for assay of FAD pyrophosphorylase were homogenized with 5 vol of 0.25 *M* sucrose and were measured as previously from this laboratory (8). Enzyme activity was expressed as nanomoles FAD synthesized per milligram protein per hour. As noted previously from this laboratory by Hunt *et al.* (1), the enzyme in Novikoff hepatoma did not differ importantly in its properties from the enzyme in normal liver. The assay for the liver enzyme was suitable for assay of the tumor enzyme with minor modifications. Protein concentrations in the tissue suspensions were determined by the biuret method (10).

Results. Treatment with pharmacological doses of thyroxine increased the activity of FAD pyrophosphorylase approximately 40% ($P < 0.001$) in livers from normal animals, as previously observed (Fig. 1). In tumor-bearing animals, the incremental increase in hepatic enzyme activity produced by thyroxine was similar ($P < 0.001$). In Novikoff hepatoma, FAD pyrophosphorylase activity was similar to that of normal liver and, as in previous studies, was slightly less than in host liver. In contrast to results in liver from either normal or tumor-bearing animals, no effect of thyroxine administration was demonstrable in Novikoff hepatoma ($P > 0.05$). Lower doses of thyroxine and shorter periods of treatment also failed to increase FAD pyrophosphorylase activity in Novikoff hepatoma.

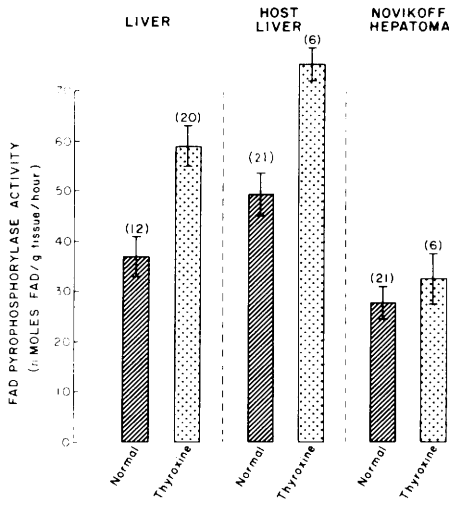

FIG. 1. Activity of FAD pyrophosphorylase in livers of normal animals, in livers of animals bearing intraperitoneal transplants of Novikoff hepatoma (host liver), and in samples of Novikoff hepatoma. Results are shown in groups of animals after daily intraperitoneal injections of pharmacological doses of thyroxine (300–500 μg/100 g body wt) for 4–5 days, and in controls treated with 0.9% NaCl solution. Data are shown as mean ± SE. Figures in parentheses refer to numbers of animals assayed.

Similar results occurred in rats treated with triiodothyronine, as shown in Table I. FAD pyrophosphorylase activity in hormone-treated rats was significantly greater than in controls ($P < 0.01$), and in tumor-bearing animals, triiodothyronine increased enzyme activity similarly ($P < 0.01$). Again, no effect of triiodothyronine administration was demonstrable in Novikoff hepatoma ($P > 0.05$).

Discussion. Mechanisms responsible for the lack of increase of activity of mitochondrial α-glycerophosphate dehydrogenase from Novikoff hepatoma in response to thyroid hormones are likely to be multiple. The Novikoff hepatoma is not unique in this respect, as Karsten *et al.* (11) have shown that in three other transplantable hepatomas thyroid hormones have no effect upon mitochondrial α-glycerophosphate

TABLE I. EFFECTS OF TREATMENT WITH TRIIODOTHYRONINE UPON ACTIVITY OF FAD PYROPHOSPHORYLASE IN LIVERS OF NORMAL RATS AND OF RATS WITH TRANSPLANTS OF NOVIKOFF HEPATOMA (HOST LIVER) AND IN NOVIKOFF HEPATOMA

Group	FAD pyrophosphorylase activity (nmole FAD/g tissue/hr)[b]		
	Liver	Host liver	Novikoff hepatoma
Control	33.7 ± 4.7	41.2 ± 2.2	25.6 ± 6.5
T_3	68.3 ± 6.9	76.9 ± 8.2	28.9 ± 5.9
Significance of difference	$P < 0.001$	$P < 0.01$	$P > 0.05$

[a] Daily intraperitoneal injections of triiodothyronine (25 μg/100 g body wt) were administered for 4 days; controls received isotonic saline of the same volume and pH.
[b] Mean ± SE, with seven or eight samples.

dehydrogenase activity. Unresponsiveness to thyroid hormones may relate to basic alterations in the mitochondria, either in their structure or function. In the Novikoff hepatoma, mitochondria have abnormal size and ultrastructure as well as striking reductions in the activities of a number of flavoprotein enzymes (12). By contrast, mitochondria from the well-differentiated Morris 7800 hepatoma, which we have shown respond to thyroid hormone (1), are structurally and functionally similar to normal mitochondria (13).

Unresponsiveness of mitochondrial α-glycerophosphate dehydrogenase activity in Novikoff hepatoma to thyroid hormone stimulation may be related to abnormalities in the regulation of synthesis of its cofactor, FAD. FAD-requiring enzymes tend to be stabilized by their cofactors, and when the rate of FAD synthesis is high, activities of FAD-dependent enzymes tend generally to increase (14, 15). In Novikoff hepatoma without thyroid hormone stimulation the rate of FAD biosynthesis relative to the concentration of FAD is already very high (16). Therefore, the holoenzyme of mitochondrial α-glycerophosphate dehydrogenase in Novikoff hepatoma may not accumulate further under stimulation with thyroid hormones because the formation of the coenzyme is already maximal, and cannot be increased further. The finding that FAD pyrophosphorylase, which synthesizes FMN from FAD, is not increased in activity in Novikoff hepatoma even by very large doses of thyroid hormones is compatible with the hypothesis that FAD synthesis is not being increased in the tumor. Under a variety of conditions, the activity of FAD pyrophosphorylase is a reliable indicator of the rate of FAD formation in tissues *in vivo* (7).

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1. Hunt, S. M., Osnos, M., and Rivlin, R. S., Cancer Res. **30**, 1764 (1970).
2. Lee, K. L., and Miller, O. N., Arch. Biochem. Biophys. **120**, 638 (1967).
3. Lee, Y. P., Takemori, A. E., and Lardy, H., J. Biol. Chem. **234**, 3051 (1959).
4. Rivlin, R. S., and Wolf, G., Nature (London) **223**, 516 (1969).
5. McCormick, D. B. *in* "Riboflavin" (R. S. Rivlin, ed.), p. 153. Plenum Press, New York (1975).
6. Yagi, K., Nagatsu, T., Nagatsu-Ishibashi, I., and Ohashi, A., J. Biochem. **59**, 313 (1966).
7. Fazekas, A. G., Pinto, J., Huang, Y. P., and Rivlin, R. S., Endocrinology **102**, 641 (1978).
8. Rivlin, R. S., and Langdon, R. S., Endocrinology **84**, 584 (1969).
9. Novikoff, A. B., Cancer Res. **17**, 1010 (1957).
10. Gornall, A. G., Bardawill, C. J., and David, M. M., J. Biol. Chem. **177**, 751 (1949).
11. Karsten, U., Sydow, G., Wollenberger, A., and Graffi, A., Acta Biol. Med. Germ. **26**, 1131 (1971).
12. White, M. T., and Tewari, K. K., Cancer Res. **33**, 1645 (1973).
13. Pedersen, P. L., Greenawalt, J. W., Chan, T. L., and Morris, H. P., Cancer Res. **30**, 2620 (1970).

14. Rivlin, R. S., Advan. Enzyme Regul. 8, 239 (1970).
15. Rivlin, R. S., Fazekas, A. G., Huang, Y. P., and Chaudhuri, R., *in* "Flavins and Flavoproteins" (T. P. Singer, ed.), p. 747. Elsevier, Amsterdam (1976).
16. Rivlin, R. S., Fazekas, A. G., and Chaudhuri, R., *in* "Frontiers of Internal Medicine" (S. H. Blondheim, ed.), p. 175. Karger, Basel (1975).

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