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Induction of Mitochondrial α-Glycerophosphate Dehydrogenase by Thyroid Stimulating Hormone and Thyroid Hormone Analogues.* (32393)

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Y-P Lee *et al*(1) observed that administration of thyroid hormone to rats led to a marked increase in the activity of liver mitochondrial alpha-glycerophosphate dehydrogenase (LM-GPDH) but caused no change in the activity of the soluble diphosphopyridine nucleotide linked GPDH. Reports from this and other laboratories indicate that the induction of LM-GPDH by thyroid hormone may result from the acceleration of enzyme protein synthesis, which is mediated through an increase of RNA synthesis, as judged by the fact that this induction is sensitive to the inhibition by ethionine, puromycin, actinomycin D, 5-fluorouracil (1-5) and fasting(5, 6). One of the characteristic features of thyroid hormone action is its biphasic effect on the body growth as well as the metabolism of some cellular constituents(7). Since the dosage of thyroid hormone used to observe the induction of LM-GPDH was much greater than the amount of thyroid hormone normally produced under physiological conditions, it is important to determine whether the observed induction is a physiological or a pharmacological effect.

In this communication, experimental results are presented which further support the view that the LM-GPDH activity is under the control of thyroid hormone in that administration of either L-triiodothyronine or thyroid-stimulating hormone (TSH) to hypophysectomized rats resulted in enzyme induction and a good correlation was found between the inducing ability of iodine containing structurally similar compounds and its hormonal activity.

Materials and methods. All animals utilized in these studies weighed 200-280 g (intact) or thyroidectomized (THX) and hypophysectomized (HYX) rats weighed 100-130 g and were obtained from the Charles River Laboratories. They were fed *ad libitum* on a Purina chow-diet and were allowed free access to drinking water. For maintenance of THX rats 1% calcium gluconate was added in the drinking water. The THX and HYX rats were not used until 4 weeks after their receipt from the supplier. During this period, the HYX rats gained essentially no weight.

3,3′,5-triiodo-L-thyronine (T_3), 3, 3′, 5,5′-tetraiodo-L-thyronine (L-thyroxine), 3, 3′, 5,5′-tetraiodo-D-thyronine (D-thyroxine), 3-iodo-L-thyronine and 3-iodo-L-tyrosine were purchased from Sigma Chemical Co. 3,5-diiodo-L-thyronine was obtained from Mann Research Laboratories. 3,5-diiodo-L-tyrosine was obtained from Nutritional Biochemicals

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Corp. 3,3'5-triiodothyroacetic acid was a product of Aldrich Chemical Co. All iodine containing compounds were prepared in alkaline-saline(8) and injected intraperitoneally to the animal at a dosage specified in the legends of the tables. TSH, a product of Armour Pharmaceutical Co., was administered intraperitoneally in saline at the dosage and frequencies specified in the legends of tables. Mitochondria were isolated from homogenates of liver, made in 0.25 *M* sucrose, according to the procedure of de Duve *et al*(9). The twice washed mitochondrial pellet was suspended in either 0.05 *M* sodium phosphate buffer, pH 7.6 or 0.25 *M* sucrose solution.

The activity of LM-GPDH was measured either colorimetrically as described by Y-P. Lee and Lardy(10) with slight modifications (11) or enzymatically as previously described (3). In the former instance, LM-GPDH activity was expressed as units per mg of mitochondrial protein (one unit of LM-GPDH activity was defined as 0.001 change in optical density per minute determined at 500 mμ under specified conditions), while in the latter case, LM-GPDH activity was expressed as μmoles of dihydroxyacetone phosphate (DHAP) formed per mg mitochondrial protein per hour at 30°C. Mitochondrial protein was determined according to the method of Lowry *et al*(12).

Results and discussion. Table I shows that the LM-GPDH level of HYX rats was decreased as compared to the level found in the intact control animal and the lowered level of LM-GPDH in HYX rats could be rapidly restored after a single injection of T_3. These findings confirm the early observation of Ruegamer *et al*(13). In addition, HYX rats responded to TSH administration by increasing the level of LM-GPDH. Quantitative differences were noted between the increase in enzyme level of HYX animals after treatment with TSH (20 m units/rat/day) for 2 days or for 6 days; the former exhibiting a 35% and the latter a 45% increase over HYX controls. When HYX rats were treated with TSH at the dose of 100 m units/rat/day, the activity of LM-GPDH increased more than that of rats treated with 20 m units/rat/day. These results indicate that the increase of LM-GPDH level in HYX rats by TSH depends on both dosage and duration of treatment with the hormone. These observations demonstrate that the endogenously produced thyroid hormone exerts an inducing action similar to the response observed when exogenous hormone, at higher dosages, is administered; and, therefore, strongly suggest that the induction of LM-PDH by thyroid hormone is the result of the physiological action of the hormone.

A number of compounds structurally related to T_3 were evaluated as possible inducing agents in an attempt to correlate the chemical structure of the compound with its inducing ability. As shown in Tables II and III. T_3 is the most potent inducer tested, although tetraiodothyroacetic acid is quite effective. L-thyroxine is about 80% as effective

TABLE I. Effect of Administration of T_3 or TSH to HYX Rats on LM-GPDH Activity.

Group	Treatment	No. of animals	Enzyme activity, units/mg mitochondrial protein	% of control	
				Group A = 100	Group B = 100
A	None	5	140 (121–148)*	100	
B	Hypophysectomy	6	60 (46– 70)	44	100
C	" + T_3	4	166 (155–176)	119	277
D	" + TSH	5	81 (76– 87)	58	135
E	" + "	5	87 (85– 90)	62	145
F	" + "	7	105 (88–131)	75	175

The rats were treated as follows: Group A received saline (euthyroid controls); group B received saline 4 wk after operation; group C received T_3 (100 μg/100 g body wt) 4 wk after operation and was sacrificed 24 hr after injection; group D received TSH (20 m units/rat/day) for 2 days and was sacrificed on the fourth day; group E received TSH (20 m units/rat/day) for 6 days and was sacrificed on the seventh day; group F received TSH (100 m units/rat/day) for 6 days and was sacrificed on the seventh day.

* Average (range).

TABLE II. Effect of Iodine Derivatives of Tyrosine and Thyronine on LM-GPDH Level of THX Rats.

Treatment†	12 hr No. of rats	12 hr LM-GPDH, μmoles × 10^{-3}/mg protein/hr	12 hr % Controls	24 hr No. of rats	24 hr LM-GPDH, μmoles × 10^{-3}/ protein/hr	24 hr % Controls
Saline (control)	4	233 (222–255)	100	4	277 (253– 297)*	100
3,3',5-Triiodo-L-thyronine	4	400 (390–430)	174	4	1136 (950–1240)	410
3,3',5,5'-Tetraiodo-L-thyronine	4	265 (222–272)	114	4	893 (790–1020)	322
3,3',5,5'-Tetraiodo-D-thyronine	4	216 (202–212)	93	4	435 (320– 530)	157
3.3',5-Triiodothyroacetate	4	341 (280–384)	147	4	951 (840–1050)	344
3,5-Diiodo-L-thyronine	3	235 (210–260)	101	3	324 (316– 344)	117
3,5-Diiodo-L-tyrosine	3	236 (226–246)	101	2	309 (310– 308)	111
3-Iodo-L-tyrosine	3	241 (218–246)	103	2	276 (251– 301)	100

* Average (range).
† 0.38 × 10^{-7} M/100 g of each compound was administered to the animal.

TABLE III. Effect of Iodine Derivatives of Tyrosine and Thyronine on LM-GPDH Level of Euthyroid Rats.

Treatment‡	No. of rats†	LM-GPDH, μmoles × 10^{-3}/mg protein/hr	% Control
Saline (control)	4	791 (768– 817)*	100
3,3',5-Triiodo-L-thyronine	6	1555 (1470–1650)	196
3,3',5,5'-Tetraiodo-L-thyronine	5	1086 (983–1145)	137
3,3',5,5'-Tetraiodo-D-thyronine	3	1012 (975–1030)	128
3,3',5-Triiodothyroacetate	3	1373 (1280–1430)	173
3,5-Diiodo-L-thyronine	3	1030 (940–1080)	130
3,5-Diiodo-L-tyrosine	2	799 (785– 812)	101
3-Iodo-L-tyrosine	2	915 (830–1000)	116

* Average (range).
† All rats sacrificed 18 hr after the injection.
‡ 1.53 × 10^{-7} M/100 g of each compound was administered to the animal.

as T_3 in a 24-hour induction period although it is only about 20% as effective as T_3 in a 12-hour induction period (Table II). This may be explained as a consequence of T_3 being less tightly bound to the thyroid hormone-binding globulin or being more permeable than L-thyroxine(14,15). D-thyroxine showed about one-fifth of the effectiveness of its L-isomer in increasing LM-GPDH 24 hours after its administration; however, D-thyroxine had essentially no effect in a 12-hour induction period. 3,5-diiodothyronine and the iodine derivatives of tyrosine tested were essentially inactive. Recently, Short and Ruegamer(16) observed that the route of the administration as well as the presence of an antithyrotoxic substance in the diet markedly affects the relative inducing activities of thyroxine analogues. Of particular interest in the present study is that the effectiveness of iodine containing structurally similar compounds on the induction of LM-GPDH activity, is in good agreement with its calorigenic effect. Such an observation along with the findings that LM-GPDH activity decreases after thyroidectomy(3,17,18) hypophysectomy (Table I) or feeding a diet containing thiouracil(11,18) and that the enzyme adaptively increases after administration of thyroid hormone to euthyroid(1,2,18) THX(3,17,18), or thiouracil-fed(11,18) rats therefore, strongly suggests that the level of LM-GPDH is controlled by thyroid hormone. The absence of induction by substrate(11) and the lack of effect of other hormones(13) on LM-GPDH activity further

indicates the specificity of the induction.

It has been emphasized that the enzyme activity assayed in tissue preparations does not necessarily indicate the enzyme concentration(19). The possibility that the increase in LM-GPDH activity by T_3 may result from the presence of an activator or the absence of an inhibitor in the T_3-treated mitochondrial preparation was tested by experiments in which varying volumes of the mitochondrial suspension from either normal or T_3-treated animals were cross-mixed or added to varying volumes of heat-treated post-mitochondrial supernatants. The results shown in Table IV reveal that the additivity of LM-GPDH activity when varying proportions of the preparations made from control or treated livers were mixed. In addition, heat-treated post-mitochondrial supernatant had no effect on the enzyme activity. The present observations further support the previous suggestion that the increased LM-GPDH after the administration of thyroid hormone does not result from changes of enzyme kinetic properties(11).

TABLE IV. LM-GPDH Activity of "Admixtures" of Normal Mitochondria and T_3-Treated Liver Mitochondria.

Source of material	LM-GPDH activity (μmoles/g/hr) Found	Expected‡
Normal animals (A)	11.2	—
T_3-treated* animal (B)	41.0	—
Mixture of A and B (1:2)	31.7	31.0
Mixture of A and B (2:1)	22.5	21.5
Mixture of A and supernatant of B† (1 ml)	11.2	11.2
Mixture of A and supernatant of B (0.5 ml)	10.8	11.2
Mixture of B and supernatant of A† (1 ml)	39.4	41.0
Mixture of B and supernatant of A (0.5 ml)	39.8	41.0

* 36 hr after T_3 administration.
† Post-mitochondrial fraction was heated for 10 min in a boiling water bath. The precipitate was removed by centrifugation and the supernatant used for the test.
‡ Expected, assuming no kinetic effect.

Summary. The level of mitochondrial L-alpha-glycerophosphate dehydrogenase of rat liver is greatly decreased after hypophysectomy. The lowered enzyme level in these animals was rapidly restored after administration of a single dose of L-triiodothyronine. Thyroid-stimulating hormone given to hypophysectomized rats, gradually increased the enzyme activity and this response depended on both the dosage and the duration of treatment. A number of iodine-containing compounds were used to evaluate the chemical specificity of structure of inducing molecules. The results showed a good correlation between the inducing ability of these analogues and its hormonal activity. The reported observations support the idea that mitochondrial L-alpha-glycerophosphate dehydrogenase activity is controlled by the thyroid hormone. The "admixture test" failed to show the presence of an activator or the absence of an inhibitor in the mitochondrial preparation obtained from L-triiodothyronine-treated animals. The present observations further support the previous suggestion that the increase of mitochondrial L-alpha-glycerophosphate dehydrogenase activity after the administration of thyroid hormone may result from acceleration of enzyme synthesis even when stimulated by physiological amounts of the hormone.

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Comparison of Anticholinergic Potency to Psychotomimetic Action of Glycolate Esters. (32394)

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Ever since Ehrlich proposed the "key and analogy" to explain the action of many drugs, the concept of drug action has been viewed in a more systematic way. According to this theory, the biologic site for attachment of a drug consisted of a configuration analogous to the chemical structure of the drug. A substance such as acetylcholine was presumed to have a corresponding "receptor site" located at nerve endings and elsewhere in the nervous system. Atropine, on the other hand, exerted its effect by blocking the action of acetylcholine.

A molecule, such as atropine, can interact with a biological receptor site in various ways (1). The tertiary nitrogen, after protoration, can electrostatically interact with an anionic group on the receptor; while the carbonyl group can undergo hydrogen bonding with such functional groups as NH_2, OH, and SH. A van der Waal interaction can also occur between the aromatic group and lipophilic regions on the receptor site(2). The steric configuration of atropine and related substances must conform to certain chemical as well as spatial requirements for activity(3).

Such blocking agents have been extremely powerful tools in the elucidation of the mechanism and site of action of chemical transmitters. It is almost a tautology to assert that before a drug can produce an effect on a biological system it has to react, or interact, with a molecule of the system; consequently, there must be similarities in chemical constitution. There is a danger, however, in assuming that a drug like atropine can only act by blocking the action of acetylcholine, thereby relegating it to the role of a "physical obstruction".

The purpose of the present experiments was to present evidence that the action of many of the so-called anticholinergic agents upon the central nervous system is independent of acetylcholine. A group of glycolic esters of aliphatic and cyclic amines have been shown to possess hallucinogenic and anticholinergic properties(4,5). These studies have indicated 1) that anticholinergic potency did not always parallel psychotomimetic potency and 2) the administration of cholinergic agents, such as diisopropylfluorophosphate, did not antagonize the central actions of the glycolate esters. Besides contributing further evidence of this nature, the present study is concerned with extending the list and types of compounds in this series which possess psychotomimetic properties.

Methods. The compounds discussed were kindly provided by L. G. Abood. Anticholinergic action was measured by use of the superperfusion technique on the isolated smooth muscle preparation(6) and by measuring the degree of mydriasis in rats. The minimal concentration necessary to produce maximal mydriasis when a solution was applied directly into the rat's eye was used as the index for mydriatic effectiveness, while the ED_{50} (effective dose for 50% blockade of acetylcholine) was used to express anticholinergic activity. Hyperactivity was deter-