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ISOPROPYLDIIODOTHYRONINE AND α-METHYLTHYROXINE: COMPARISON OF THEIR *IN VITRO* AND *IN VIVO* EFFECTS WITH THOSE OF THYROID HORMONES

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Abstract—The *in vivo* and *in vitro* effects of the two thyroid hormone analogs 3,5-diiodo-3′-isopropyl-L-thyronine (IPT_2) and of α-methyl-DL-thyroxine (MT_4) have been compared to those of the thyroid hormones 3,5,3′-triiodo-L-thyronine (T_3) and thyroxine (T_4).

Against purified glutamate, isocitrate and alcohol dehydrogenases IPT_2 and MT_4 exert inhibitory effects which are quite similar to those exerted by T_3 and T_4. The effects of the analogs on isolated mitochondria follow closely those of the hormones i.e. they uncouple phosphorylations and inhibit electron transfer along the respiratory chain.

When thyroidectomized animals are treated with analogs (5 nmoles/100 g/day for 15 days) their effects on two accepted parameters of thyromimetic activity, metabolic rate (BMR) and α-glycerophosphate (GPD) induction, are quite different. After IPT_2-treatment, the rise in MR and GPD indicates a hyperthyroid state, whereas MT_4-treatment results in very incomplete compensation of the hypothyroid state. However, treatment of the thyroidectomized animal with IPT_2 or MT_4 has the same effect on hepatic mitochondrial glutamate dehydrogenase as treatment with T_4.

A relation between thyroid state and adrenal tyrosine hydroxylase (TH) activity has been established; TH activity which decreases in the thyroidectomized animal, is brought back to normal by administration of T_4 and above normal by that of IPT_2.

Thyroid hormones exert an inhibitory effect *in vitro* on a large number of purified enzymes [1–6] and affect *in vivo* the activity of a variety of tissue enzymes, enhancing the activity of some and decreasing that of others [7–12]. There appears to be no obvious relation between the effects observed *in vitro* and those seen in the treated animal [13, 14].

Iodinated phenols lacking a *para*-hydroxylated diphenylether moiety manifest none of the effects exerted by thyroid hormones on enzymes [15–17] while structural analogs of thyroid hormones such as the formic acetic and propionic derivatives of 3,5,3′-triiodo-L-thyronine show all the effects exerted by the parent compound *in vitro* or in the treated animal [18–20].

It appeared of interest to study the effect of 3′-isopropyl-3,5-diiodo-L-thyronine (IPT_2) and that of α-methyl-DL-thyroxine (MT_4) [21] two thyroid hormone analogs vastly different from those investigated previously. The isopropyl analog is characterized by the replacement of the 3′iodine, i.e. of the iodine ortho to the phenolic hydroxyl group by the more lipophilic isopropyl group [22, 23]. The effect of IPT_2 on basal metabolism and on mitochondrial respiration are of the same order as those of 3,5,3′-triiodo-L-thyronine but its effects as an *in vitro* enzyme inhibitor as well as its *in vivo* effects on tissues enzymes have not been investigated; α-methylthyroxine is known to be ineffective as a thyromimetic but no systematic study of its effects on enzymes *in vivo* or *in vitro* has been undertaken.

In a first series of *in vitro* assays the effects of the isopropyl analog and of α-methylthyroxine were compated to those of 3,5,3′-triiodo-L-thyronine and of L-thyroxine. The enzymes chosen for study were glutamate dehydrogenase (E.C.1.4.1.3), isocitrate dehydrogenase (E.C.1.1.1.42) and alcohol dehydrogenase (E.C.1.1.1.1), enzymes which had been shown in this laboratory to be effectively inhibited by thyroid hormones and their acid analogs [13, 14]. Simultaneously, the effects of the isopropyl analog and of α-methylthyroxine on the oxidative phosphorylation of rat liver mitochondria was investigated.

During the *in vivo* assays the effects of the isopropyl analog and of α-methylthyroxine treatment on liver mitochondrial glycerophosphate dehydrogenase and on glutamate dehydrogenase were measured. This led us to compare in thyroidectomized animals the calorigenic effect of isopropyldiiodothyronine, α-methylthyroxine or thyroxine with the effect exerted by these compounds on the activities of liver glycerophosphate dehydrogenase, an effect considered as a valid test of thyromimetic activity [27–29] and of glutamate dehydrogenase, another enzyme shown to be affected by thyroid hormones [7–11].

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Since tyrosine hydroxylase is the rate-limiting step of catecholamine biosynthesis [30, 31] and since many adrenergic effects are in many ways dependent upon thyroid status [32–34], it seemed appropriate to investigate the effect of L-thyroxine and of isopropyl-diiodo-L-thyronine administration on the adrenal tyrosine hydroxylase activity of thyroidectomized animals.

MATERIALS AND METHODS

3,5,3′-Triiodo-L-thyronine (T_3) and L-thyroxine (T_4) were obtained as the sodium salts from the Sigma Chemical Company. 3′-Isopropyl 3,5-diiodo-L-thyronine (IPT_2) was a gift from Dr. E. C. Jorgensen* and α-methyl-DL-thyroxine (MT_4) a gift from Chemie Grünenthal. Rotenone and oligomycin were furnished by Sigma, iodonitrophenyltetrazolium by Biochim, NADPH and ADP by Boehringer and 6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$) by Calbiochem. L-3,5-[H^3]tyrosine (53 mCi/μmole) was an Amersham product. PPO and dimethyl POPOP were Merck products.

Pig heart isocitric dehydrogenase (ICDH, threo-D-isocitrate NADP oxydoreductase) and horse liver alcohol dehydrogenase (ADH) were Boehringer products while bovine liver L-glutamate dehydrogenase (GLDH) was obtained from Sigma. Catalase was prepared according to Lallemand and Martin [35] and was diluted from a suspension containing 350,000 i.u. and 137.5 mg/ml. Dihydropteridine reductase was prepared from sheep liver following the procedure of Kaufman [36] through the first two ammonium sulfate precipitation steps.

Assay of pure enzymes. Glutamate dehydrogenase activity was followed at in 0.2 M potassium phosphate buffer, pH 7.6, in the presence of 15 mM glutamate, 0.4 mM NAD^+ and 0.4 μg enzyme protein/ml [37].

Alcohol dehydrogenase activity measurements were made in a 0.1 M pH 8.5 sodium pyrophosphate buffer containing 10 mM ethanol, 2% (w/v) semicarbazide, 0.7 mM NAD^+ and 6.6 μg enzymatic protein/ml [38].

Isocitrate dehydrogenase activity measurements were carried out in a medium containing 1 mM sodium isocitrate, 0.58 mM $MnCl_2$ and 0.42 mM $NADP^+$ in a pH 7.6 0.2 M potassium phosphate buffer [39]. The concentration of enzyme protein was of 40 μg/ml.

The reduction of the coenzymes was followed at 340 nm in a Beckman DB spectrophotometer; assays in the presence of IPT_2, MT_4, T_3 and T_4 were carried out at five different effector concentrations.

Assays of isolated mitochondria. Rat liver mitochondria were prepared by the method of Beattie [40]. Oxygen uptake measurements were performed in a Gilson oxygraph at 25° with vibrating platinum electrode. The State 3 and State 4 respiration of the mitochondria was studied in a medium containing 3 mM $KHPO_4$, 13 mM K_2HPO_4, 26 mM NaCl, 58 mM KCl, 6 mM $MgCl_2$ and 12 mM, NaF at a pH of 7.35. Succinate (6.7 mM) in the presence of rotenone (1,5 μM) and glutamate–malate (16 mM) were used as substrate and ADP (320 μM) was used to initiate State 3 respiration; the amount of mitochondrial suspension used in each assay was 1.5 mg protein/ml incubation medium. The iodinated products, solubilized in 10^{-2} N NaOH, were introduced in the oxygraphic cell during State 3 or State 4 respiration in a volume not exceeding 80 μl. In experiments dealing with the effects of the iodinated analogs in inhibited or uncoupled oxidative phosphorylation the iodinated analogs was introduced after the addition of the inhibitor oligomycin (5 μg dissolved in 25 μl ethanol) or of the uncoupling agent 2,4-dinitrophenol (50 μM). The final volume of incubation in the oxygraphic cell was 1.6 ml. The addition of the solvents i.e. 80 μl 10^{-2} N NaOH or 20 μl ethanol was found to have no effect on the O_2 consumption.

In vivo *assays.* Male Wistar rats were used in all experiments. Thyroidectomy was performed surgically on animals weighing 60–80 g. The animals were divided in five experimental groups: normal, thyroidectomized and thyroidectomized treated with IPT_2, MT_4, and T_4, respectively. Each group was made up of eight animals housed in not more than four animals per cage. Treated rats received a daily intraperitoneal (i.p.) injection of 5 nmoles/100 g body wt for 15 days; the two control groups received a saline injection.

At the end of the treatment period the basal metabolic rate of each animal was measured in a metabolic chamber at 28° connected to a Beckman No. 777 oxygen analyzer. Animals were sacrificed by decapitation 24 hr after the last injection.

The liver was removed immediately upon sacrifice and placed in ice-cold 0.25 M sucrose buffered to pH 7.4 with 2 mM Tris. Mitochondria were isolated from each separate liver according to Beattie [40] and suspended in 2 ml 0.25 M sucrose; the protein concentration was of about 20 mg/ml. An aliquot of the mitochondrial suspension served for the assay of α-glycerophosphate dehydrogenase (GDP) activity and another aliquot, to which Triton-X-100 (1%, w/v) was added, served for the assay of glutamate dehydrogenase (GLDH). Protein was measured on a third aliquot, after addition of desoxycholate, by the method of Gornall [41] with bovine serum albumin as standard.

The adrenal glands of each animal, isolated immediately upon sacrifice, were weighted and homogenized in a Teflon Potter homogenizer in 1.0 ml ice cold 5 mM Tris, pH 7.4, containing 0.1% Triton-X-100. The homogenate was centrifuged at 20,000 *g* for 20 min and the supernatant fraction frozen until used for the assay of tyrosine hydroxylase (TH); proteins were measured on this fraction according to the method of Lowry [42].

Measurements of enzyme activity. Mitochondrial GLDH activity was measured 15 min after the addition of Triton X-100 to the mitochondrial suspension. The enzymatic reduction of 0.4 mM NAD^+ was followed at 340 nm in the presence of potassium glutamate in potassium phosphate buffer at pH 7.6 [37]. The activity is expressed as nmoles glutamic acid oxidized per min per mg mitochondrial protein.

Mitochondrial GPD was measured according to the method of Gardner [43]: the incubation medium contained 25 μl 0.5 M glycerophosphate, 125 μl 0.4%

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Table 1. Inhibition of some purified dehydrogenases by thyroxine, α-methyl-DL-thyroxine 3,5,3'-triiodo-L-thyronine and 3'-isopropyl-3,5-diiodo-L-thyronine

Inhibitor	I_{50}* Beef liver glutamate dehydrogenase	I_{50} Pig heart isocitrate dehydrogenase	I_{50} Horse liver alcohol dehydrogenase
L-Thyroxine	0.50×10^{-5} M	1.0×10^{-4} M	1.8×10^{-4} M
α-Methyl-DL-thyroxine	1.0×10^{-5} M	8.5×10^{-5} M	inhibition = 20% with 1.8×10^{-4} M
3,5,3'-Triiodo-L-thyronine	0.8×10^{-5} M	2.1×10^{-4} M	6.1×10^{-5} M
3'-Isopropyl-3,5-diiodo-L-thyronine	0.6×10^{-5} M	2.3×10^{-4} M	1.6×10^{-4} M

* I_{50}: Concentration necessary to inhibit enzyme activity by 50 per cent in the present experimental conditions.

(w/v) aqueous iodonitrophenyltetrazolium, 25 μl 0.5 M potassium phosphate containing 0.01 M KCl, 50 μl 0.03 M phosphate buffer and 20 μl of mitochondrial suspension containing 0.4 mg protein. Assays carried out without protein and without substrate served as blanks. The enzyme reaction proceeded for 30 min at 37° and was stopped by the addition of 1.0 ml M acetic acid. The color was extracted by 2.5 ml ethyl acetate and the organic layer was then separated by centrifugation and read in a spectrophotometer at 500 nm. Enzyme activity is expressed in 500 nm absorbance units per min per mg protein.

Adrenal TH activity was measured according to the method of Shiman, Akino and Kaufman [44] modified to be of first order with regard to time and to enzyme protein. To 100 μl of adrenal homogenate supernatant were added 50 μl of dihydropteridine reductase preparation [36], 50 μl of catalase containing 7,000 i.u., 50 μl of 10 mM aqueous NADPH and 50 μl of $DMPH_4$ in 1 mM HCl. After equilibration in a shaking water bath for 7.5 min at 37°, 100 nmoles tyrosine (0.25 pCi) and 30 μmoles phosphate buffer, pH 6.0, were introduced in a volume of 200 μl. After 15 min at 37° the enzyme reaction was stopped by rapid congelation in alcohol-dry ice. The solution was lyophilized at 10^{-2} Torr and the lyophilized water collected in liquid nitrogen in 30-ml standard tapered traps. The assay was performed in triplicate and 100 μl aliquots of Tris–Triton buffer, handled as the supernatant fractions, served as blanks. A 400 μl of the lyophilized water was transferred to a liquid scintillation vial containing 15 ml of a toluene–triton cocktail containing 0.4% PPO (w/v) and 0.01% dimethyl POPOP (w/v) and counted in a Packard Tricarb liquid scintillation counter.

RESULTS

In vitro *inhibition of pure enzymes*. The values of I_{50}, the inhibitor concentration inhibiting the enzyme by 50 per cent in the experimental conditions used, are gathered in Table 1. It is seen that the effect of IPT_2 on ICDH is very close to that of T_3 and that MT_4 has an effect of the same order of magnitude as T_4 on this enzyme. The α-methyl substituted MT_4 has half the inhibitory activity of T_4 on GLDH but the replacement of iodine by isopropyl at 3' has little effect on inhibitory activity. However IPT_2 is a markedly weaker inhibitor of ADH than T_3 and the inhibitory effect of MT_4 is very weak at the I_{50} of T_4.

In vitro *mitochondrial effects*. The oxygraph tracings allowed us to establish the rates of oxygen uptake by hepatic mitochondria placed in different respiratory states; a sample experimental tracing is given in Fig. 1. The effects of the analogs investigated

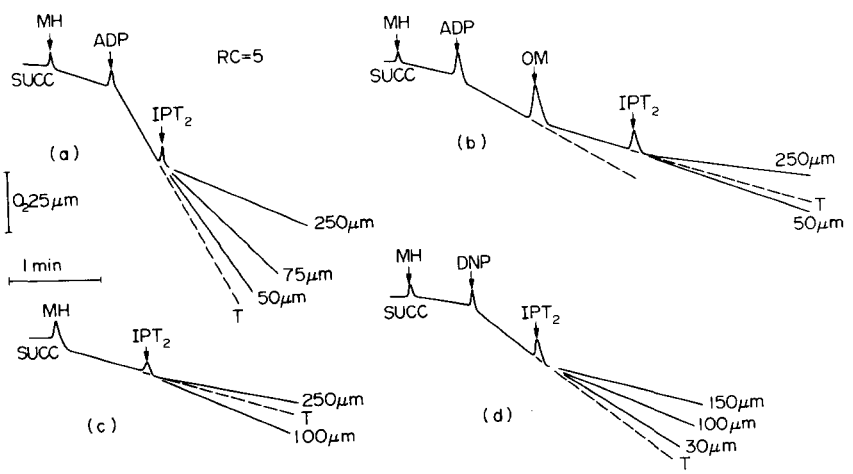

Fig. 1. Oxygen electrode tracings representing the effect of IPT_2 on mitochondrial respiration with succinate 6.7 mM as substrate in the presence of rotenone (1.5 μM) (1.52 mg mitochondrial protein/ml) (a) State 3; (b) State 3 in the presence of oligomycin (5 μg) (OM); (c) State 4; (d) State 4 in the presence of 2–4 dinitrophenol (50 μM) (DNP).

are expressed as percentage change in respiratory rate observed after addition of the analog. The four plots of Fig. 2 represent the effects obtained in function of the concentration of iodinated effectors with succinate as substrate: the first one (A) is concerned with State 3, the second (B) with State 4, the third (C) shows the effects on State 3 in the presence of oligomycin and the fourth (D) the effects on State 4 uncoupled by dinitrophenol.

Assays performed with glutamate–malate gave similar results. One sees from these representations a marked similarity between IPT_2 and T_3 as well as between MT_4 and T_4. All these substances have an inhibitory effect on State 3 while State 4 respiration is stimulated by weak doses of iodinated thyronines but inhibited by greater concentrations. However, while the optimal concentration needed to stimulate State 4 and to inhibit State 3 by 50 per cent are very close to each other for T_3 and T_4, MT_4 is more stimulating than inhibitory and IPT_2 has an inhibitory effect which is more marked than its stimulating effect. Finally, State 3 mitochondrial respiration in the presence of oligomycin is stimulated to a much smaller degree by IPT_2 than by T_3.

In vivo *effects on hepatic mitochondria.* The glycerophosphate dehydrogenase and glutamate dehydrogenase activity of hepatic mitochondria as well as basal metabolic rate data obtained 24 hr before sacrifice are presented in Table 2. Determinations were carried out on each animal and the table indicates mean as well as statistical spread and significance.

A good correlation is seen between variations in metabolic rate and in GPD activity although variations in GPD activity appear much more sensitive than those of the metabolic rate in the whole animal. Thus, while overall variations in metabolic rate vary over a twofold range, GPD activity decreases to one-fourth of its activity upon thyroidectomy while treatment of the thyroidectomized animal with T_4 increases its activity by a factor of 10 and with IPT_2 by a factor of 20. MT_4 has no effect on metabolic rate but increases the GPD activity of the thyroidectomized animal by a factor of 2, i.e. to half that of the normal animal. The effects of analogs on GLDH are quite significant but less pronounced: thyroidectomy leads, as observed previously in this laboratory [13], to a lowering of enzyme activity but treatment with T_4, IPT_2 and even MT_4 leads, in the present experimental conditions, to an increase in activity to a level above that seen in the normal animal. Table 2 shows that MT_4, which has no detectable effect on oxygen uptake and produces but a weak increase in GPD activity has an effect on GLDH similar to that of IPT_2 or T_4.

In vivo *effects on adrenal tyrosine hydroxylase.* The activity of this enzyme, expressed in Table 3 as nmole DOPA formed per hr per pair of glands is also related to adrenal protein and to animal weight as these two parameters are known to vary considerably with the thyroid state [33].

The overall TH activity, as expressed in nmoles DOPA formed (Table 3), is significantly decreased after thyroidectomy (about 40 per cent). The treatment of the thyroidectomized animal with T_4 compensates for the decrease in overall TH activity but the TH activity expressed in nmoles per mg of adrenal protein remains below that of the normal animal. Treatment of the thyroidectomized animal with IPT_2 results in a 15 per cent increase in overall TH activity and to a specific activity equal or superior to that of the normal animal. If one relates TH activity to body weight the same type of variations is observed.

The decrease in adrenal TH activity which is seen to occur after thyroidectomy closely correlates with the decrease in metabolic rate: thus the ratio of adrenal TH activity (related to body weight) to metabolic rate is approximately equal in the normal and in the thyroidectomized rat. The treatment of thyroidectomized animals resulted, in our experimental conditions, in increases in adrenal activity which

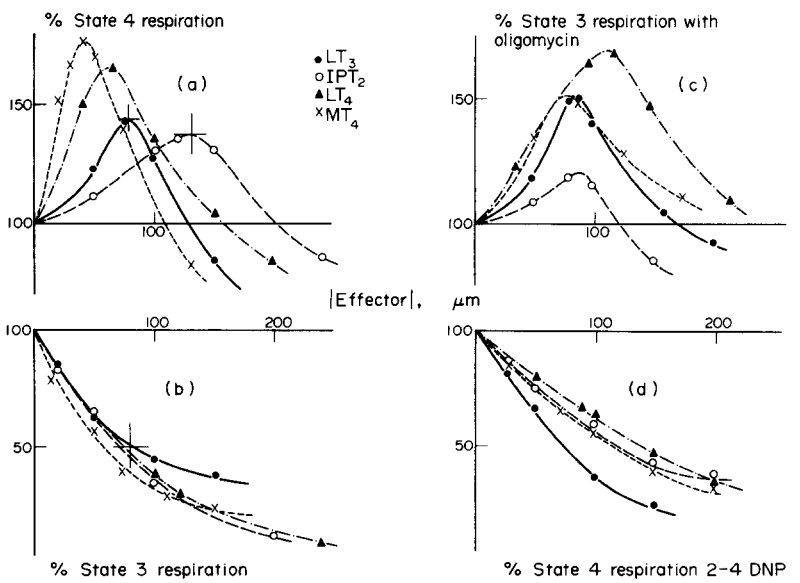

Fig. 2. Plots showing the effects of T_3, IPT_2, T_4 and MT_4 on the respiration of rat liver mitochondrial in the presence of succinate. Control values (100%) were obtained in the absence of effectors: (A) State 4; (B) State 3; (C) State 3 with oligomycin (5 μg); (D) State 4 with 2-4 DNP (50 μM).

Table 2. Effects of thyroid states and of the treatment of thyroidectomized animals with thyroid analogs on the hepatic mitochondrial L-α-glycerophosphate and L-glutamate dehydrogenase activities

Treatment group	L-α-glycerophosphate dehydrogenasee (ΔA/min/mg/ mitochondrial protein)	Mitochondrial L-glutamate dehydrogenase (nmoles glutamic acid/ min/mg protein)	Basal metabolism (ml O_2/100 g body wt/min)
I Normals	0.031 ± 0.008	134.2 ± 9.2	2.10 ± 0.099
II Thyroidectomized	0.0075 ± 0.001*	94.2 ± 11.7†	1.71 ± 0.13
III Thyroidectomized treated with L-thyroxine	0.075 ± 0.009‡	155.5 ± 16.1§	2.76 ± 0.15
IV Thyroidectomized treated with isopropyldiiodo-L-thyronine	0.146 ± 0.022‡	145.5 ± 12.7§	3.70 ± 0.14
V Thyroidectomized treated with α-methyl-DL-thyroxine	0.015 ± 0.0025§	153.9 ± 23.0‖	1.69 ± 0.095

Treatment was 5 nmoles/100 g/day for 15 days
‡ $P < 0.001$, † $P < 0.05$ } compared to normal animals
‡ $P < 0.001$, § $P < 0.02$, ‖ $P < 0.05$ } compared to thyroidectomized animals

appeared a more sensitive index than the effect on the metabolic rate.

DISCUSSION

The *in vitro* inhibitory effects of IPT_2 and MT_4 against GLDH, ICDH and ADH are similar to those of T_3 and T_4. For each enzyme, the concentration that inhibit the enzyme activity by 50 per cent (I_{50}) are, in the experimental conditions used, of the same order of magnitude for all iodinated products although these products have very different thyromimetic activities. It appears appropriate to recall that the formic, acetic and propionic derivatives of T_3 have the same *in vitro* inhibitory [13, 14] effects at roughly the same concentrations. Although MT_4 behaves as the other effectors towards other enzymes, it inhibits horse liver alcohol dehydrogenase but very weakly.

The effects of IPT_2 and MT_4 on the oxidative phosphorylation activities of isolated mitochondria are quite similar to those of thyroid hormones. In State 4 they increase mitochondrial oxygen uptake at low concentration but decrease it at higher concentration. In State 3 the only effect observed is an inhibition proportional to the dose. The effects obtained in the active state in the presence of oligomycin are the same as those observed in the controlled state. Finally, IPT_2 and MT_4 have an inhibitory effect on mitochondrial respiration in the presence of 2,4-dinitrophenol. Thus the effect exerted by IPT_2 and MT_4 on the isolated mitochondria is in all aspects similar to that of thyroid hormones [45–47]: like these, IPT_2 and MT_4 uncouple oxidative phosphorylation and simultaneously inhibit electron transfer of the respiratory chain. The replacement of the 3'-iodine and the presence of an α-methyl group bring no basic change in the effects exerted on pure enzymes or on isolated

Table 3. Effects of thyroid states and of the treatment of thyroidectomized animals with L-thyroxine and isopropyldiiodo-L-thyronine on adrenal tyrosine hydroxylase activity.

Group	Body wt (g)	Adrenal protein (mg/pair)	Tyrosine hydroxylase activity: nmoles/ pair/hr	Tyrosine hydroxylase activity: nmoles/mg protein/hr	Tyrosine hydroxylase activity: nmoles/100g body wt/hr basal metabolic rate
Normal	263 ± 14	4.27 ± 0.15	38.9 ± 1.5	9.13 ± 0.39	7.04
Thyroidectomized	191 ± 11*	3.08 ± 0.21*	22.7 ± 1.1*	7.40 ± 0.36†	6.95
Thyroidectomized treated with L-thyroxine	156 ± 11	4.63 ± 0.33‡	38.1 ± 2.05‡	8.22 ± 0.6	8.84
Thyroidectomized treated with isopropyldiiodo-L-thyronine	159 ± 11	4.74 ± 0.30‡	46.9 ± 4.2‡	9.89 ± 0.57§	7.97

* $P < 0.01$, † $P < 0.05$ } Compared to normal animals
‡ $P < 0.001$, § $P < 0.02$ } Compared to thyroidectomized animals

L-Thyroxine and isopropyldiiodo-L-thyronine were injected i.p. at a dose of 5 nmoles/100 g/day for 15 days. Results are expressed ± S.D.

mitochondria. Since the acid analogs of T_3 have an inhibitory effect similar to that of parent compound it appears that the 3,5-diiodo-4'-hydroxydiphenylether moiety is essential for the *in vitro* effects seen in this series of substances while other substituents play a secondary role. On the other hand it appears that they are very effective *in vivo* probably because of different cellular permeability.

In vivo, specific parameters of thyroid hormone activity such as metabolic rate and GPD induction respond very differently when the thyroidectomized animal is treated with IPT_2, MT_4 or T_4. A daily dose of 50 nmoles/kg of T_4 restores the basal metabolic rate and the hepatic GPD activity of the thyroidectomized animal to the euthyroid level. A similar treatment with IPT_2 goes largely beyond a simple compensation, metabolic rate and GPD activity being superior to that of the normal animal. After MT_4 treatment however, the *in vivo* activities observed clearly demonstrate incomplete compensation of the hypothyroid state. The activity of liver mitochondrial GLDH appears independent of other *in vivo* parameters of thyromimetic activity: MT_4 and IPT_2 have the same effect on this enzyme which therefore appears to be an unreliable index of thyroid activity.

The results of our experiments establish a relationship between thyroid state and adrenal tyrosine hydroxylase activity: the enzyme is definitely less active in the thyroidectomized animal. Because of body and adrenal weight variations as well as of metabolic rate variations in function of the thyroid state the results of Table 3 are related to mg adrenal protein, to body weight and to the metabolic rate of the animals before sacrifice. From these expressions it is seen that the thyroidectomy-induced 40 per cent drop in TH activity of the adrenal pair is only of 20 per cent when related to adrenal protein or to body weight. When the thyroidectomized animal receives T_4, the total activity is very close to normal but, because of the smaller weight of the treated animals the specific activity expressed per body weight shows an increase of 40 per cent above normal; likewise, the specific activity of the IPT_2 treated animal is twice that of the normal animal. The ratio of TH specific activity per body weight and metabolic rate are virtually similar for normal and thyroidectomized animals but distinctly elevated for the T_4 and IPT_2 treated groups; this points to a parallel between changes in TH activity and in metabolic rate of the hypothyroid animal which does not persist in hyperthyroid states. Thus T_4 and IPT_2 increase adrenal TH activity: the mechanism of this increase has not been investigated and one cannot assert that an enzymatic induction is involved. It is however, of interest within the framework of thyroadrenergic relationships [32].

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