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Different Effects of Methylmercaptoimidazole and Propylthiouracil on Thyroidal ^{131}I Release in Rats on ClO_4^-

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ABSTRACT. The administration of propylthiouracil to rats is followed by a prompt increase in circulating TSH (1,2). To test further the idea that this is related to the extrathyroidal effects of PTU on L-thyroxine (L-T_4), we have screened the effects of several other antithyroid drugs on TSH release. We were especially interested in those drugs, such as thiourea and methylmercaptoimidazole (MMI), known to lack extrathyroidal effects on L-T_4 metabolism. The change occurring after a single injection of the drug in the rate of thyroidal ^{131}I release of rats on ClO_4^- was taken as an indirect measure of a possible change in circulating TSH. Contrary to what we expected on the basis of the idea indicated above, we found that MMI behaved similarly to PTU, inasmuch as it promptly dis-

charged ^{131}I from the ClO_4^- -blocked thyroid. To see whether this similarity might be only apparent, the experiment was repeated in hypophysectomized rats on ClO_4^- and exogenous TSH. It was found that under such conditions PTU had no effect, whereas MMI continued to increase the rate of thyroidal ^{131}I release. Some findings reported here indicate that MMI also has extrathyroidal effects on the distribution of iodide in thyroidectomized, L-T_4 maintained rats. Present findings, moreover, confirm previous reports which indicate that MMI, in contrast to PTU, has no effect on the deiodination of L-T_4 or on the effectiveness of this hormone as regulator of TSH secretion. The latter was assessed by the antigoiter assay. (*Endocrinology* **83**: 671, 1968)

IT HAS BEEN proposed in this laboratory (1, 2) that there is an early release of thyrotrophic hormone (TSH) following administration of 6-propyl-2-thiouracil (PTU) which is related to the depression of extrathyroidal deiodination and effectiveness of L-thyroxine (L-T_4) caused by the drug.

According to this proposal, thiourea and 1-methyl-2-mercaptoimidazole (MMI), which do not have such extrathyroidal effects on L-T_4 deiodination (3-5), should not induce an early release of TSH from the pituitary. Using rates of thyroidal ^{131}I (I^*) release in rats on ClO_4^- as an indirect measure of changes in circulating TSH, a preliminary screening experiment confirmed this assumption in the case of thiourea. The ip injection of 7.5 mg MMI, however, resulted in a prompt increase in the rate of

thyroidal release. The result obtained with MMI appeared contradictory to the above proposal. The following possibilities were investigated: a) MMI interferes with the extrathyroidal metabolism of L-T_4 but the effect is so transient or so small it might be overlooked with usual techniques; it might decrease the effectiveness of the hormone without affecting its deiodination. b) The effect of MMI on thyroidal I^* release does not reflect an increase in circulating TSH. c) The initial proposal from this laboratory (1, 2) is not tenable.

Materials and Methods

Young male or female rats of a Wistar strain weighing 85-140 g were fed a medium-residue, low-iodine diet of the Remington type. Distilled water was used. The procedures for isotopic equilibration of thyroidectomized (T) rats with I^* -labeled (L-T_4^*) and for the measurement of thyroidal I^* disappearance rate in animals receiving ClO_4^- to block I^* recycling have already been described (2, 3).

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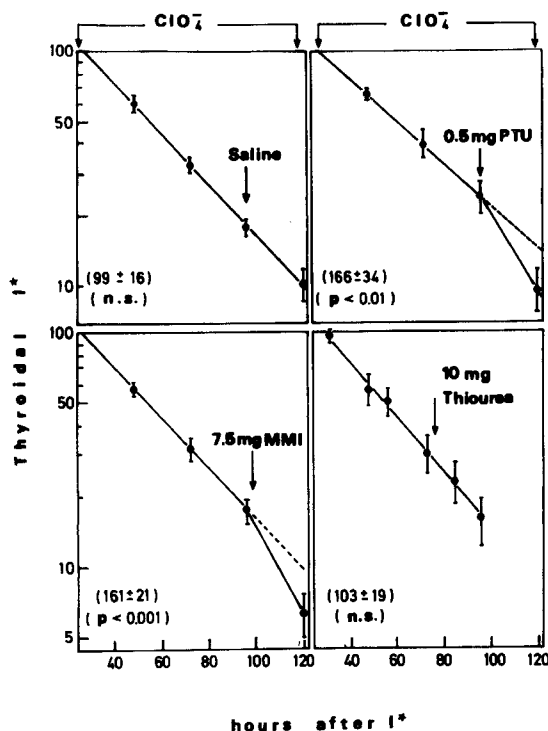


FIG. 1. Effects of PTU (0.5 mg), MMI (7.5 mg) or thiourea (10 mg) on the thyroidal I* release rate in rats injected with I* iodide and treated with 160 mg ClO_4^- /rat/day starting 24 hr after injection of the tracer. Data are expressed as per cent of the 24 hr thyroidal I* content, the mean (\pm SE) being shown for 8 rats/group, except for 5 injected with thiourea. Data in brackets indicate the mean (\pm SD) values of the individual changes observed in the thyroidal I* release rate after the injection of saline, PTU, MMI or thiourea: The slope during the last 24 hr was calculated as a percentage of the previous rate. The probability that the mean value is the same as 100% is also given, n.s. indicating that $p > 0.05$. The experiment with thiourea was carried out on a different occasion than the one shown here for rats injected with saline, PTU or MMI. A group of 5 rats injected with saline and another injected with 0.5 mg PTU were studied concomitantly as controls, the corresponding changes in the thyroidal I* release rates being $99 \pm 10\%$ and $150 \pm 21\%$, respectively.

The effects of MMI on the rate of extrathyroidal disappearance of either L-T₄ or I⁻ were studied following 2 procedures, one using \bar{T} rats, the other intact animals on ClO_4^- . In both cases 5 days or more prior to onset of the experiment the animals were accustomed to individual cages adequate for collection of the excreta. In the first case, the \bar{T} rats were injected ip with 3 μg L-T₄/rat/day. At time 0,

either 1 μCi , 3 μg L-T₄* or 1 μCi , 3 μg I* iodide were injected into the femoral vein under light ether anesthesia. At the same time, half the group received saline ip, the other animals 7.5 mg MMI diluted in saline. Collection of urine and feces was started. The animals were killed after different intervals by exsanguination under light ether anesthesia. Most of the organs were dissected and counted in a well scintillation counter. After removing the intestinal tract, stomach and skin, the skeleton and muscles (referred to as carcass) were homogenized mechanically and aliquots were counted. In the second case, that is, when intact rats were used, they received 160 mg ClO_4^- daily for 4 days by stomach tube. At time 0, immediately after the last treatment with ClO_4^- , they were injected iv either with 1 μCi , 3 μg L-T₄* or 1 μCi , 3 μg I* iodide, half the animals of each group receiving saline and the other half 7.5 mg MMI in saline. The same procedure was then followed as for the \bar{T} rats.

To study which level of plasma PBI was attained when L-T₄ was injected in amounts adequate to prevent goiter in animals treated chronically with 1 mg of PTU/rat/day or 7.5 mg MMI/rat/day, female rats weighing 100–120 g were separated into 9 groups of 6 rats each and fed the Remington diet. One group served as untreated controls; 4 groups received 1 mg PTU/rat/day and the remaining four groups 7.5 mg MMI/rat/day. The amount of daily ration was such that the rats could eat *ad lib.* with a minimum of waste. From the onset one of the groups from each set on goitrogens received either saline or 0.25, 0.50, 0.75 μg L-T₄/rat/day ip. After 12 days the rats were killed. Immediately thereafter another experiment was carried out using 9 groups of animals under similar conditions, except that the groups on goitrogens received 0, 1.0, 2.0 or 3.0 μg L-T₄/rat/day. The iodine content of the Remington diet was about 0.04 $\mu\text{g}/\text{g}$, whereas it had been about 0.09 $\mu\text{g}/\text{g}$ for the previous experiment. The thyroids were weighed on a torsion balance and the plasma was pooled for each group and frozen for determination of the plasma PBI. All ¹²⁷I determinations were carried out by a modified Zak procedure (6).

To measure the thyroidal I* disappearance rate in hypophysectomized (\bar{H}) rats, the animals were obtained from Academisch Ziekenhuis, Leiden, Holland, and kept in our animal quarters about 20 days before starting the experiment. They were fed the diet suggested by Shaw and Greep (7) for \bar{H} rats and drank saline instead of distilled water. Three days before time 0, the animals were injected ip with 1 U USP of TSH/rat/day and 2 days later with

10 μCi I^* iodide. Twenty-four hr later the I^* over the neck region was about 20% of the tracer dose. From this moment I^* recycling was blocked with 160 mg ClO_4^- /rat/day given by stomach tube under the light ether anesthesia used for counting the neck region. The experiment was thereafter continued as for intact rats, the only difference being a daily ip injection of 0.25 U USP of TSH/rat/day. After sacrifice, the pituitary area was explored and the weight of the thyroid, adrenals, testis and kidneys was measured in all animals. These organs were significantly smaller in H rats than in normal controls. The animals were discarded if visual inspection of the pituitary area or a much larger weight of the organs, especially the testis, suggested the presence of functioning pituitary tissue.

Results and Comments

A single ip injection of 7.5 mg MMI into rats with thyroids previously labeled with I^* and on ClO_4^- to block recycling of iodide induces a prompt increase in the rate of thyroidal I^* disappearance comparable to that effected by 0.5 mg PTU (Fig. 1). A single injection of 5 mg MMI under the same experimental conditions had a borderline effect on thyroidal I^* disappearance, none being detected with 2.5 mg or lower doses. A single 10 mg injection of thiourea had no effect.

Daily administration of 7.5 mg MMI/rat does not change the daily urinary excretion of I^* iodide in $\bar{\text{T}}$ rats isotopically equilibrated with L-T_4^* (Fig. 2). No clear-cut effect was obtained even when the dose of MMI was increased to 25 mg/rat/day. For comparison, the intense effect of 1.0 mg PTU/rat/day in a group studied simultaneously is also shown.

Because the urine had been collected every 24 hours, we could not discard the possibility of a transient effect which would be detected only at shorter intervals. To check this point, the effect of 7.5 mg MMI on the rate of disappearance of L-T_4^* was studied in $\bar{\text{T}}$ rats receiving L-T_4 . Animals were killed at 4, 6, 16 and 24 hours and at all times the plasma PBI* and the radioactivity in whole blood, carcass, kidneys, heart, spleen, skin, liver, intestines and

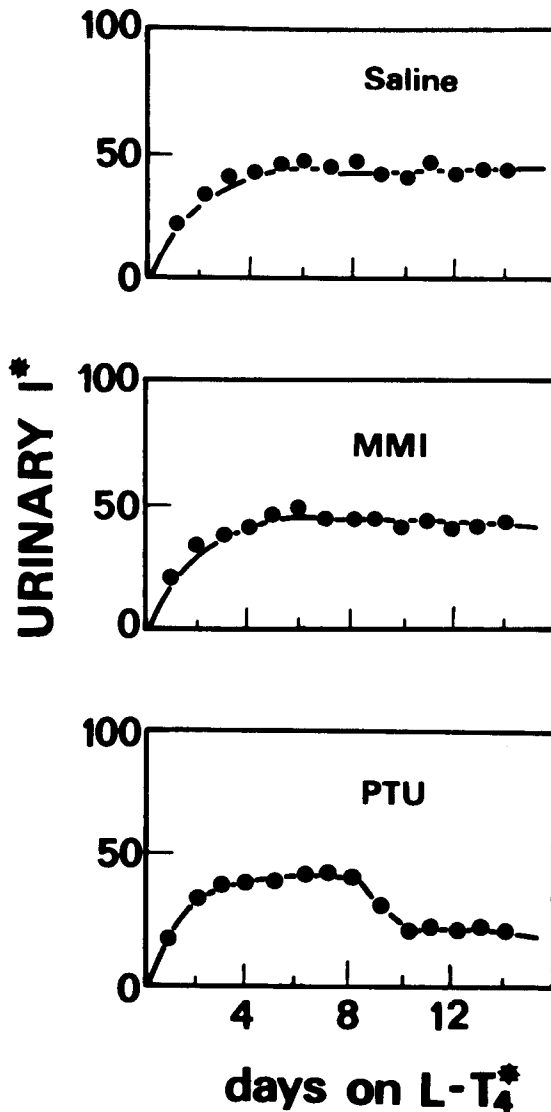


FIG. 2. Effect of MMI and PTU on the I^* urinary excretion in thyroidectomized rats isotopically equilibrated with 3 μg , 1 μCi L-T_4^* /rat/day. From day 8 on 1 mg PTU was given/rat/day. On days 8, 9 and 10, 7.5 mg MMI was given; 15 mg on days 11 and 12; 25 mg the last 2 days. Controls received saline from day 8 onward. Data are mean values of urinary I^* content (5 rats/group) expressed as per cent of daily dose of hormonal I^* . At all points the SE was smaller than the symbol employed.

feces were the same for both groups. At 4 and 6 hours it was slightly higher in the stomach of the MMI-treated rats as compared to their controls, the difference hav-

TABLE 1. I* urinary excretion in thyroidectomized rats, maintained with 3 µg L-T₄/rat/day and iv injected at time 0 with 3 µg, 1 µCi L-T₄/rat, and either 7.5 mg MMI/rat or saline

Hr after injection of L-T* and MMI or saline	I* excretion into Urine + Bladder (as % of the L-T ₄ * dose: mean ± SD)		Statistics p
	Controls (4 rats/group)	MMI (4 rats/group)	
4	5.4 ± 0.8	3.4 ± 0.9	.02-.01
6	12.0 ± 1.2	6.5 ± 0.7	<.001
16	19.3 ± 2.0	18.4 ± 1.3	NS†
24	25.2 ± 2.3	25.2 ± 2.4	NS

† Not significant: p > 0.05.

ing disappeared by 16 hours. Table 1 summarizes the differences found in urinary I* iodide excretion: at 4 and 6 hours the I* iodide excreted into the urine plus that still retained in the bladder was significantly lower in the MMI-treated rats than in the controls. These differences had, however, disappeared at 16 and 24 hours. Such results could indicate that MMI has a transient but significant inhibitory effect over the extrathyroidal metabolism of L-T₄, or that it alters the distribution of I* iodide, proceeding in these animals from deiodination of the hormone and from the I* iodide originally contaminating the L-T₄* preparation (about 5–10%). For this reason, the effect of 7.5 mg MMI on the peripheral disappearance of I* iodide was studied in T rats on L-T₄. As in the previous experiment using L-T₄*, the radioactivity in the whole

blood, plasma PBI, carcass, kidneys, heart, spleen, liver, intestines and feces was the same in MMI-treated rats and in controls. However, urinary I* iodide excretion in rats on MMI was half that of their controls (Table 2). This decrease is very nicely compensated for by an increase in the radioactivity of the stomach of rats receiving MMI in such a way that addition of the I* in the urine + bladder + stomach yields the same value for both experimental groups. These results support the conclusion that MMI does not have extrathyroidal effects on the deiodination of L-T₄, but does alter the extrathyroidal distribution of iodide. Table 2 also shows the results obtained when we determined the effect of MMI on the distribution of L-T₄* and of I* iodide in intact rats which had been for four days on ClO₄⁻. This was done

TABLE 2. I* distribution in thyroidectomized rats and in intact rats on ClO₄⁻ six hours after iv injection of 3 µg, 1 µCi I* iodide or 3 µg, 1 µCi L-T₄*/rat and either 7.5 mg MMI or saline (results expressed as % of dose: mean ± SD)

Groups No. of rats	I* given as I ⁻				I* given as L-T ₄	
	(T)		(ClO ₄ ⁻)		(ClO ₄ ⁻)	
	Control 4	MMI 4	Control 5	MMI 5	Control 5	MMI 5
Urine + Bladder p	48.5 ± 8.6 <.001	24.8 ± 6.6	75.9 ± 3.6	75.9 ± 4.6 NS†	18.1 ± 2.0	18.6 ± 0.1 NS
Feces + Intestines p	4.15 ± 0.95	4.83 ± 1.75 NS	2.19 ± 0.68	1.85 ± 0.54 NS	20.9 ± 3.5	20.6 ± 1.4 NS
Stomach p	7.1 ± 1.1 <0.001	25.2 ± 3.5	0.20 ± 0.13 <0.001	1.00 ± 0.26 <0.001	0.53 ± 0.08 0.05	0.71 ± 0.10 0.02
Thyroid p	—	—	1.35 ± 0.52 <0.001	0.20 ± 0.08 <0.001	0.49 ± 0.11	0.13 ± 0.03 <0.001

† Not significant: p > 0.05.

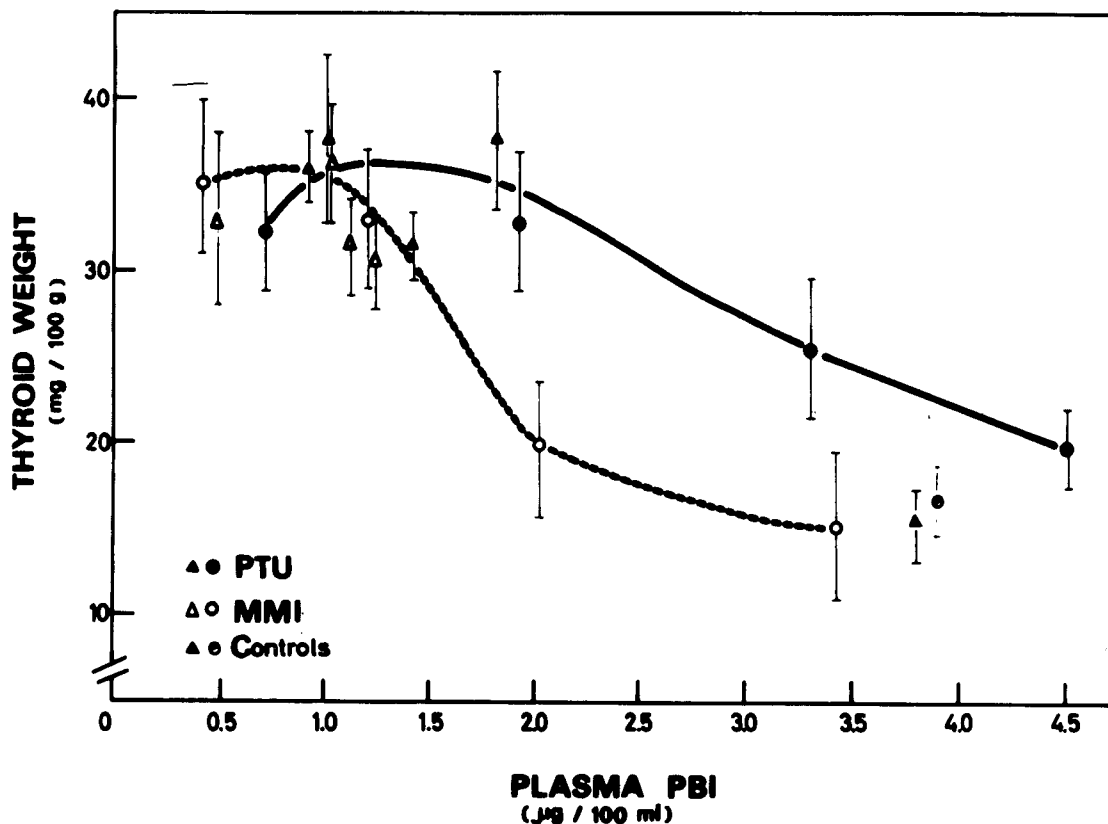


FIG. 3. Effect of different doses of L-T₄ on the thyroid weight and plasma PBI of rats on 7.5 mg MMI or 1 mg PTU/rat/day for 12 days. Data are mean (\pm SD) values of the thyroid weights of 6 rats/group and the mean value obtained with pooled plasma for the PBI. The triangles and circles identify data from 2 different experiments. The first 4 data on the left of the figure correspond to groups on goitrogens receiving no exogenous L-T₄. The doses of the hormone progress from left to right.

in order to see whether the effects of MMI on extrathyroidal I* distribution observed in the \bar{T} rats on L-T₄ could also have been playing some role under the conditions we had used to study the effect of MMI on thyroidal I* release. The extrathyroidal effects of MMI on I* iodide distribution were no longer detectable in the presence of ClO₄⁻. This anion is known to displace iodide from extrathyroidal sites (8). The radioactivity of the thyroids of the rats on ClO₄⁻ was lower, and that of the stomach higher, when MMI had been given.

MMI might, however, have an effect on the potency of L-T₄ as regulator of TSH secretion or a direct effect on TSH release by the pituitary. The first point was inves-

tigated by determining how much exogenous L-T₄ restores to normal the size of the thyroid gland of rats on MMI and to see whether this dose ensures a normal PBI. Results illustrated in Fig. 3 show that thyroid weights of rats on MMI reverted to the control value with the same L-T₄ doses resulting in a normal plasma PBI. On the contrary, when goiter was induced with PTU, a higher dose of L-T₄ was needed to prevent it and this occurred at a higher PBI than that of controls. The second point indicated above was studied by comparing the effect of both MMI and PTU on thyroidal I* release in hypophysectomized (\bar{H}) rats receiving exogenous TSH and on ClO₄⁻ to block radioiodide recy-

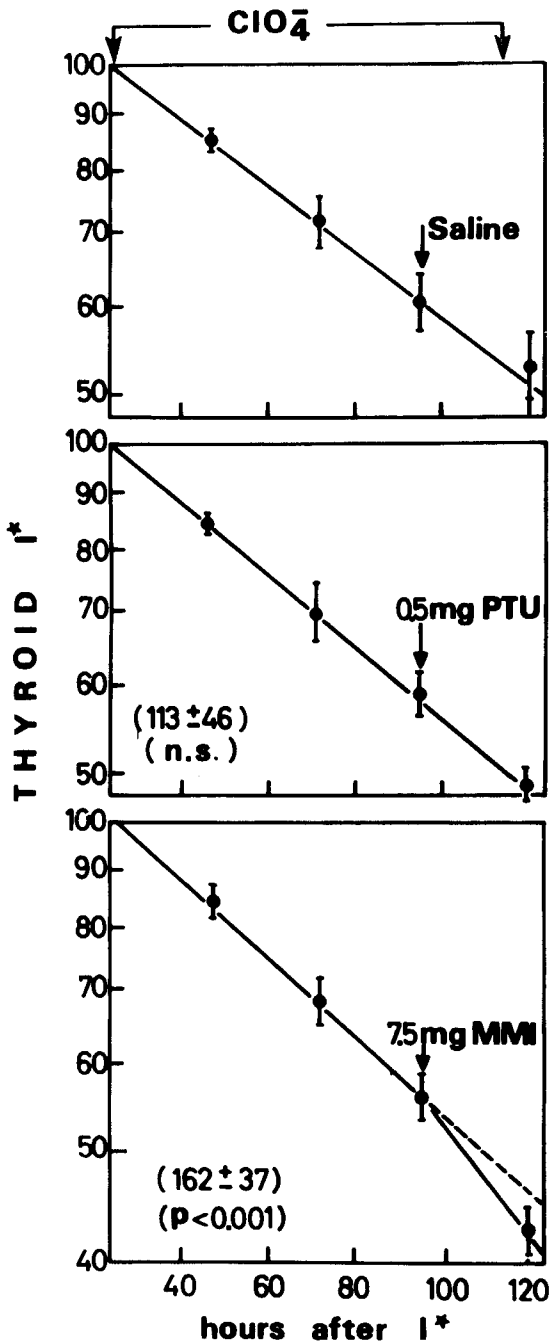


FIG. 4. Effect of PTU (0.5 mg) and MMI (7.5 mg) on the thyroidal I^* release rate in hypophysectomized rats injected with exogenous TSH and receiving 160 mg ClO_4^- /rat/day starting 24 hr after injection of I^* . Data are expressed as in Fig. 1, with 12 rats/group receiving saline or PTU and 15 receiving MMI. The results correspond to 3 different experiments, each comprising groups of 4-5 rats on saline, PTU and MMI.

cling. Fig. 4 shows that, in the ClO_4^- -treated \bar{H} rats receiving exogenous TSH and saline, there was a slight tendency of the thyroidal I^* release rate to decrease on the last day of the experiment. This did not occur in the rats receiving 0.5 mg PTU instead of straight saline, the thyroidal I^* release rate being the same as on the day prior to injection of the drug. Contrariwise, 7.5 mg MMI increased thyroidal I^* release from the ClO_4^- -blocked glands to a degree similar to that in intact animals.

Discussion

The present findings confirm those of other authors that MMI does not affect the extrathyroidal deiodination of $L-T_4$ (4, 5) or the potency of $L-T_4$ as regulator of TSH secretion, as measured by the antigoiter assay (9). These findings are in conceptual agreement with the demonstration that MMI does not alter the effectiveness of $L-T_4$ in other tests, such as maintenance of hepatic intramitochondrial alpha-glycerophosphate-dehydrogenase activity (9).

It therefore appeared unlikely that the effect of MMI on the thyroidal I^* release in rats on ClO_4^- involved an increase in circulating TSH. In direct confirmation of this point is the present finding that MMI increases the rate of thyroidal I^* release in rats on ClO_4^- despite hypophysectomy. This shows that a direct effect of MMI on the thyroid was involved which could be due to 1) a synergic action with ClO_4^- in blocking radioiodide recycling, 2) displacement of radioiodide liberated during intrathyroidal deiodination or 3) some other cause. Present findings obtained in the \bar{H} rats again confirm that at least a major part of the prompt effect of PTU on thyroidal I^* release in intact rats on ClO_4^- involves a response by the pituitary (1, 2) and that it is not merely due to blocking of the reutilization of intrathyroidally formed I^* iodide via a mechanism insensitive to ClO_4^- . This conclusion agrees with that drawn by Onaya and Halmi (9). The results obtained from the goiter-prevention assay support

the idea that this effect of PTU involves a decrease in extrathyroidal L-T₄ activity (10). In connection with the latter point, Jagiello and McKenzie (11) had previously shown that the dose of L-T₄ reverting PTU-induced goiter and the high plasma TSH to normal resulted in a higher than normal plasma PBI. It has been suggested (12) that in such an experimental setup this might have been due to the circumstance that only L-T₄ was substituting for the normal thyroidal secretion which involves also L-T₃: a higher plasma PBI might have been needed in the rats on exogenous L-T₄ alone than that of the control rats to ensure the same metabolic response. However, the present experiments comparing the effects of L-T₄ in MMI-induced vs. PTU-induced goiter indicate this is an unlikely explanation: with MMI the dose of L-T₄ ensuring a normal thyroid weight results in a normal PBI.

MMI has extrathyroidal effects on the distribution of radioiodide, which disappear if ClO₄⁻ is administered.

All these findings indicate that there are differences between these two widely used antithyroid drugs, MMI and PTU, as regards both their extrathyroidal effects on L-T₄ and on I⁻. Some of them also suggest

that the actions of these two drugs at the thyroidal level are not precisely the same.

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