Intrathyroidal Recycling of Iodide in the Rat: Effects of Goitrogens[1] (37281)

TOSHIMASA ONAYA, TAKASHI YAMADA, AND NICHOLAS S. HALMI

Department of Medicine, Institute of Adaptation Medicine, Shinshu University School of Medicine, Matsumoto 390, Japan; and Department of Anatomy, University of Iowa, Iowa City, Iowa 52240

In 1960, Mayberry and Astwood (1) found that thyroidal ^{131}I in intact rats is lost at a faster rate when further uptake of ^{131}I by the thyroid is prevented by propylthiouracil (PTU) than when it is blocked by ClO_4^-. Therefore, it has been suggested that some of the intrathyroidally formed I^- is reutilized by organic binding without involvement of ClO_4^--sensitive transport (2). On the other hand, Escobar del Rey *et al.* (3–5) have reported that PTU administration results very soon in acceleration of the rate of thyroidal ^{131}I release. Although they suggested that this PTU effect might be due to an increased TSH secretion by the pituitary, a direct evidence for this view is lacking because of insensitivity of bioassay technique. More information was therefore needed on how intrathyroidally formed I^- recycles and how this recycling is affected by different goitrogens. In the present study, we have tested what PTU or ClO_4^- did on thyroidal ^{131}I release when TSH is exogenous, and also demonstrated an early response of thyroidal endocytosis to methylthiouracil (MTU).

Materials and Methods. Young male rats of the Wistar or Holtzman strain (150–180 g) were used. When a low iodine diet was given, it was a homemade diet (6) or the Remington type test diet distributed by Nutritional Biochemicals, Inc., Cleveland, OH (iodine content < 0.1 $\mu g/g$). Hypophysectomized rats were obtained from Hormone Assay Laboratories, Chicago, IL, 10 days after the operation. The rate of thyroidal ^{131}I release was measured as described previously (6). The NIH-S-3 ovine TSH (0.05 to 1.0 unit) was injected sc twice a day into hypophysectomized rats. PTU was injected sc daily in 10% gum acacia, MTU, which was distributed by Chugai Pharmaceutical Co., ip in 2% (20 mg/ml) solution, $NaClO_4$ or $KClO_4$ (10 to 20 mg) ip in 1 ml saline. As an index of endocytosis, intracellular colloid droplets were enumerated as previously described (7). Statistical analysis of the significance of difference between groups or between before and after treatment in the same group was done by means of Student's t test (or with paired observations). A p value $<$ 0.05 was considered statistically significant.

Results. Expt 1. Effects of ClO_4^- and MTU on thyroidal ^{131}I release in intact rats. The half-life of thyroidal radioactivity in groups A (11 animals) and B (10 animals) was 130 $\pm$ 11 and 133 $\pm$ 9 hr (mean $\pm$ SE), respectively, before $KClO_4$ or MTU injections (Fig. 1). Seventy-three hours after the initial neck count, the animals of group A received 10 mg $KClO_4$, while the animals of group B received 10 mg $KClO_4$ and 40 mg MTU. As shown in Fig. 1 (top), an acceleration of thyroidal ^{131}I release rate occurred during the drug administration in both groups. The half-life of thyroidal radioactivity during drug treatment was 53 $\pm$ 4 hr in group A and 32 $\pm$ 3 hr in group B. These values were significantly different from their initial values ($p < 0.001$). However, the acceleration was significantly greater in group B than in group A ($p < 0.005$).

Expt 2. Effect of MTU on thyroidal ^{131}I release in thyroxine-treated intact rats. Nine animals received 20 μg T_4 ip daily beginning 24 hr after ^{131}I (30 μCi) administration. In

[1] Supported by U.S. Public Health Service Grant AM-02463.

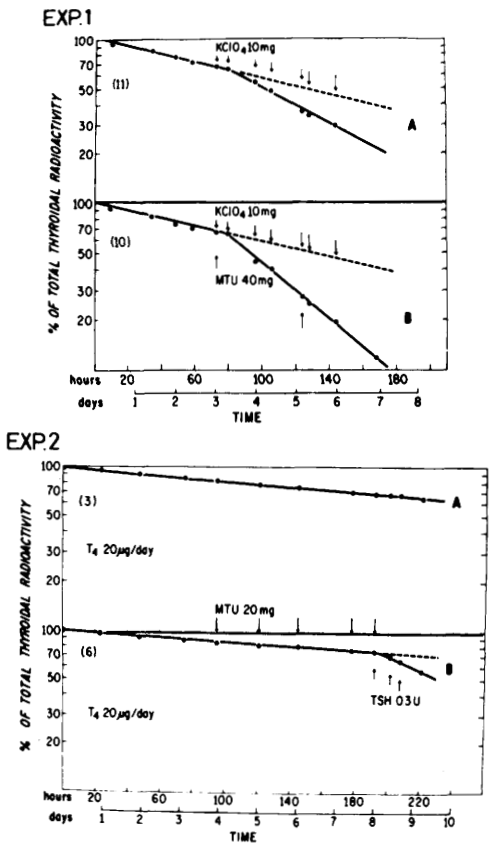

FIG. 1. Expt 1: Effects of ClO_4^- and MTU on thyroidal ^{131}I release in intact rats. Animals were fed a low iodine diet for 7 days before ^{131}I. Data shown for the release rate in Figs. 1 and 2 are mean values obtained in the number of rats shown in parentheses. Expt 2: Effect of MTU on thyroidal ^{131}I release in thyroxine-treated intact rats.

3 control rats, thyroidal ^{131}I release rate was slow but exponential throughout the measurement (Fig. 1 bottom A). In 6 animals, thyroidal ^{131}I release was also measured for 95 hr during T_4 administration. Beginning 95 hr after the initial neck count, 6 animals were given 20 mg MTU at 14 to 33 hr intervals in addition to T_4. Thyroidal ^{131}I release was not altered by administration of MTU, however (Fig. 1 bottom B). TSH (3 $\times$ 0.3 U) accelerated the rate of release of thyroidal ^{131}I.

Expt 3. Effects of PTU or ClO_4^- on thyroidal ^{131}I release in TSH-treated, hypophysectomized rats. Two units of TSH were injected sc 24 hr before and simultaneously with 40 μCi ^{131}I. After allowing uptake of ^{131}I by the thyroid for 48 hr, animals were given 1 unit TSH every 12 hr. Forty-eight hours after the initial neck count, 5 mg PTU injections were started in group A (8 animals) and 20 mg $NaClO_4$ in group B (8 animals). The half-life of thyroidal radioactivity in group A was 88 $\pm$ 6 and 50 $\pm$ 5 hr, respectively, before and during PTU injection, while it was 78 $\pm$ 4 and 49 $\pm$ 3 hr in group B, respectively (Fig. 2 top A and B). The release rate after increase was the same in A and B groups.

Expt 4. Effect of PTU plus ClO_4^- on thyroidal ^{131}I release in TSH-treated hypophysectomized rats. Hypophysectomized animals were treated with 0.2 unit TSH twice a day for 4 days before and simultaneously with 30 μCi ^{131}I. After allowing uptake of ^{131}I by the thyroid for 48 hr, animals were given 0.2 unit TSH every 12 hr. Forty-eight hours af-

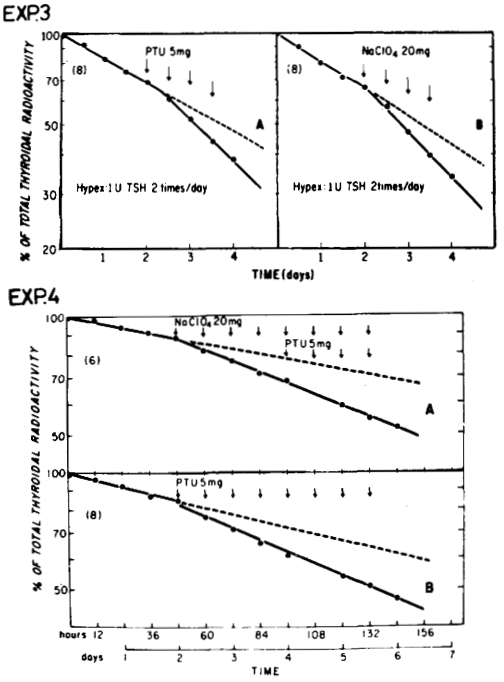

FIG. 2. Expt 3: Effect of PTU or ClO_4^- on thyroidal ^{131}I release in TSH-treated hypophysectomized rats. Expt 4: Effect of $NaClO_4$ plus PTU on thyroidal ^{131}I release in TSH-treated hypophysectomized rats.

ter the initial neck count, 20 mg $NaClO_4$ injections were started in group A (6 animals) and 5 mg PTU in group B (8 animals). Forty-eight hours after the initial dose of $NaClO_4$, 5 mg PTU injections were also started in group A in addition to $NaClO_4$ injections. The half-life of thyroidal radioactivity in group A was 225 ± 26 and 131 ± 15 hr, respectively, before and during $NaClO_4$ injections. However, further administration of PTU failed to alter this increased rate of thyroidal ^{131}I release. The half-life of thyroidal radioactivity in group B was 216 ± 26 and 118 ± 9 hr, respectively, before and during PTU injections. No difference in the release rate was found between A and B groups.

Expt 5. Effects of MTU or methimazole on colloid droplet formation in rat thyroid gland. MTU or methimazole (5 mg) caused colloid droplet formation in rat thyroid gland with a peak stimulation at 2 to 4 hr, suggesting that the pituitary secretes TSH in response to goitrogen within a short time. In contrast, less than 50 droplets/25 follicles were found in the control thyroid.

Discussion. Iodide liberated from intrathyroidal deiodination of free iodotyrosines has been thought to be reutilized for further hormone synthesis. However, it is not known how much iodide liberated would be reutilized or whether reutilizable iodide will mix with iodide newly trapped from the blood. Halmi and Pitt-Rivers (9) and Nagataki and Ingbar (10) have suggested the existence of the second iodine pool which is available for intrathyroidal recycling of iodide and not influenced by ClO_4^-. Although this hypothesis is attractive, subsequent studies have shown that recycling of iodide liberated from free tyrosines through the stimulation of TSH will be blocked by ClO_4^- or large doses of iodide coming from the blood (14–15).

In our present study, ClO_4^- apparently augmented release of ^{131}I from prelabeled thyroid. Since primary action of ClO_4^- is to inhibit iodide uptake by thyroid cell but not to block organic binding of iodine, this increase of thyroidal ^{131}I release may be largely due to the block of recycling of iodide derived from organic sources rather than to an acute increase of TSH secretion. Theoretically, it is possible that iodide derived from organic source first gets out into the extracellular space within the thyroid where it mixes with the iodide coming from the blood and in turn is taken up by other thyroid cells in a manner similar to so called "iodide trapping." If this is the case, ClO_4^- would interfere with the uptake of iodide from organic sources as well as iodide coming from the blood. A part of such a view has already been suggested by Urquhart (15). A similar sequence of events has also been suggested by Rosenberg *et al.* (14–16) who thought that the iodide from organic sources and the iodide from the blood will mix with each other in the extracellular space within the thyroid. All the data thus gathered suggested that iodide of thyroid cell → extracellular space within the thyroid → thyroid cell system constitutes the most, if not all, of so called "intrathyroidal recycling of iodide." Interestingly, ClO_4^- was less effective than PTU or MTU in interfering with recycling of iodide in intact animals. In contrast, ClO_4^- was equally effective as PTU or MTU in TSH-treated hypophysectomized animals. As previously suggested (17), this may mean that these goitrogens block intrathyroidal iodide recycling on one hand and augment TSH secretion on the other. In support of this concept, our data clearly indicated that PTU and MTU apparently caused an acute increase of TSH secretion as evidenced by a rapid increase in intrathyroidal colloid droplets.

Summary. Since ClO_4^- accelerated thyroidal ^{131}I release in intact rats or in hypophysectomized rats treated with TSH, and since it failed to do so in T_4-treated rats, it seemed that ClO_4^- interfered with the recycling of iodide derived from free iodotyrosines. Similarly, MTU did not increase the rate of release of ^{131}I in T_4-treated rats, but did significantly so in TSH-treated, hypophysectomized rats. It thus seemed that intrathyroidally recycling iodide was largely from free iodotyrosines produced by hydrolysis of thyroglobulin. ClO_4^- was similarly effective as MTU and PTU in TSH-treated hypophysectomized rats, but it was less ef-

fective in intact rats. This difference was due to the fact that more TSH was secreted in response to PTU or MTU in intact rats thus producing a large amount of iodotyrosines through the hydrolysis of thyroglobulin.

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Received Jan. 23, 1973. P.S.E.B.M., 1973, Vol. 143.