Recovery of Thyroid Hormone Biosynthesis after a Single Injection of Methylthiouracil as Judged by Thyroidal Radioiodine Uptake, Thyroidal Protein-Bound Iodine, Thyroidal Radioiodinated Amino Acids and Thyroglobulin in the Rat*

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Synopsis

In an attempt to assess the synthesis of thyroid hormone in the rat after a single large dose of methylthiouracil, radioiodine was injected 0 to 164 hrs. after administration of 20 mg methylthiouracil (MTU). Thyroidal radioiodine uptake, thyroidal PB¹³¹I, thyroidal radioiodinated amino acids and thyroidal content of thyroglobulin were measured.

When radioiodine was injected at 0 time, 80 % of total thyroidal radioiodine was in organic form at 24 hrs., while thyroidal radioiodine uptake was extremely low because of rapid loss of ¹³¹I⁻ from the blood in MTU treated animals. Thyroidal PB¹³¹I does reflect MIT but not T₄ and T₃. On the other hand, when radioiodine was injected 4 hrs. before autopsy, all thyroidal radioiodine uptake, thyroidal PB¹³¹I and thyroidal T₄ and T₃ returned to normal between 3 and 4 days, indicating a good correlation among different thyroid parameters. DIT/MIT ratio, however, showed some delay in its return. Thyroidal content of thyroglobulin decreased when thyroid hormone synthesis was blocked by MTU, but returned to normal when synthesis of thyroid hormone was normal. It is suggested that methylthiouracil acts on the thyroid in two ways; one, blocking oxydative enzyme and the other, depressing thyroglobulin content.

It has been established for some time that antithyroid drugs of the thianamide series interfere with synthesis of thyroid hormones, primarily by inhibiting both the oxidation of inorganic iodide and its subsequent incorporation into tyrosyl radicals (Greer *et al.*, 1964). In addition to blockade of oxidation of iodide, Roche (Roche, 1951) have reported a decrease of thyroidal content of thyroglobulin after a long-term administration of antithyroid drug. Blocking action of antithyroid drugs on thyroid hormone synthesis is more complicated, since it

has been demonstrated that the smaller quantities of antithyroid drugs than used customarily to induce goiter will preferentially inhibit the diiodination of tyrosine and coupling of iodinated tyrosine radicals to form thyroxine and triiodothyronine, while monoiodination of tyrosine actually may be increased as the production of other iodinated amino acids is being reduced (Slingerland *et al.*, 1959; Richards and Ingbar, 1959; Iino *et al.*, 1961). Thus, when a single large dose of antithyroid drug is administered, a number of parameters of the thyroid function may initially and uniformly be affected in a large measure, because of a marked depression of thyroid hormone synthesis. However, when amounts of circulating antithyroid drug decrease progressively with time, discrepancies in thyroid parameters may

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be developed, because of partial block of thyroid hormone synthesis. Such a sequence of hypothetical events has not been studied in detail in man and experimental animals, however.

The present experiment is designed to study such conditions by administering a single large dose of methylthiouracil and by measuring thyroïdal radioiodine uptake, thyroïdal protein-bound iodine and thyroïdal radioiodinated amino acids in the rat. Attempt is also made to study whether or not thyroïdal content of thyroglobulin is affected by a single large dose of methylthiouracil.

Materials and Methods

One hundred and seventy two adult male Wistar rats, weighing approximately 200 g, were used in this experiment. Animals were fed with a pellet diet* and water *ad lib*. Tracer dose (5 to 20 μ c) of carrier free radioiodine (Na^{131}I) were injected intraperitoneally 0 to 164 hrs. after a single injection of 20 mg methylthiouracil (MTU). The animals were sacrificed 4 to 48 hrs. after injection of radioiodine. The thyroid was cleanly dissected and thyroïdal radioactivity was determined using a scintillation counter. The thyroids were homogenized in a glass homogenizer and hydrolyzed with 0.5 ml of pronase solution (10 mg pronase/10 mg thyroid weight) at pH 7.4 at 37°C for 12 hrs. Portions of the digests were subjected to ascending paper chromatography in butanol-acetic acid-water (4:1:5) and butanol-ethanol 0.5 *N* ammonium (5:1:2). For the analysis of thyroïdal protein bound iodine (PB^{131}I), portions of homogenates were subjected to ascending paper chromatography in 2 solvent systems. Iodide, moniodotyrosine (MIT), diiodotyrosine (DIT), thyroxine (T_4) and triiodothyronine (T_3) were employed as carriers. The chromatographed strips were then put on x-ray film to obtain radioautographs. The strips were then cut into 5 pieces for ^{131}I analysis depending on the radioautogram. The ^{131}I content of the various components was expressed as percentage of the total ^{131}I found on each strip. No attempt was made to calculate absolute amounts of intrathyroïdal iodinated amino acids. Strips with carrier iodide, MIT, DIT, T_4 and T_3

were stained with ceric sulfate arsenious acid reagent. For the analysis of thyroïdal proteins, the thyroids were homogenized by adding physiologic saline to obtain at least 0.5% protein concentration. Portions of these homogenates were subjected to ultracentrifugation as reported previously (Ui and Tarutani, 1961).

Results

Experiment 1. Effect of a single injection of 20 mg methylthiouracil on thyroïdal radioiodine uptake

As an initial step, the thyroïdal radioiodine uptake was used to evaluate blocking action of MTU on thyroid hormone synthesis. Ninety animals were divided into 17 equal groups. Animals of 8 groups received 20 mg MTU and radioiodine i.p. at 0 time and were sacrificed 15 mins., 1, 2, 4, 8, 12, 24 and 48 hrs. after MTU administration. Control animals of 9 groups received saline and radioiodine simultaneously and were killed 15 and 30 mins., 1, 2, 4, 8, 12, 24 and 48 hrs.

Fig. 1. Effect of a single injection of 20 mg MTU on thyroïdal radioiodine uptake. Circles and vertical lines indicate mean $\pm$ SE. Radioiodine and methylthiouracil (MTU) are injected at 0 time.

thereafter. The results were shown in Figure 1. In the control groups, thyroïdal radioiodine uptake increased progressively with time until 4 hrs., but was constant thereafter. In sharp contrast to this pattern, thyroïdal radioiodine uptake in MTU treated groups was markedly

* Iodine content of this diet was approximately 200 – 300 μ g ^{127}I /kg.

depressed throughout 24 hrs. at the level of approximately 0.3 %.

Since thyroidal radioiodine uptake was governed by thyroidal trapping ability of radioiodine as well as blood concentration of ^{131}I blood concentration of ^{131}I was measured in MTU treated groups. As shown in Figure 2, a rapid loss of ^{131}I from the blood did occur in MTU treated animals. The ^{131}I concentration at 24 hrs. was approximately 1/8 to 1/10 of the concentration at 2 hrs.

Fig. 2. Disappearance of ^{131}I from the blood in MTU J treated animals. Radioiodine and MTU are injected at 0 time.

Experiment 2. Effect of a single injection of 20 mg methylthiouracil on thyroidal protein bound iodine

In our next step, blocking action of MTU on thyroid hormone synthesis was assessed by measuring intrathyroidal PB^{131}I . The thyroid homogenates were obtained from the animals of experiment 1, and PB^{131}I as the percentage of total radioactivity was measured by paper chromatography. As shown in Figure 3, more than 95 % of thyroidal radioactivity was PB^{131}I as early as 15 mins. and approximately 99 % was PB^{131}I later than 8 hrs. in the control groups. As expected, a negligible amount of organic iodine was found 15 mins.

Fig. 3. Thyroidal PB^{131}I as the percentage of total thyroidal ^{131}I is indicated. Circles and vertical lines indicate mean $\pm$ SE. Radioiodine and MTU are injected at 0 time.

after MTU and radioiodine administration. However, PB^{131}I increased progressively with time and more than 80 % of thyroidal radioiodine was organically bound at 24 hrs. in MTU treated animals. A further increase of PB^{131}I was found and its value reached approximately normal at 48 hrs. in MTU treated animals.

Experiment 3. Effect of a single injection of methylthiouracil on thyroidal radioiodinated amino acids

In our third step, measurement of intra-thyroidal radioiodinated amino acids was used to assess blocking action of MTU on thyroid hormone synthesis. The digests of thyroid homogenates were obtained from animals of experiment 1, and percentage of thyroidal radioiodinated amino acids was analyzed by paper chromatography. As shown in Figure 4, approximately 32 % of total thyroidal radioactivity was MIT at 1 hr. in the control animals. The percentage decreased gradually thereafter and reached plateau (28 %) at 12 hrs. Synthesis of DIT was also rapid and DIT constituted 47 % of total thyroidal radioactivity at 1 hr. This percentage was roughly

Fig. 4. Changes in intrathyroidal iodinated amino acids in various time intervals after ^{131}I injection in the HID fed rat. Radioactivity of MIT, DIT (diiodotyrosine), T₄ (thyroxine), T₃ (triiodothyronine) is expressed as per cent of total thyroïdal radioactivity. Circles and vertical lines indicate mean $\pm$ SE. Radioiodine and MTU are injected at 0 time.

Fig. 5. Formation of thyroïdal iodinated amino acid after a single injection 20 mg MTU. Circles and vertical lines indicate mean $\pm$ SE. Radioiodine and MTU are injected at 0 time.

constant throughout 48 hrs. Synthesis of T₄ increased progressively throughout 48 hrs. On the other hand, T₃ synthesis reached plateau as early as 8 hrs.

In sharp contrast to control animals, only a negligible amount of MIT was found 1 hr. after MTU and radioiodine administration. No other radioiodinated amino acids were detectable at this time (Fig. 5). This strong inhibition of thyroïd hormone synthesis did not continue for extended time, however. For instance, MIT increased gradually with time by 12 hrs. and rapidly thereafter. At 24 hrs. MIT constituted more than 50 % of thyroïdal radioactivity. DIT was detectable at 4 hrs. and increased gradually with time. T₄ was detectable at 8 hrs. and increased similarly with time.

Experiment 4. Effect of a single injection of methylthiouracil on thyroïdal radioiodine uptake, thyroïdal organic iodine and radioiodinated amino acids when assessed 4 hrs. after radioiodine administration

In our fourth step, thyroïdal radioiodine uptake, thyroïdal organic iodine and thyroïdal radioiodinated amino acids were measured to assess effect of a single injection of methylthiouracil on thyroïd hormone synthesis by injecting radioiodine 4 hrs. before sacrifice. Eighty two animals were divided into 14 groups. The animals of eleven groups received a single injection of 20 mg MTU at 0 time. Radioiodine was injected at 0, 4, 8, 12, 16, 20, 44, 68, 92, 116, and 164 hrs., and animals

Fig. 6. Thyroïdal radioiodine 4 hour uptake after a 20 mg MTU injection. Circles and vertical lines indicate mean $\pm$ SE. Radioiodine is injected 4 hrs. before autopsy.

Fig. 7. Changes in intrathyroidal iodinated substances following a single large dose of MTU. Radioiodine is injected 4 hrs. before autopsy. Circles and vertical lines indicate mean $\pm$ SE.

were sacrificed 4 hrs. after radioiodine administration. As shown in Figure 6, 4 hrs. thyroidal radioiodine uptake was 5 % in the 3 control groups. In MTU treated animals, however, thyroidal radioiodine uptake was markedly low at 4, 8, 12, 16, 20 and 24 hrs. The uptake increased gradually with time thereafter, and reached normal value between 3 and 4 days. Furthermore, the uptake exceeded the control value at 4 and 5 days. The uptake at 7 days was approximately normal, however.

When thyroid homogenate was analysed by paper chromatography, only iodide and original substance (PB^{131}I) were found. When MTU was given, the pattern of intrathyroidal

iodide was very similar to that found in Figure 7, and thus alterations of intrathyroidal PB^{131}I were not indicated here. However, it should be noted that thyroidal PB^{131}I increased with time and reached normal value between 3 and 4 days.

When thyroidal digests were analysed by paper chromatography, more than 95 % of thyroidal radioiodine was iodide 4 hrs. after administration of MTU and radioiodine (Fig. 7). As expected, amounts of iodide decreased sharply with time and reached control value between 3 and 4 days. On the other hand, thyroidal radioiodinated amino acids increased progressively with time. The most striking is the rapid increase of MIT, which constituted

more than 40 % of total thyroidal radioiodine at 2, 3 and 4 days. This percentage of MIT decreased slightly between 4 and 5 days and reached normal value at 5 days. DIT increased less rapidly and reached normal value at 5 days. T_4 and T_3 increased slowly with time and reached normal value between 3 and 4 days.

As one of the criteria of thyroid hormone synthesis, DIT/MIT ratio was calculated from the data of Figure 7. As shown in Figure 8, DIT/MIT ratio reached normal value at 5 days.

Fig. 8. DIT/MIT ratio. Control value is indicated on the right hand. Circles and vertical lines indicate mean $\pm$ SE.

Experiment 5. Effect of a single injection of methylthiouracil on thyroglobulin

Thyroid proteins were analysed using ultracentrifugation technique. Nine mins. after reaching 50740 rpm, the protein patterns were photographed. Figure 9 indicates such protein patterns in control (A), 4 (B), 24 (C) and 40 (D) hrs. after a single injection of 20 mg methylthiouracil. In the control group, a striking peak of 19 S thyroglobulin was found. The peak of 19 S fraction decreased somewhat at 4 hrs. The decrease was clearly shown at 24 hrs. In addition, 27 S fraction decreased at 4 hrs. and was undetectable thereafter. Interestingly, the peak of 19 S fraction returned to normal at 40 hrs.

Fig. 9. Ultracentrifugation patterns of thyroidal proteins. A, control group; B, 4 hrs. after MTU; C, 24 hrs. after MTU; D, 40 hrs. after MTU. From left to right, 27S, 19S and 4S.

Discussion

A number of criteria have been used to assess blocking action of antithyroid drugs on thyroid hormone synthesis. Since antithyroid drug inhibits synthesis of organic iodine which normally constitutes more than 98 % of thyroidal iodine, it is theoretically possible that administration of antithyroid drug results in a decrease of thyroidal radioiodine uptake (Iino *et al.*, 1961). In fact, thyroidal radioiodine uptake was low for up to 24 hrs. in our present experiment in which 20 mg MTU was administered simultaneously with radioiodine. If this decrease of thyroidal radioiodine uptake accurately reflects the magnitude of blocking action of antithyroid drug on thyroid hormone synthesis, then the measurement of thyroidal radioiodine uptake might be the most simplest technique to assess antithyroid action. However, question arises as to its significance. Since intrathyroidal antithyroid drug can easily be reached out (Shimoda and Greer, 1966), continuation of a low uptake for 24 hrs. should indicate that an appreciable amount of antithyroid drug is still circulating at 24 hrs. This seems unlikely in view of the fact that disappearance of antithyroid drug from the blood is rapid (Maloof and Soodak, 1957). To solve this discrepancy, we have measured blood $^{131}\text{I}^-$ in MTU treated animals, since thyroidal radioiodine uptake is governed by both thyroid ability for trapping iodine and amounts of circulating iodide. It is clearly shown that $^{131}\text{I}^-$ disappeared quite rapidly from the blood and its concentration at 24 hrs. was about 1/8 to 1/10 of initial concentration in MTU treated animals. This agrees well with the report by Brown (Brown, 1956). If iodide trapping ability of the thyroid did not significantly differ between control and MTU treated animals, such a rapid decrease of blood $^{131}\text{I}^-$ should result in a decrease of thyroidal radioiodine uptake. Therefore, it seems that thyroidal radioiodine uptake does not exactly reflect blocking action of MTU on thyroid hormone synthesis when the uptake is

measured long time after administration of radioiodine.

To settle such a question, a direct measurement of organic iodine in the thyroid may be in order. As shown in Figure 3, thyroidal PB^{131}I increased progressively with time and constituted more than 80 % of total thyroidal radioiodine at 24 hrs., while thyroidal radioiodine uptake remained low throughout 24 hrs. Since percentage of intrathyroidal organic iodine is governed by MTU but not by the reduction of circulating $^{131}\text{I}^-$ produced by the drug, our data suggest that thyroidal PB^{131}I is more reliable index than thyroidal radioiodine uptake in MTU treated animals.

Since our purpose is to study which parameters of thyroid activity is the reliable index for measuring blocking action of antithyroid drug on thyroid hormone synthesis, and since thyroidal PB^{131}I is comprised of hormones (T_4 and T_3) and its precursor (MIT and DIT), we have further studied the reliability of PB^{131}I as the criteria of thyroid hormone synthesis after a single injection of 20 mg

MTU. As shown in Figure 5, thyroidal PB^{131}I only reflects MIT at 4 hrs. Even at 24 hrs., thyroidal PB^{131}I well reflects MIT but not T_4 and T_3 . Thus it can be concluded that blocking effect of antithyroid drug on thyroid hormone synthesis can only be evaluated by measuring thyroidal T_4 and T_3 . However, measurement of intrathyroidal hormones is time consuming and not possible in *in vivo* experiment. In view of these reasons, we have further attempted to re-evaluate thyroidal radioiodine uptake and thyroidal PB^{131}I as the criteria of thyroid hormone synthesis. In order to minimize a possible error produced by rapid disappearance of iodide, radioiodine was injected 4 hrs. before autopsy. Here we found that thyroidal uptake initially suppressed returned to normal by 4 days and exceeded normal value thereafter. A low value of thyroidal PB^{131}I also returned to normal by 4 days. Furthermore, percentage of intra-

thyroidal T_4 and T_3 returned to normal by 4 days. Thus, thyroidal radioiodine uptake and thyroidal $PB^{131}I$ well reflect synthesis of thyroid hormones when radioiodine is injected 4 hrs. before measurement. However, when judged by DIT/MIT ratio, the ratio was still abnormal at 4 days and normalized at 5 days, indicating some delay of this parameter.

Roche (Roche, 1951) have reported a decrease of thyroidal content of thyroglobulin when antithyroid drug was administered for a long period. The significance of this decrease remained unsolved. However, our present study clearly indicated that 20 mg MTU actually depressed thyroidal content of thyroglobulin in which iodination of tyrosyl residue took place. This decrease seems not to be an accidental occurrence, since thyroglobulin content decreased when hormone synthesis is depressed by MTU and returned to normal when hormone synthesis is actually normal. Thus, it seems reasonable that antithyroid drug, at least MTU, acts on the thyroid in two ways; one blocking oxydative enzyme and the other depressing thyroglobulin content.

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