Hormonal control of manganese transport in the mouse thyroid

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Received 11 February 1991; accepted 2 September 1991

Abstract. The present study deals with a possible mechanism controlling the transport of manganese (Mn), an essential trace element, from the circulation to the thyroid. Mice were pretreated with propylthiouracil (PTU) or triiodothyronine (T_3), and a measurement of the thyroid:serum concentration ratio (T/S) of radioactive manganese (^{54}Mn) was carried out. The T/S of ^{54}Mn was greatly enhanced by PTU, but reduced by T_3. Several methods were used to demonstrate that the T/S of ^{54}Mn depends upon the level of thyroid-stimulating hormone (TSH) in the serum. First, bovine TSH was injected into mice; an increase in the T/S resulted. Secondly, serum thyroxine and T_3 levels measured by radioimmunoassay (RIA) suggested that PTU produced an increase in serum TSH and T_3 a decrease. However, direct measurement of mouse TSH by RIA for rat TSH failed to produce proof of any changes in TSH level, owing to poor cross-reactivity. Taking all the information into account, it is concluded that Mn-transport into the thyroid is controlled by the thyroid state.
Key words. Manganese; thyroid; thyroxine; triiodothyronine; metal transport.

Manganese (Mn) is distributed ubiquitously throughout the body. It is an essential trace element which plays an important role in a variety of physiological functions[1]. Substantial amounts of Mn have been found in some endocrine glands[2,3], but the function of Mn in these glands is not well understood. The thyroid is one of the glands which tend to accumulate Mn[2,3], but the significance of this accumulated Mn is unknown. The authors have demonstrated that excess Mn induced goiter in the thyroid of female mice[4], and that some pathological conditions altered the distribution of Mn in the thyroid of experimental animals[5] and in the human thyroid[6]. These observations indicate that Mn-transport might be controlled by the state of the thyroid. Further study is necessary to investigate what conditions are required for Mn-transport into the thyroid.

The purpose of the present study was to explain the relationship between Mn-uptake by the thyroid and the thyroid state. To this end, the uptake of radioactive manganese (^{54}Mn) by the thyroid was examined after propylthiouracil (PTU), a typical antithyroid drug, or 3,3',5-triiodothyronine (T_3), a physiologically active thyroid hormone, had been administered to the mice to modify their thyroid state.

Materials and methods

ddY Mice of both sexes, weighing 22–25 g, were fed a normal pellet diet and given tap water ad libitum. Mice were divided into four groups. In the first group, PTU was given in the drinking water (0.5 mg/ml) for six days. The daily ingested amount of PTU was approximately 8 mg/100 g body weight (bw)/day (d). In the second group a saline solution was given. The third group was given 20 μg/100 g bw/d of T_3 intraperitoneally for 6 days, and the fourth group was given the vehicle of the hormone. The duration of the drug treatment and the drug doses were determined from the results of preliminary tests. Special care was taken to choose conditions under which thyroid function was sufficiently influenced by the drugs, but the weight of the gland was not changed a great deal, because, as can be seen in the definition of the T/S (see below), the change in the weight would significantly affect the value of the T/S.

After the six-day drug treatment, 2–3 μCi of ^{54}Mn was injected intraperitoneally. At 24 h, under anesthesia, the animals were killed and their thyroid glands and blood processed. The thyroid:serum ratio (T/S) of ^{54}Mn was measured by the method of Halmi et al.[7], except that ^{54}Mn was measured instead of radioactive iodide. T/S was defined as the ratio of the radioactivity in 100 mg thyroid tissue (T) to that in 100 μl serum (S). Gamma rays emitted at 0.86 MeV were counted by Aloka auto well-type gamma scintillation counter with a counting efficiency of 57%.

The contribution of serum thyroid-stimulating hormone (TSH) to the Mn-uptake by the thyroid was assessed in three ways. First, at the time of ^{54}Mn administration to the mice, 100 mU of bovine TSH (bTSH, Sigma, St. Louis, USA) was co-injected intravenously, according to the method of Gafni and Gross[8], and the T/S of ^{54}Mn at 1 h was determined. Second, levels of serum thyroxine (T_4) and triiodothyronine (T_3) were measured by radioimmunoassay (RIA) as a parameter for monitoring the thyroid state, which reflects the serum TSH level. Third, an attempt was made to measure the TSH by RIA, with a rat anti-TSH antibody provided by NIADDK (Bethesda, USA), although the antibody was poorly cross-reactive to the mouse TSH. The significance of the data was statistically evaluated by Student's unpaired t-test.

Results

^{54}Mn-uptake was significantly affected by the pretreatment with either PTU or T_3. Figure 1 shows the effect of a 6-day treatment with PTU on the T/S of ^{54}Mn in both male and female mice. The T/S rose substantially in the

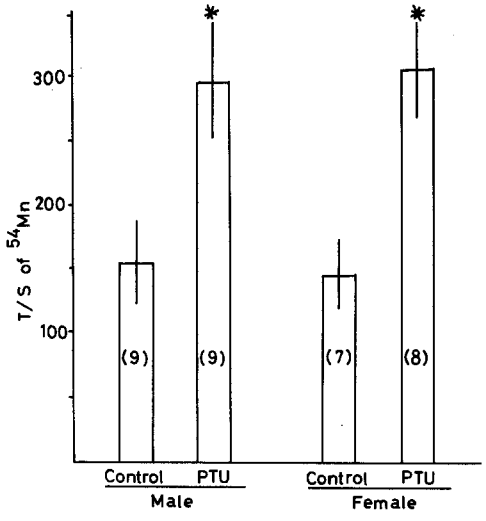

Figure 1. Effect of propylthiouracil on the T/S of ^{54}Mn. Values represent the mean ± SD of the number of animals indicated in parentheses. (*) stands for $p < 0.001$.

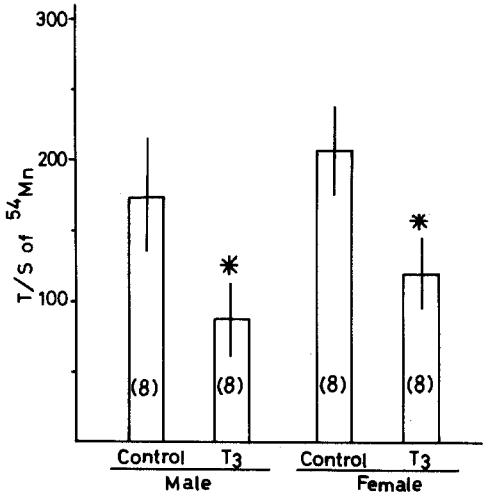

Figure 2. Effect of triiodothyronine on the T/S of ^{54}Mn. Explanations are the same as for fig. 1.

PTU-treated group, compared with that in the control. The thyroid weight of male mice was slightly increased by PTU but that of female mice was unchanged by it: for males, 3.2 ± 0.4 mg in the control, 3.8 ± 0.5 mg in the PTU group (the mean ± SD, n = 9 for each group, $p < 0.01$): for females, 2.8 ± 0.1 mg in the control, 2.9 ± 0.4 mg in the PTU group (n = 7 and 8, respectively, p: not significant). When PTU administration was extended to 14 days, the average weight of thyroids rose to over 150% of that of the control, and the T/S became slightly smaller than that at 6 days, mainly owing to the increase in thyroid weight (data not shown).

Figure 2 presents the effects of a six-day treatment with T_3 on the T/S of ^{54}Mn of both male and female mice. The T/S values were reduced substantially under the impact of T_3. In a first test, 2 µg/100 g bw/d T_3 was used; in this test the T/S values deviated widely, suggesting that the dose was insufficient (data not shown). The results in figure 2 were with a ten times higher dose of T_3 than in the first trial. When the dose was increased to 50 µg/100 g bw/d, no further reduction in the T/S value was observed. The thyroid weights of T_3-treated mice in both sexes were unchanged under the conditions used. Hormonally inactive r-T_3 was also tested in the dose range of 5–20 µg/100 bw/d for 6 days: no inhibitory effect on the T/S ratio of ^{54}Mn was observed (data not shown).

It had been suggested that the increase in the T/S value for ^{54}Mn due to PTU, and its decrease due to T_3, were caused by the changes in serum TSH level evoked by the drugs. Therefore the effect of a direct injection of bTSH on the T/S was examined at 1 h, where the serum TSH level after a single injection was expected to be nearly at its highest[8]. The mean values ± SD of the T/S ratio were 108.3 ± 45.4 (n = 6) for the TSH-group, and 46.8 ± 29.0 (n = 6) for the control ($p < 0.05$).

Serum T_4 and T_3 levels are presented in the table. Administration of PTU resulted in a significant reduction in the T_4 level, and T_3 also reduced it significantly. However, the authors consider that the causes of the reduction are different in the cases of PTU and of T_3. This will be discussed further in a later section. Serum T_3 levels in female mice were not drastically altered by either treatment, although they were reduced to some extent in male mice by either PTU or T_3 treatment. Furthermore, an attempt was made to measure TSH by RIA with a rat anti-TSH antibody; the values obtained were

Serum thyroxine and triiodothyronine concentrations. Values represent the mean ± SD of the number of animals indicated in parentheses. (*) and (**) stand respectively for $p < 0.01$ and $p < 0.001$.

Hormone	Sex	Control	Treatment PTU	T_3
T_4 (µd/dl)	Male	3.76 ± 0.57 (n = 8)	1.64 ± 0.28** (n = 9)	< 0.02** (n = 6)
	Female	3.07 ± 0.29 (n = 9)	1.46 ± 0.29** (n = 8)	0.25 ± 0.21** (n = 6)
T_3 (ng/dl)	Male	121.2 ± 11.3 (n = 10)	101.4 ± 16.2* (n = 10)	94.7 ± 9.5** (n = 6)
	Female	128.0 ± 7.56 (n = 10)	123.7 ± 7.09 (n = 8)	136.0 ± 14.7 (n = 5)

0.57 ± 0.21 ng/ml in the control (n = 5), 0.83 ± 0.10 ng/ml in the PTU-treated (n = 6) and 0.69 ± 0.21 ng/ml in the T_3-treated group of male mice (n = 5) (statistically insignificant in any combinations).

Discussion

The present study indicated the possibility that Mn-uptake by the thyroid is controlled by the thyroid state, which is regulated by drugs and hormones: when the serum T_4 level was reduced by PTU, ^{54}Mn-uptake from the circulation to the thyroid was stimulated, whereas when the hormone level was suppressed by the external T_3 supply, the uptake was inhibited. The degree of the increase in the uptake due to PTU (96.1% in male mice) far exceeded the increase of the thyroid weight caused by the drug (18.8%). The decrease in ^{54}Mn uptake due to T_3 was not accompanied by any observable changes in the thyroid weight. This indicated that the changes in the uptake were not dependent on the thyroid mass, but on changes in physiological functions caused by secondary effects of drugs. Although a direct effect of PTU or T_3 on Mn-transport has not been ruled out, the authors consider that the changes in T/S ratio caused by PTU or T_3 are effected via hormonal control, most probably the serum TSH level; this is because an inactive T_3 analog, r-T_3, did not change the T/S ratio of ^{54}Mn.

The authors tried to demonstrate the involvement of TSH in Mn-uptake by the thyroid in three different ways. First, the injection of bTSH gave rise to an increase in ^{54}Mn-uptake. This may be considered good evidence in support of a role for TSH in Mn-transport. Evidence that bTSH does have an effect on the mouse thyroid is provided by the observation that radioiodide uptake by primarily cultured mouse thyroid epithelial cells was greatly enhanced by the addition of bTSH (authors, unpublished data). Secondly, the serum thyroid hormone level, which also reflects thyroid state, was measured. PTU reduced the serum T_4 level. This may have been the result of PTU blocking T_4 synthesis in the thyroid, so that the serum T_4 level was reduced. This would have been followed by an enhanced TSH secretion from the pituitary[9]. Although the thyroid weight was not much increased by a six-day treatment with PTU, the duration ought to have been long enough to affect the thyroid function.

Administration of excess T_3 for six days also reduced serum T_4 level. This is a different mechanism of T_4 reduction from that due to PTU, because excess T_3 suppresses TSH secretion from the pituitary[10], and thus reduces T_4 release from the thyroid. This, in turn, may cause the depletion of circulating T_4. Deficiency of T_4 supply to the peripheral tissues, where the deiodination of T_4 to T_3 is active[11], will result in a low level of serum T_3. Therefore, the changes in serum T_4 due to PTU or T_3, shown in the table, are considered to be related to the corresponding TSH levels. According to experiments by the authors[12] and others[13], serum T_3 levels are in many cases less sensitive to drug effects than are levels of serum T_4. As the table shows, some reductions in T_3 were observed in male mice when PTU or even excess T_3 was administered. This may have been the result of a limited supply of T_3, consequent upon a serum T_4 level kept down by PTU or excess T_3, and also of T_3 having a very much shorter metabolic half-lifetime in the blood compared with that of T_4[14].

The third method tried was a direct assay of serum TSH. This was attempted using a RIA with a rat anti-TSH antibody, but the results were obscure, owing to the insufficient cross-reactivity. In experiments with rats given PTU or excess T_3, the TSH values were 3.6 ± 0.4 for the control 22.5 ± 1.8 for the PTU group and 2.1 ± 0.6 ng/ml for the T_3 group (n = 5 for each group, authors' unpublished results). Therefore, it is possible that TSH values were also altered in mice, although this could not be clearly demonstrated.

The question as to whether or not an observed effect of PTU or T_3 on ^{54}Mn uptake is specific to Mn was not answered in the present study. However, transport of other ions can be affected by the thyroid state; for example, lithium, which is also accumulated in the thyroid, has been demonstrated by the authors[12] to be reduced by PTU and increased by T_4; that is, the effects are opposite to those on Mn. Thus, the regulatory mechanisms for metal ion transport in the thyroid may operate independently for each metal.

Mn binds to α_2-globulin and/or transferrin[15,16] in the circulation and is transferred to the peripheral tissues. There is not sufficient evidence to show whether Mn-transport across the cell membranes takes place via specific carrier proteins, or whether Mn-transport requires energy. It is known that iodide concentration by the thyroid requires energy, generated by the Na^+, K^+-ATPase system. Iodide transport reacted similarly to Mn-uptake in response to PTU and thyroid hormone; iodide transport was stimulated by PTU and inhibited by thyroid hormone. Na^+, K^+-ATPase and Mg^{++}-ATPase in the membrane fraction of the thyroid were not affected by Mn (authors, unpublished data), but in vivo iodide transport was inhibited by excess Mn[3,17]. These observations make it unlikely that there is co-transport of Mn with iodide. The driving force for Mn transport against its concentration gradient in the thyroid – and perhaps in some other endocrine glands – still remains to be elucidated.

When $MnCl_2$ was injected into rats, only a small fraction of the Mn^{2+} could be recovered in tissues[2]. Thus, the valence state of Mn is likely to be rapidly changed to other valence states in which Mn can function physiologically[2]. As the author's group has recently demonstrated[18], there are fundamental differences between the biological and chemical behavior of Mn in the liver and in the thyroid. Moreover, there is a drastic alteration in Mn distribution in tissues under pathological conditions induced by chemicals[19], in diabetes in experimental ani-

mals [5], and in human thyroid cancer [6]. There is no doubt that Mn moves and functions dynamically in the body. To understand the real roles of Mn in the body it is very important to know the controlling mechanisms of its transport from the circulation. In the present study it has been possible to bring to light at least one of the regulatory mechanisms by which Mn-uptake by the thyroid is controlled.

Acknowledgment. The authors are indebted to Ms Ryoko Murakami and Ms Chiaki Adachi for their technical assistance.

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0014-4754/92/030262-04$1.50 + 0.20/0

Effect of stress on choline acetyltransferase activity of the brain and the adrenal of the rat [1]

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Received 25 May 1991; accepted 16 September 1991

Abstract. Choline acetyltransferase (ChAT) activity was determined in cerebral cortex, hypothalamus, hippocampus, cerebellum, medulla oblongata, midbrain and adrenal gland of rats exposed to acute or chronic stress. The exposure of animals to acute immobilization and cold stress (4 °C) for one hour resulted in a significant decline of ChAT activity in all brain regions examined except for the medulla oblongata. Moreover, the exposure to acute stress resulted in significant increase of the same enzyme in the adrenal gland. However, chronic exposure of animals to cold stress (4 °C) for 7 days resulted in no significant changes of ChAT activity in all tissues examined except for a decline in the midbrain and an increase in the medulla oblongata. The administration of corticosterone (2.0 mg/kg) 1 h prior to sacrificing caused an effect similar to that of acute stress on ChAT activity in all brain regions except for the hypothalamus and the cerebellum. It was concluded from this experiment that stress-induced changes in the ChAT activity of specific brain regions might be mediated by the adrenal steroids.
Key words. Choline acetyltransferase; cerebral cortex; hypothalamus; hippocampus; cerebellum; medulla oblongata; midbrain; adrenal gland.

Stress is known to lead to a series of biochemical, physiological, and behavioral changes mediated through the neuroendocrine system that alter normal homeostasis [3–7]. Factors such as stress, drugs, anesthesia and hormones have been shown to have significant effects on the nervous system performance [7–13]. It has been proposed that the effects of an acute and chronic stress are partially mediated by the central muscarinic system with capacities to activate the adrenergic nervous system [3, 14]. The cholinergic regions of the central nervous system play an essential role in the capability of living organisms to cope with external or internal demands, particularly when the limits of tolerance tend to be exceeded [8, 15, 16]. For example, it was shown that an acute swimming stress in the rat produces changes in cholinergic muscarinic receptors selective for certain regions of the central nervous system [15]. A neurotransmitter, such as acetylcholine (ACh) is known to produce behavioral, neuroendocrine, cardio-