Studies on the Mechanism of Inhibitory Action of Excess Iodide on Thyroid Hormone Secretion (37631)

MASAO OHTAKE, TOSHIMASA ONAYA, AKIRA SATO, AND TAKASHI YAMADA

Department of Medicine, Institute of Adaptation Medicine, School of Medicine, Shinshu University, Matsumoto, Japan

It seems well-established that the administration of excess iodide inhibits thyroid hormone secretion in hyperthyroid patients (1–5) and in experimental animals as well (6–14). However, the use of radioiodine for measuring thyroid hormone release makes the interpretation somewhat difficult, since the behavior of radioiodine may not accurately indicate the behavior of the stable iodine when excess iodide is administered. Yamamoto *et al.* (14) in our laboratory have recently shown that excess iodide inhibits the colloid droplet formation induced by TSH or dibutyryl cyclic AMP in rats and mice that are fed a low iodine diet, suggesting that excess iodide manifests its inhibitory action on thyroid hormone secretion at a site subsequent to generation of cyclic AMP. However, the mechanism which inhibits the action of cyclic AMP is not known at present. In 1946, De Robertis and Nowinski (15) suggested that excess iodide may inhibit the activity of proteolytic enzyme(s) which is probably responsible for the release of the follicular colloid. More recently, Takeuchi *et al.* (16) have reported that the depression of the proteolytic activity in the thyroid of iodide-treated animals appears to play an important role in inhibiting the release of thyroid hormone. There were some aspects of the problems, however, which had not been well investigated. Among them are: (a) excess iodide stabilizes thyroglobulin by increasing the iodine content and thereby making it resistant to proteolytic enzyme(s); (b) iodide directly inhibits thyroidal proteolytic enzyme activity: and (c) excess iodide manifests its action in the form of iodide or after conversion to some form of organic iodine. The studies reported herein were undertaken to solve these problems.

Materials and Methods. Fifty eight male Wistar rats, weighing approximately 140 g, and 50 male DDY mice, weighing 30 g were used in these experiments. In Expt 1, 10 μCi of ^{131}I were administered 24 hr before autopsy to 6 rats fed a low iodine diet (LID) for 7 days. The thyroids were pooled and homogenized in a glass tissue grinder with 5 ml of saline–Tris buffer (pH 8.0) containing 1 m*M* methimazole (MTZ). Graded doses of pronase (0.001 to 1.0 mg) were added to 1 ml of thyroid homogenates, and the mixtures were incubated for 6 hr at 37°. Portions of each hydrolysate were fractionated by ascending paper chromatography in *n*-butanol–ethanol–0.5 *N* NH_3 (5:1:2) solvent system (BEA) (8). The radioactivity which migrated from the origin was expressed as a percentage of the total radioactivity of the paper strip, and this percentage was regarded as indicating the proteolytic activity.

In Expt 2, 20 rats were fed LID for 10 days and were divided into 4 equal groups (A–D). A group served as the control and was injected with saline once daily for 5 days. Ninety minutes after the last dose of saline, autopsies were performed. B group received saline once daily for 5 days, while C group received saline similarly for 4 days and 1 mg KI on the fifth day. D group received 1 mg KI once daily for 5 days. One hour after the last dose of KI or saline, TSH (20 mU) was administered and autopsy was performed 30 min later. One thyroid lobe served to measure the intrathyroidal colloid droplet formation and the other lobe was used to test the *in vitro* proteolytic activity. The techniques for measuring intrathyroidal colloid droplets were reported previously (17).

In Expt 3, 8 rats were divided into 2 equal groups. The animals were fed LID for 7 days before the experiment, and were injected with saline or 1 mg KI once daily for 3 days. TSH (20 mU) was injected 1 hr after the last injections of KI or saline and autopsy was performed 10 min later. Autoproteolysis of thyroid protein was measured in the presence or absence of reduced glutathione (GSH), using the technique reported by Alpers, Robbins and Rall (18) and Takeuchi *et al.* (16).

In Expt 4, 3 subexperiments were performed. In group 1, 28 rats were fed LID for 7 days and were divided into 6 subgroups (A–F). The animals were injected once daily for 3 days with saline (A, B), 2 mg KI (C), 5 mg propylthiouracil (PTU) (D), 5 mg PTU + 2 mg KI (E) and 40 mg PTU + 2 mg KI (F). One hour after the last dose, 20 mU TSH was administered and autopsy was performed 30 min later. In group 2, 23 male mice were fed LID for 5 wk before the experiment, and were divided into subgroups (A–E). The animals received a single injection of saline (A, B), 2 mg KI (C), 1 mg MTZ (D) or 1 mg MTZ + 2 mg KI (E), and 4 mU of TSH was administered 1 hr after the injection of the test materials. Thirty minutes after TSH injection, autopsy was performed. In group 3, 27 male mice were fed LID for 6 wk before the experiment, and were divided into 5 subgroups (A–E). The animals were injected once daily for 2 days with saline (A, B), 2 mg KI (C), 0.1 mg MTZ + 2 mg KI (D) and 3 mg MTZ + 2 mg KI (E). One hour after the last dose, 4 mU TSH were injected and autopsy was performed 30 min later. Intrathyroidal colloid droplets were then analyzed.

PTU and MTZ were obtained from Tokyo Tanabe Pharmaceutical Co. For injection, PTU was suspended in 5% gum acacia and injected subcutaneously. Bovine TSH (Thytropar) was purchased from the Armour Pharmaceutical Co.

Results. Expt 1. Effect of graded doses of pronase on hydrolysis of thyroglobulin previously labeled with ^{131}I. As shown in Fig. 1, 0.001 mg pronase did not display any hydrolytic activity, as evidenced by the fact

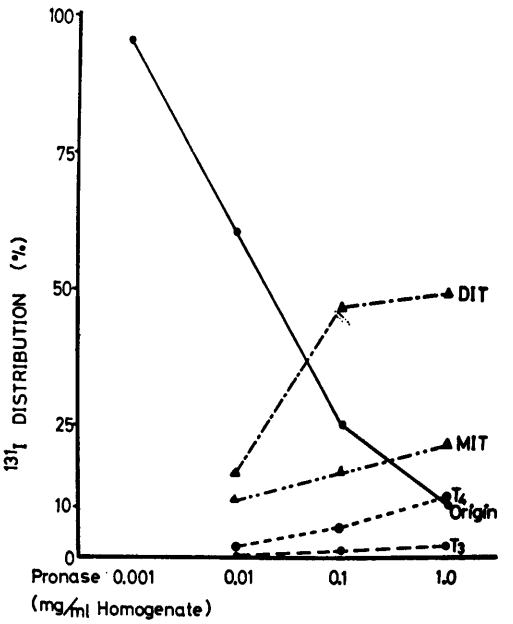

FIG. 1. Effect of various concentrations of pronase on ^{131}I distribution among iodoaminoacids in rat thyroid homogenate. Animals were fed a low iodine diet for 7 days. Six rat thyroid lobes which had been labeled with 10 μCi of ^{131}I for 24 hr were homogenized and pooled for digestion. Digestion with pronase was performed at 37° for 6 hr in the presence of methimazole (10^{-2} *M*).

that almost all the radioactivity remained at the origin. However, the radioactivity remaining at the origin decreased progressively with increasing concentrations of pronase. Conversely, the labeled amino acids released increased progressively with increasing doses of pronase. About 50% proteolysis was found at the concentration of 0.01 mg pronase/1 ml homogenate. In the subsequent experiment, this concentration of pronase (0.01 mg) was used.

Expt 2. Effects of excess iodide on intrathyroidal colloid droplet formation produced by TSH and in vitro *stability of iodoprotein against pronase.* As shown in Table I, only a small number of intrathyroidal colloid droplets were found in saline-treated control. Administration of TSH apparently produced an increase of intrathyroidal colloid droplet formation (Group B), but this increase could be significantly depressed by a single injection of 1 mg KI (Group C of Table I). By contrast, repeated injections of 1 mg KI had

TABLE I. Effect of Excess Iodide on the Stability of Iodoprotein Against Proteolytic Activity of Pronase.[a]

Group	Treatment	No. of animals	Colloid droplets/25 follicles	p value	Proteolytic activity (%)
A	Saline only	5	20 ± 18[b]		55.8 ± 2.0
B	Saline + TSH (20 mU)	5	936 ± 170		56.6 ± 1.6
C	KI (1 mg) + TSH (20 mU)	5	310 ± 125	B–C $p < 0.01$	53.2 ± 0.7
D	KI (1 mg) for 5 days + TSH (20 mU)	5	719 ± 103	C–D $p < 0.05$	53.0 ± 1.1

[a] Animals were fed a low iodine diet for 2 wk. A = saline was injected once daily for 5 days; B = saline was injected similarly for 5 days; C = saline was injected once daily for 4 days and KI (1 mg) was injected on the fifth day; D = KI (1 mg) was injected once daily for 5 days. One hour after the last dose of saline (B) or KI (C, D), 20 mU of TSH in 0.5 ml saline were injected, and the animals were killed 30 min later.

[b] Mean ± SE.

no effect on an increase in the colloid droplet formation produced by TSH (Group D).

Proteolysis of thyroglobulin by pronase was also compared. In the saline-injected control group, 55.8% of iodinated protein was proteolyzed by pronase (0.01 mg). This proteolysis produced by pronase did not significantly differ from group to group regardless of whether colloid droplet formation was stimulated or not.

Expt 3. Effect of in vivo *administration of excess iodide on autoproteolytic activity of the thyroid* in vitro *in the presence or absence of reduced glutathione.* TSH was administered singly or in combination with excess iodide to produce a condition in which the excess iodide blocks thyroid hormone secretion. Administration of KI did not depress autoproteolytic activity in the absence of GSH, nor did it affect the autoproteolytic activity in the presence of GSH (Table II).

Expt 4. Effect of propylthiouracil or methimazole on KI inhibition of colloid droplet formation induced by TSH in rats and mice. In group 1, only a small number of colloid droplets were found in the control thyroid (Table III, 1A). Administration of TSH apparently increased intrathyroidal colloid droplet formation (1B), but this increase was blocked by 3 injections of excess iodide (1C). Small dose of PTU did not increase intrathyroidal colloid droplet formation (1D). This dose of PTU did not interfere with the inhibitory action of KI on an increase in the colloid droplet formation produced by TSH (1E). However, a large dose of PTU apparently depressed the inhibitory action of excess iodide on the colloid droplet formation (1F). In group 2, TSH again produced a marked increase of intrathyroidal colloid droplet formation (2B), but this increase was depressed by a single injection of

TABLE II. Effect of Excess Iodide on Autoproteolytic Activity in Rat Thyroids in the Presence or Absence of Reduced Glutathione.[a]

Group	Treatment	No. of animals	Autoproteolytic activity (%) GSH (—)	Autoproteolytic activity (%) GSH (+)	p value
A	LID, saline for 3 days + TSH (20 mU)	4	31.6 ± 3.0[b]	49.7 ± 2.1	$p < 0.005$
B	LID, KI (1 mg) for 3 days + TSH (20 mU)	4	31.7 ± 1.5	51.5 ± 1.4	$p < 0.001$

[a] Animals were fed a low iodine diet (LID) for 7 days. TSH was injected 1 hr after the last injection of KI or saline. Autopsy was performed 10 min after TSH injection. GSH = reduced glutathione (final concn, 0.05 *M*).

[b] Mean ± SE of the mean.

TABLE III. Effect of Propylthiouracil or Methimazole on Iodide Inhibition of Colloid Droplet Formation Induced by TSH in Rats and Mice.[a]

Group	Treatment	No. of animals	Colloid droplets/ 25 follicles	p value
1. rat, LID for 7 days				
A	Saline	5	36 ± 12[b]	
B	Saline + TSH (20 mU)	4	235 ± 43	B–A $p < 0.001$
C	KI (2 mg) for 3 days + TSH (20 mU)	5	10 ± 3	B–C $p < 0.001$
D	PTU (5 mg) for 3 days	5	49 ± 13	
E	PTU (5 mg) for 3 days + KI (2 mg) for 3 days + TSH (20 mU)	5	37 ± 19	B–E $p < 0.001$
F	PTU (40 mg) for 3 days + KI (2 mg) for 3 days + TSH (20 mU)	4	115 ± 30	C–F $p < 0.01$
2. mice, LID for 5 wk				
A	Saline	5	22 ± 13	B–A $p < 0.001$
B	Saline + TSH (4 mU)	4	819 ± 16	B–A $p < 0.001$
C	KI (2 mg) + TSH (4 mU)	4	323 ± 64	B–C $p < 0.01$
D	MTZ (1 mg)	5	2 ± 1	
E	MTZ (1 mg) + KI (2 mg) + TSH (4 mU)	5	592 ± 65	C–E $p < 0.05$
3. mice, LID for 6 wk				
A	Saline	5	16 ± 11	
B	Saline + TSH (4 mU)	6	1107 ± 73	B–A $p < 0.001$
C	KI (2 mg) for 2 days + TSH (4 mU)	6	502 ± 73	B–C $p < 0.001$
D	MTZ (0.1 mg) for 2 days + KI (2 mg) for 2 days + TSH (4 mU)	5	351 ± 32	
E	MTZ (3 mg) for 2 days + KI (2 mg) for 2 days + TSH (4 mU)	5	688 ± 78	D–E, B–E $p < 0.01$

[a] PTU and MTZ injected subcutaneously, TSH was injected intraperitoneally 1 hr after the last dose of injections. Autopsy was performed 30 min after TSH administration. PTU = propylthiouracil, MTZ = methimazole.

[b] Mean ± SE of the mean.

2 mg KI (2C). MTZ apparently depressed the action of KI on the colloid droplet formation induced by TSH (2E). In group 3, TSH also markedly elevated the number of intrathyroidal colloid droplets (3B), but this increase was decreased by excess KI (3C). A small dose of MTZ did not depress this KI action (3D), but a large dose of MTZ depressed the action of KI on colloid droplet formation (3E).

Discussion. Since colloid droplet formation is an initial step essentially involved in the secretion of thyroid hormone (19, 20), a decrease of colloid droplet formation in response to excess iodide undoubtedly indicated the site of action of excess iodide in depressing thyroid hormone secretion. Unfortunately, however, the exact biochemical processes involved in engulfment of thyroglobulin at the apical cell surface and the eventual formation of intrathyroidal colloid droplets are not known at present and any explanations produced are merely speculative. However, it should be noticed that lysosomal membrane stabilizers such as chlorpromazine and propranolol will block the colloid droplet formation produced by TSH and dibutyryl cyclic AMP (17, 21). This may mean that lysosomal enzyme is in some way related with the engulfment of thyroglobulin at the apical cell surface. Further experiments are required to clarify whether or not excess iodide similarly stabilizes lysosomal membrane and thus inhibits the formation of colloid droplets. Whatever the exact mechanism of action of excess iodide may be, it is not established whether iodide itself blocks thyroid hormone secretion or whether iodide manifests its action after

conversion to some form of organic iodine. To settle this question, we have made experiments with small and large doses of PTU or MTZ, and found that large doses of PTU and MTZ actually did interfere with the action of excess iodide. This finding is in keeping with our previous report (17) that excess iodide fails to retard the thyroidal radioiodine release under the influence of large doses of PTU. These findings thus offer the explanation that iodide manifests its action on thyroid hormone secretion after its conversion to some form of organic iodine.

In addition to the action on colloid droplet formation, excess iodide may play an additional role in the hydrolysis of the colloid droplets already accumulated within the thyroid cells. From a number of experimental studies (22, 23), it can be suspected that by enriching the iodine content of the thyroglobulin, thyroglobulin becomes resistant to hydrolytic enzyme(s). To test this hypothesis, the response of thyroglobulin to pronase was compared in normal and iodide-treated animals. As far as the exogenous hydrolytic enzyme was concerned, excess iodide did not seem to block thyroid hormone secretion by making thyroglobulin unresponsive to hydrolytic enzyme(s).

Finally we considered the possibility that excess iodide blocks the hydrolytic enzyme activity in the thyroid, since Takeuchi *et al.* (16) have presented evidence that excess iodide interferes with the action of thyroidal hydrolytic enzyme activity. In contrast to the report by Takeuchi *et al.*, excess iodide does not seem to affect the thyroidal hydrolytic enzyme activity as evidenced by a similar autoproteolytic activity in normal and iodide-treated thyroids. However, it is still possible that a depression of protease activity produced by excess iodide is too small to detect the difference under limited experimental conditions. To facilitate enzyme activity somewhat, we have measured autoproteolytic activity under the influence of GSH, since GSH has been known to render thyroglobulin more susceptible to the action of proteolytic enzyme possibly by reducing the disulfide bonds (22). Despite this modification of experimental procedure, we again failed to find any effect of excess iodide on proteolytic enzyme activity at least *in vitro*.

Summary. In order to study the mechanism of inhibitory action of excess iodide on thyroid hormone secretion, effects of excess iodide on colloid droplet formation, susceptibility of thyroglobulin to exogenous protease and thyroidal hydrolytic enzyme activity were studied under several experimental conditions. Excess iodide was first converted to some form of organic iodine and then inhibited colloid droplet formation, an initial step of thyroid hormone secretion. Since this effect of excess iodide was very similar to those produced by lysosomal membrane stabilizers, a possible role of excess iodide on lysosomal membrane was suggested. Administration of excess iodide did not stabilize the thyroglobulin against an exogenous protease, pronase. Excess iodide did not affect thyroidal hydrolytic enzyme activity as evidenced by a normal autoproteolytic activity. It is suggested that excess iodide depressed the thyroid hormone secretion primarily by interfering with the engulfment of thyroglobulin at the apical cell surface and eventual formation of intrathyroidal colloid droplets, without affecting the nature of thyroglobulin or proteolytic enzyme activity.

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