Effects of Goitrin on *in vitro* Hormone Biosynthesis by the Chick Thyroid

Yukio AKIBA and Tatsuro MATSUMOTO

Faculty of Agriculture, Tohoku University Sendai-shi 980

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Abstract The effect of graded doses of goitrogen such as goitrin, PTU, AITC and KClO_4 , on the *in vitro* biosynthesis of thyroid hormone was studied in chicks. Thyroid lobes dissected from the chick fed goitrogen or without dietary goitrogen were incubated in 3 ml KRP buffer, pH 7.4 at 37°C with 20 μCi carrier-free ^{131}I . Thyroidal ^{131}I uptake was depressed by 10^{-5}M of KClO_4 but not significantly depressed by 10^{-6} to 10^{-4}M of goitrin, PTU and AITC. Thyroidal ^{131}I uptake *in vitro* by the thyroid lobes of chicks fed goitrin or PTU was accelerated in comparison with that without goitrogen. Ratios of MIT/DIT were elevated and iodothyronine synthesis was depressed in proportion to concentration (10^{-7} – 10^{-4}M) of goitrogen added. Monoiodination of tyrosine in the thyroid was depressed at the level of 10^{-5}M of KClO_4 and 10^{-4}M of goitrin or PTU. *In vitro* iodothyronine biosynthesis was depressed by 10^{-6}M of goitrin or PTU in the thyroid of chicks fed with or without goitrin or PTU.

It has been established that (–)-5-vinyl-2-oxazolidinethione, which is subsequently called goitrin, and isothiocyanate are contained in rapeseed¹⁾ and these substances have antithyroid properties on animals^{2–4)}. Goitrogenic effects of goitrin have been studied by AKIBA and MATSUMOTO in respect to ^{131}I metabolism⁵⁾, thyroid hormone biosynthesis^{6–9)} and the relative activity of goitrin to propyl-thiouracil (PTU) in growing chicks¹⁰⁾.

The purpose of the work described below is to compare the effects of goitrin with those of PTU, allylisothiocyanate (AITC) and potassium perchlorate (KClO_4), on *in vitro* thyroid hormone biosynthesis by the chick thyroid.

Materials and Methods

Animal and diet. Day-old Single-Comb White Leghorn male chicks were housed in group wire cages for 14 to 16 days under free-access to feed and water, and controlled light and temperature (22°C). Basal diet was composed of 68% yellow corn, 17% soybean meal, 6% casein, 5% alfalfa meal, and minerals and vitamins. The basal diet (iodine content 230 $\mu\text{g}/\text{kg}$) was fed in trials 1 and 2 and the iodine supplemented basal diet (iodine content 670 $\mu\text{g}/\text{kg}$) was fed in trial 3. Metabolizable energy and crude protein content were 2850 kcal/kg and 20.2%, respectively.

***In vitro* incubation of chick thyroid.** Chicks were killed by heart puncture and each thyroid lobe was carefully dissected without any damage on the tissue. Then thyroid lobes (average weight: 6 mg) were placed individually in 3 ml of Krebs-Ringer phosphate buffer (KRP buffer, pH 7.4) containing 20 μCi carrier-free ^{131}I . They were incubated in a shaking water bath at 37°C with air as the gas phase.

At the end of incubation, each thyroid lobe was removed from the flask, held with a fine forceps and rinsed with small amount of KRP buffer to remove the adherent ^{131}I .

Analysis. The radioactive iodine content in the thyroid was determined by counting each lobe individually in a well-type scintillation counter after placing it in a test tube. To determine the intrathyroidal composition of radioiodine, thyroid lobes were homogenized and digested in Tris buffer at pH 8.4 containing 0.5% pancreatin and 0.01 M methimazole. Aliquots of the hydrolysate were subjected to ascending paper chromatography in a solvent system, *n*-butanol-absolute ethanol-0.5N- NH_4OH . Detailed procedure was previously described in our reports^{4,6}.

Chemicals. The goitrin used in this experiment was prepared from rapeseed (*Brassica napus*) by the method of ASTWOOD *et al.*¹¹ PTU was purchased from Sigma Chemical Company, Mo. U.S.A., and AITC and KClO_4 were purchased from Wako Pure Chemicals Ind., Osaka, Japan. Radioactive iodine (^{131}I) was obtained from Dainabot Radioisotope Lab., Tokyo, Japan.

Results

Relation of incubation time to in vitro thyroid hormone biosynthesis (Trial 1).

Individual thyroid lobes were incubated for 1, 2, 3 and 6 hours in KRP buffer. As illustrated in Fig. 1, thyroidal ^{131}I uptake was elevated as a function of time and reached the highest level of 20% of administered ^{131}I at 6 hours after. Percentage of iodide- ^{131}I for total thyroid ^{131}I gradually decreased until approximately equilibrated state was obtained at 3 hours of incubation. Percentage of iodothyronines increased as a function of time and biosynthesized iodothyronine- ^{131}I was approximately 2.5% for total thyroid ^{131}I at 3 and 6 hours of incubation. In views of the above results, it is concluded that 3 hour incubation is appropriate to investigate *in vitro* thyroid hormone biosynthesis in chicks.

Effectes of graded doses of goitrogen on in vitro thyroid hormone biosynthesis (Trial 2).

The thyroid lobes of the chicks fed the relatively low iodine basal diet were incubated in KRP buffer for 3 hours. Effects of goitrin on *in vitro* thyroid hormone biosynthesis are illustrated in

Fig. 1. Relation between incubation time and thyroid function *in vitro*. A. Thyroidal ^{131}I uptake (% of dose). B. Percentage of iodide- ^{131}I of total thyroid ^{131}I . C. Percentage of iodothyronines- ^{131}I of total thyroid ^{131}I . Vertical bars indicate standard deviation of the means of 4 samples.

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Fig. 2. Effects of graded doses of goitrin on thyroid ^{131}I metabolism *in vitro*. A. Thyroidal ^{131}I uptake (% of dose). B. Iodide- ^{131}I (% of total thyroid ^{131}I). C. Iodothyronines- ^{131}I (% of total thyroid ^{131}I). D. MIT- ^{131}I /DIT- ^{131}I ratios.

Fig. 2. Thyroidal ^{131}I uptake was approximately 7% when incubated in the medium without goitrin. In the medium of 5×10^{-5} to 5×10^{-4} M goitrin, a slightly decreasing, but not statistically significant, trend in thyroidal ^{131}I uptake was observed. Percentage of iodide was not influenced by 5×10^{-7} to 5×10^{-5} M goitrin, and was markedly elevated in the medium containing 5×10^{-4} M goitrin. Biosynthesis of iodothyronines decreased in proportion to the goitrin concentration in the medium, and in 5×10^{-4} M goitrin, it showed to be about one-third of that of the lobes without goitrin. The ratios of monoiodotyrosine (MIT)/diiodotyrosine (DIT) were significantly and progressively elevated in the medium of 5×10^{-7} to 5×10^{-4} M goitrin.

Fig. 3. Effects of graded doses of PTU on thyroid ^{131}I metabolism *in vitro*. A. Thyroidal ^{131}I uptake (% of dose). B. Iodide- ^{131}I (% of total thyroid ^{131}I). C. Iodothyronines- ^{131}I (% of total thyroid ^{131}I). D. MIT- ^{131}I /DIT- ^{131}I ratios.

As shown in Fig. 3, no marked changes were observed in thyroidal ^{131}I uptake by the addition of PTU except a slight decrease in $5 \times 10^{-4}\text{M}$ PTU. Elevated percentage of iodide (80% of total thyroid ^{131}I) was found in $5 \times 10^{-4}\text{M}$ PTU and it was higher than that in $5 \times 10^{-4}\text{M}$ goitrin (Fig. 2). Iodothyronine biosynthesis were depressed by the addition of PTU and were almost blocked in the medium of $5 \times 10^{-4}\text{M}$ PTU.

Thyroidal ^{131}I uptake was increased by the addition of AITC until the concentration of $2 \times 10^{-4}\text{M}$, and over this concentration it began to decrease as illustrated in Fig. 4. Addition of AITC to the incubation medium resulted in a significant depression of iodothyronine biosynthesis. It is clearly demonstrated that elevation of MIT/DIT ratio by the addition of AITC was smaller than that by the addition of goitrin or PTU.

Fig. 4. Effects of graded doses of allylthiocyanate on thyroid ^{131}I metabolism *in vitro*. A. Thyroidal ^{131}I uptake (% of dose). B. Iodide- ^{131}I (% of total thyroid ^{131}I). C. Iodothyronines- ^{131}I (% of total thyroid ^{131}I). D. MIT- ^{131}I /DIT- ^{131}I ratios.

In the medium containing 2×10^{-5} and $2 \times 10^{-4}\text{M}$ KClO_4 , thyroidal ^{131}I uptake were significantly depressed and percentage of iodide- ^{131}I was significantly depressed in comparison with the lobes in medium without goitrogen (Fig. 5). These phenomena were clearly different from those obtained by the addition of goitrin, PTU or AITC. Similarly, biosynthesis of iodothyronines were markedly depressed by adding KClO_4 . Elevation of MIT/DIT ratio by KClO_4 was relatively smaller than that by other goitrogens though the ratios were elevated progressively.

Effects of graded doses of goitrin or PTU on in vitro thyroid hormone biosynthesis in the lobes of chicks fed goitrin or PTU.

Thyroid lobes of the chicks fed the diet containing 0.05% goitrin or 0.01% PTU for 14 days were incubated with graded level of goitrin or PTU. Fig. 6 shows that thyroid lobes of the chicks fed goitrin incorporated ^{131}I were twice as much as those of the chicks fed no goitrin, and in the medium containing $2 \times 10^{-4}\text{M}$ goitrin thyroidal ^{131}I uptake was significantly depressed. Percentages of iodide- ^{131}I were slightly elevated in thyroid of the chicks fed goitrin and in the medium containing lower concentrations of goitrin, and were greatly elevated in the medium containing $2 \times 10^{-4}\text{M}$ goitrin (Fig. 6B). Biosynthesis of iodothyronines was depressed

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Fig. 5. Effects of graded doses of KClO₄ on thyroid ¹³¹I metabolism *in vitro*. A. Thyroidal ¹³¹I uptake. B. Iodide-¹³¹I (% of total thyroid ¹³¹I). C. Iodothyronines-¹³¹I (% of total thyroid ¹³¹I). D. MIT-¹³¹I/DIT-¹³¹I ratios.

Fig. 6. *In vitro* effects of graded doses of goitrin on ¹³¹I metabolism in thyroid of goitrin-fed chicks. A. Thyroidal ¹³¹I uptake (% of dose). B. Iodide-¹³¹I (% of total thyroid ¹³¹I). C. Iodothyronines-¹³¹I (% of total thyroid ¹³¹I). D. MIT-¹³¹I/DIT-¹³¹I ratios. ○.....Thyroid of chicks fed no goitrin. ●.....Thyroid of chicks fed goitrin.

by goitrin feeding and was gradually depressed by the addition of goitrin in the incubation medium. Ratios of MIT/DIT were not changed by feeding of goitrin or in the incubation medium of 5×10⁻⁶M goitrin in comparison with that of thyroid lobes fed without goitrin though the ratio was elevated in the medium containing 5×10⁻⁵M goitrin.

Increase of thyroidal ¹³¹I uptake in lobes of the chicks fed PTU and decrease of that in the medium containing 5×10⁻⁴M PTU in comparison with that without goitrogen are shown

Fig. 7. *In vitro* effects of graded doses of PTU on ^{131}I metabolism in thyroid of PTU-fed chicks. A. Thyroidal ^{131}I uptake (% of dose). B. Iodide- ^{131}I (% of total thyroid ^{131}I). C. Iodothyronines- ^{131}I (% of total thyroid ^{131}I). D. MIT- ^{131}I /DIT- ^{131}I ratios. ○.....Thyroid of chicks fed no PTU. ●.....Thyroid of chicks fed PTU.

in Fig. 7. These changes are similar to the changes by the goitrin addition. Percentages of iodide were increased in the medium containing 5×10^{-6} and $5 \times 10^{-5}\text{M}$ PTU and were markedly elevated in the medium of $2 \times 10^{-4}\text{M}$ PTU as compared with that of lobes without PTU. *In vitro* iodothyronine biosynthesis was significantly reduced by feeding of 0.01% PTU and was strongly blocked by the addition of PTU in the incubation medium. It was also demonstrated that feeding of PTU significantly elevated the ratio of MIT/DIT in the thyroid lobes comparing to the feeding of goitrin.

Discussion

It has been reported that appreciable quantity of labelled iodothyronines is consistently formed by rat whole thyroid lobes under *in vitro* incubation⁽¹²⁾. In this experiment, KPR buffer was used as an incubation medium and Ca^{++} content in buffer was controlled to 1/3 of that of the original composition. It is considered that effective comparison of goitrogenic activity may be performed by these chick thyroid lobes under *in vitro* incubation although biosynthesis of iodothyronines was somewhat lower than that observed in the experiment of SHIMODA and GREER⁽¹²⁾ in rats. Then the first research was performed to determine adequate incubation time, and it was concluded that 3 hours were most adequate in the present study since synthetic ability of thyroid hormone reached equilibrated state at 3 hour incubation and standard deviation became larger at 6 hour incubation. MACK⁽¹³⁾ has stated that thyroidal ^{131}I uptake by the whole thyroid lobe was greater than that of sliced thyroid under *in vitro* condition. In view of the above considerations, *in vitro* incubation of chick whole thyroid lobes was performed in KRP buffer at 37°C for 3 hours.

Addition of KClO_4 strongly blocked thyroidal ^{131}I uptake at the level of 10^{-5}M . This phenomenon *in vitro* is in good agreement with many previous reports^(14,15) that incorporation of iodine into thyroid is inhibited by the administration of KClO_4 through competition with iodine. In incubated thyroid lobes of the chicks fed 0.05% goitrin or 0.01% PTU, thyroidal ^{131}I uptake was

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elevated and further increase was observed by addition of these goitrogens in the medium. These results are in agreement with the report of LAGUE and TIENHOVEN¹⁶⁾ that thyroidal ¹³¹I uptake was elevated under *in vitro* incubation by pretreatment of thyroid with methimazole, a goitrogen. Furthermore, these results are in good agreement with our previous data^{4,5)} that feeding of goitrin or PTU for 14 days elevated thyroidal ¹³¹I uptake twice as much as that of without goitrogen in growing chicks.

Organification of iodine (monoiodination of tyrosine) in the thyroid lobes was strongly depressed by addition of high concentration of goitrin or PTU (10^{-4} M) as shown in Figs. 2 and 3. Addition of KClO₄ over 10^{-5} M reduced monoiodination of tyrosine but 10^{-4} M AITC did not inhibit it. Therefore, it may be confirmed that depressing effect of goitrogens on monoiodination of tyrosine was most striking in KClO₄, followed by goitrin or PTU and AITC was the weakest among the goitrogens tested in the present study.

It has been known that ratios of MIT/DIT in thyroid gland reflect synthetic ability of thyroid hormone. It was shown in chick thyroid incubated *in vitro* that biosynthesis of hormones depressed by addition of 10^{-6} M of goitrin or PTU and was depressed in proportion to the concentration of goitrin or PTU. On the other hand, these gradual depression with the concentration of goitrogen added was not observed by the addition of AITC. This may suggest that antithyroid activity of AITC was less potent in comparison with that of goitrin or PTU. Elevated ratios of MIT/DIT in the lobes incubated in the KClO₄ medium may indicate that KClO₄ inhibited not only incorporation of iodine into the thyroid but also the biosynthesis of thyroid hormone, although RICHARD and INGBAR¹⁷⁾ showed that composition of iodine in thyroid in rats was not influenced by the administration of KClO₄. The above suggestion may be supported by the depression of iodothyronine synthesis in the thyroid incubated in the KClO₄ medium as shown in Fig. 5-C.

As stated above, some phenomena induced by the addition of goitrin or PTU in the incubation medium, such as depression in iodothyronine synthesis and iodination of MIT, were in good agreement with our previous *in vivo* findings obtained in chicks fed goitrin or PTU for 14 days. In our previous paper¹⁰⁾, it has been stated that the relative potency of goitrin in chicks was estimated to be about one-tenth that of PTU in respect to thyroid hormone synthesis when goitrogens were fed to day-old chicks for 14 days. In *in vitro* incubated chick thyroid, it can be indicated that the difference in the antithyroid activity between goitrin and PTU on chick thyroid under *in vitro* incubation was not so much as that in *in vivo* studies.

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In vitro 培養ひな甲状腺のホルモン合成に 対する Goitrin の作用

秋葉 征夫・松本 達郎

東北大学農学部, 仙台市 980

In vitro 培養ひな甲状腺の甲状腺ホルモン合成に対する Goitrin の作用を PTU (propyl-thiouracil), AITC (allyl-isothiocyanate) および KClO_4 (potassium perchlorate) の 3 種の抗甲状腺物質と比較検討した。14～16 日令の白色レグホーン種雄ひなの甲状腺を傷つけないように採取し、 $20\mu\text{Ci}$ の ^{131}I を含む pH 7.4 の KRP buffer (3 ml) に入れ、 37°C , 3 時間振盪培養した。また、0.05% Goitrin または 0.01% PTU を 14 日間給与したひなの甲状腺を用い同様の試験を行なった。培養液中の抗甲状腺物質の濃度は $10^{-7}\sim 10^{-4}\text{M}$ に調製した。甲状腺

^{131}I とり込みは、 KClO_4 で 10^{-5}M , Goitrin と PTU では 10^{-4}M の濃度で抑制されたが、 $10^{-6}\sim 10^{-4}\text{M}$ の AITC では全く抑制されなかった。Goitrin または PTU 投与ひな甲状腺の ^{131}I とり込みは無給与ひなの約 2 倍に増加した。甲状腺内の MIT/DIT 比は抗甲状腺物質の添加濃度に比例して増加し、Iodothyronines の合成割合は比例的に減少する傾向がみられた。甲状腺内での Monoiodination は 10^{-5}M の KClO_4 および 10^{-4}M の Goitrin および PTU で抑制された。