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EFFECT OF THIONAMIDES ON ADENYL CYCLASE ACTIVITY AND PHOSPHOLIPOGENESIS IN RAT THYROIDS

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Abstract—The effects of two thionamide drugs, propylthiouracil (PTU) and methylmercaptoimidazole (MMI), are studied on adenyl cyclase (AC) activity and phospholipogenesis (PLGS), in rat thyroids. Both these manifestations of TSH-action are stimulated in the thyroids of rats fed MMI, while depressed in thyroids of rats fed PTU. Besides, induction of larger goitres in the rats fed PTU is accompanied with the lesser gain in the final body-weight after 6 weeks, when compared with the age-paired rats of MMI or control groups. There seems to exist an inverse relationship between the thyroid-weight and body-weight of rats from control, MMI and PTU groups. The differential action of these anti-thyroid drugs is probably due to the different endocrine status of the animals.

Antithyroid drugs of thionamide series, mainly propylthio-uracil (PTU) and methimazole (MMI), are generally used for the management of thyrotoxic patients. Although their mechanism of action is not clearly understood, it is believed that these drugs not only block the hormone biosynthesis in the thyroid gland, but also prevent its peripheral utilisation [1, 2]. More recently Morris and Hager [3] and Taurog [4] have proposed that these thionamides interfere with the action of thyroidal peroxidase, a key enzyme responsible for hormone biosynthesis.

Low iodine diet or antithyroid drugs including thionamides have been used for the production of goiter in experimental animals. Since administration of the goitrogen results in elevated levels of circulating thyrotrophin* (TSH) [5–7], the thyroids of PTU treated rats, have been used, as a model system for studying the bio-chemical manifestations of *in vivo* endogenously raised TSH-action. The available reports concerning adenyl cyclase-cyclic AMP (AC-cAMP) system, in thyroids of PTU treated rats, are at variance [8, 9]. Moreover, there are no reports on thyroidal AC-cAMP system, or other bio-chemical manifestations of TSH-action, in animals treated with MMI, except for the fact that MMI induces smaller goiters in rats, in spite of comparably raised levels of TSH in circulation [5, 6].

It was therefore, felt necessary to study whether bio-chemical changes observed in thyroids of rats treated with PTU and MMI, were the direct reflections of TSH actions, or due to the drug effect *per se*.

In the present communication, we report our findings of AC-activity and phospholipogenesis (PLGS), an early and delayed manifestations of TSH-action [10, 11] in the thyroids of rats fed PTU and MMI. Both the drugs have also been studied for their goitrogenic effect on thyroid-weight and general body-growth.

*Although we have not been able to determine the circulating TSH levels in rats because of the practical difficulties (in obtaining rat-TSH and its anti-sera) evidence for the raised levels of circulating TSH in rats fed PTU has already come from the studies of Alexander *et al.* and Jolin *et al.* [5, 6].

MATERIALS AND METHODS

ATP, ADP, AMP, c-AMP and adenosine were obtained from Sigma Chemical Co., U.S.A. ATP-8-[^{14}C], 50 mCi/mM (Tracer Lab, U.S.A.), [^{3}H]cyclic AMP-2340 mCi/mM (Amersham, U.K.); Dowex 50-H^{+} (50 × 8): 200–400 mesh size (J. Baker Chemical Co., NJ), silica gel G (E. Merck, Germany); Isobutyric acid (Riedel, Germany) and bovine TSH (Armour, U.S.A.) were used. PTU was obtained from Koch Light laboratories and MMI from Schuchardt Münchan (Germany). All other reagents used, were of analytical grade.

Young male rats of Wistar strain weighing about 140–150 g were taken for this study. They were divided into three groups, each with 12–16 rats (1) Control group was fed normal colony diet (2) PTU group was fed the normal diet with 0.1% PTU and (3) MMI group was also fed the normal diet with 0.1% MMI. After feeding for 6 weeks, the thyroid lobes of these rats were removed under ether anaesthesia. The thyroid lobes from each group were collected separately in chilled solution of 0.25 M sucrose. These were then trimmed, dried with blotting paper, weighed and further taken for studying AC-activity and PLGS.

(a) *Adenyl cyclase assay.* AC-activity was measured in 9000 *g* pellet of thyroid homogenate [8] according to the method of Krishna *et al.* [12] and also by the method developed in our laboratory for the separation of cAMP from other adenine nucleotides [13]. Protein was determined by Lowry's method [14].

(b) *Phospholipogenesis.* Thyroid slices (equal weights) from rats of control, PTU and MMI groups were taken in 3 ml incubation mixture containing Tris–Cl buffer, pH 7.4 (50 mM); NaCl (131 mM); KCl (5 mM); $MgSO_4$ (1.2 mM); $CaCl_2$ (0.8 mM); D-glucose 1 mg/ml and TSH, 100 mU/ml or albumin (equivalent

Table 1. Effect of PTU and MMI on adenyl cyclase activity in rat thyroids *AC-activity = cAMP – pmoles/m protein/min

	Basal	% of control	TSH (100 mU/ml)	% of control
Control	107 ± 3.0	100	201 ± 11.0	187
PTU	15 ± 0.8	14 ($P < 0.005$)	20 ± 2.2	20
MMI	156 ± 9.0	146 ($P < 0.005$)	176 ± 22	165

* Results are Mean ± S.E.M. of three experiments (each done in triplicate). Thyroid lobes from 8–12 rats (each group) were pooled for each experiment and homogenised separately in chilled sucrose solution (0.25 M). The 9000 *g* pellet was suspended in 0.8–1 ml Tris–Mg buffer and assayed immediately for AC-activity [12].

to the concn of TSH-protein) in basal experiments, with 15–20 μCi of carrier free $KH_2^{32}PO_4$. These were then incubated at 37° in a metabolic shaker for 2 hr under the atmosphere of oxygen. The thyroid slices were then washed with normal saline and 0.1 M KH_2PO_4 (5 times each with 10 ml solution) to get rid of any adhering radioactivity, and finally suspended in 10 ml solution of chloroform–methanol (2:1). Total phospholipids were then extracted according to the method of Folch *et al.* [15]. Stable phosphorus was determined as reported earlier [16].

RESULTS

(a) *Adenyl cyclase activity.* Table 1 shows the AC-activity in thyroids of rats fed PTU and MMI in diet for 6 weeks. Both these thionamide drugs have been shown to raise the circulating TSH levels resulting in the endogenous stimulation of the thyroid gland. However it is worth noting that PTU treatment had lowered AC-activity (14 per cent of control), while MMI treatment had raised AC-activity (146 per cent of control) in these thyroids. Although *in vitro* addition of TSH (100 mU/ml) to the incubating thyroid slices from the control rats, stimulated AC-activity, it failed to elicit any effect in thyroids of PTU and MMI treated rats.

(b) *Phospholipogenesis.* As seen from Table 2, PLGS is reduced (41 per cent of control) in the thyroids of rats fed PTU; and enhanced in those of MMI group (221 per cent of control). Extra addition of TSH (100 mU/ml) to the incubating thyroid slices did not show any stimulation, in the thyroids of rats fed either PTU or MMI, though PLGS was stimulated by TSH in thyroids of control group.

(c) *Effect of PTU and MMI on thyroid-weight and final body-weight.* Figure 1 shows the relationship between the thyroid-weight and final body-weight after 6 weeks duration of treatment with thionamide drugs. Though PTU feeding has induced larger goiters, these rats had comparatively smaller gain in their body-weight after 6 weeks; while those with smaller goiters due to MMI feeding or no goiters (in control rats) had better increase in their body-weight. There seems to be an inverse relationship between the thyroid-weight and body-weight.

DISCUSSION

The findings reported here indicate that both PTU and MMI have different effects on AC-activity, PLGS, thyroid-weight and body-weight, though both the thionamides have been fed at the same dosage (0.1%) in diet for the same duration.

AC-activity and PLGS are believed to be an early and delayed manifestations, respectively, of TSH action on the thyroid gland [10, 11]. Both AC-activity and PLGS are enhanced in the thyroids of rats fed MMI. This observation can be readily explained on the basis of the raised circulating levels of TSH in these animals. In contrast to the effect of MMI, AC-activity and PLGS were lowered in the thyroids of rats fed PTU. Granner *et al.* [8] have also reported lowered AC-activity in the thyroids of PTU treated rats. These observations are difficult to explain at present. As will be discussed later, PTU, but not MMI, has been shown to have some extrathyroidal effects [6, 18], which are not attributable to its antithyroid action. The lowering of the thyroidal AC-activity and PLGS in PTU treated rats, may be because of some yet unknown effects of this drug.

Both AC-activity and PLGS are enhanced with *in vitro* addition of TSH (100 mU/ml) in thyroids of control rats, but failed to show any rise in the thyroids of either PTU or MMI treated rats. It is conceivable that these thyroids were maximally stimulated by an

Table 2. Effect of PTU and MMI on phospholipogenesis in rat thyroids *^{32}P incorporation into phospholipids = c.p.m./μg phosphorus

	Basal	% of control	TSH (100 mU/ml)	% of control
Control	2345 ± 159	100	4331 ± 209	184
PTU	965 ± 43	41 ($P < 0.005$)	896 ± 37	38
MMI	5182 ± 280	221 ($P < 0.005$)	4760 ± 402	203

* Results are Mean ± S.E.M. of three experiments (each in triplicates). Thyroid lobes from 12–16 rats (each group) were pooled separately in chilled sucrose solution (0.25 M). Equal weights of thyroid slices (50 m) from each group were incubated in Tris–HCl buffer medium at 37° under oxygen for 2 hr with $KH_2\ ^{32}PO_4$ for PLGS.

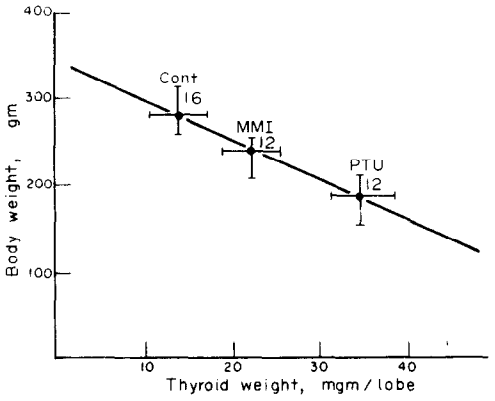

Fig. 1. The relationship between thyroid-weight and body-weight in rats from control, PTU and MMI groups. Increased goitrogenesis is accompanied with lower gain in body-weight. (Figures in parentheses show the number of rats in each group).

endogenously raised levels of TSH due to goitrogen-feeding, and hence could not be stimulated further. Failure of *in vitro* TSH, to augment glucose-1-[^{14}C] oxidation in thyroids of PTU treated rabbits has been shown by Field *et al.* [19]. Unresponsiveness of the thyroids to TSH has also been observed by Zakarija *et al.* [20] in PTU treated rats.

The results described in section (c), show that PTU is more potent goitrogen, as judged by the induction of larger goiters in these rats compared to those treated with MMI. It is generally believed that production of goiter is the result of chronic stimulation of the thyroid gland by elevated levels of circulating TSH. PTU, apart from raising TSH levels, has also been shown to affect other extra-thyroidal hormones such as insulin and growth hormone (GH). Involvement of insulin, GH and possibly thyroid hormone have been implicated in the intensification of goitrogenesis [6, 7, 18]. Though we have not been able to measure the levels of these hormones (due to the lack of the availability of the standards), it is very likely that the larger goiter size in rats fed PTU, is a result of the combined action of all these hormones. Since MMI has been shown to have no such extrathyroidal effect like PTU, smaller goiters are produced in these animals.

Induction of larger goiters by PTU treatment is accompanied with significantly lesser gain in the body-weight, than for age-paired rats of MMI and control groups. As is clear from Fig. 1, there appears to exist an inverse relationship between the thyroid-weight and body-weight of these rats. This further suggests that the intensification of goitrogenesis is growth related presumably through the concerted action of TSH and GH.

In conclusion our findings reveal that the differential effects of these thionamides are mainly because of different mechanisms of action on the thyroid and particularly extrathyroidal tissues; and the endocrine state of rats may not be the same when different antithyroid drugs are used.

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