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Studies on the effects of omeprazole on thyroid function in the rat

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Abstract—The effects of omeprazole (an H^+, K^+-ATPase inhibitor) on thyroid parameters in rats have been examined. SK&F Wistar rats were dosed orally with omeprazole (up to 500 mg kg^{-1}) or vehicle. Treatment for 7 or 14 days resulted in generally decreased plasma T_3 concentrations in males (with little change or slight increases in females) and increased serum TSH concentrations (22%–68% increases). No changes were detected in thyroid ^{125}I uptake or organification. Liver 5′-deiodinase activity was decreased in male rats after 7 days treatment. Thyroxine clearance was not altered after a single dose of omeprazole. In-vitro studies showed omeprazole to be only a weak inhibitor of TSH-stimulated ^{125}I organification in cultured porcine thyrocytes. It is concluded that omeprazole has weak effects on the pituitary-thyroid-liver axis, its main action being to inhibit the peripheral deiodination of thyroid hormones.

Omeprazole is an inhibitor of gastric acid secretion belonging to a new class of compounds with a mechanism of action at the level of H^+, K^+-ATPase in the parietal cell (Wallmark et al 1983).

Toxicological studies on omeprazole at high doses in rats (138–414 mg $kg^{-1}day^{-1}$) have shown changes in peripheral thyroid hormone metabolism with no pathological changes in the thyroid gland (Ekman et al 1985). Plasma triiodothyronine concentrations were decreased, TSH was increased, and thyroxine was unchanged. As well as a reported inhibitory effect on liver 5′-deiodinase activity (Ekman et al 1985), omeprazole could potentially have direct effects on thyroid hormone synthesis in the thyroid gland in view of the H^+, K^+-ATPase immunoreactivity reported in porcine thyroid tissue (Saccomani et al 1979) and the importance of ATPases in controlling thyroid function.

It is known that many agents can perturb thyroid gland function by effects on any of a number of homeostatic processes both within the hypothalamic-pituitary-thyroid axis and through changes in thyroid hormone metabolism (see Atterwill & Brown 1988; Zbinden 1987). Therefore, to fully evaluate the mechanism of the changes produced by omeprazole we have studied its effects on a number of thyroid parameters using both in-vivo and in-vitro models.

Materials and methods

Thyroid hormone measurements and iodine accumulation. SK&F Wistar rats were dosed with omeprazole (500 mg kg^{-1}) or vehicle control. Ten males and 10 females were dosed by gavage daily for 1, 3, 7 or 14 days. At each time 5 males and 5 females were selected for plasma T_3 and T_4 determinations (first subset) while the remaining 5 males and 5 females were selected for plasma TSH determination and ^{125}I-accumulation studies (second subset). Plasma T_3 and T_4 concentrations were measured using Amerlex total T_3 and total T_4 radioimmunoassay kits (Amersham International, Amersham, UK). Reagents for radioimmunoassay of rat TSH were kindly provided by NIH, Bethesda, Maryland, USA.

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For the first subset, 6 h after the final dose (on Days 1, 3, 7 or 14) 2 mL blood samples were obtained from a lateral tail vein for estimation of plasma T_3 and T_4. At necropsy 18 h later (i.e. 24 h after the final dose) a further blood sample was obtained from the vena cava.

For the second subset, 24 h after the final dose, blood samples (2 mL) were obtained from a lateral tail vein for estimation of serum TSH concentrations. These animals then received 1 μCi of Na ^{125}I intraperitoneally. Three hours later the rats were killed by cervical dislocation. The thyroid glands (on a section of trachea) and a 0·5 mL blood sample were obtained from each animal and the gamma emission counted. ^{125}I-Accumulation was then expressed as i) % total ^{125}I-accumulation in the thyroid glands and ii) tissue to blood ratio.

Deiodinase measurements. SK&F Wistar rats (6 males and 6 females per treatment group) were dosed with omeprazole (414 mg kg^{-1}) or vehicle control by oral gavage daily for 7 days. Twenty four hours after the last dose the rats were killed by cervical dislocation. Serum samples were obtained for estimation of T_3, T_4 and TSH. Liver and kidney samples were used for estimation of 5^1-deiodinase activity (see Jones et al 1987).

Perchlorate discharge test. The perchlorate discharge test measures the efficiency of the thyroid iodide organification mechanism (Atterwill et al 1987) and has been used clinically to assess such conditions as Hashimoto's disease or the effectiveness of antithyroid drug treatment e.g. carbimazole. Perchlorate is a competitive anionic inhibitor of TSH-stimulated thyroidal iodide transport into the follicular cells. If free iodide is backed up within the cells (due to a blocked organification mechanism) following perchlorate administration, there is a diffusional discharge of iodide. The magnitude of this discharge is a measure of the degree of organification 'block'.

Male SK&F Wistar rats (12 per group) were dosed with omeprazole (500 mg kg^{-1}) or vehicle control by oral gavage daily for 7 days. Twenty four hours after the last dose each animal was given 1 μCi Na ^{125}I intraperitoneally. Six hours later half the animals from each group received 10 mg kg^{-1} $KClO_4$ intraperitoneally (see Atterwill et al 1987), and 2·5 min later all rats were killed by cervical dislocation. The thyroid glands (on a section of trachea) and 1 mL blood sample were obtained from each rat and the gamma emission counted.

In-vitro studies with cultured porcine thyrocytes. Porcine thyrocytes were cultured and the cells assessed for their capacity to accumulate and organify ^{125}I as described by Brown et al (1986). The effect of omeprazole (0·02–2000 μM) was compared to that of the positive control antithyroid compounds propylthiouracil (PTU; 0·1–100 μM) and methimazole (0·02–300 μM).

Results

Thyroid hormone measurements and iodine accumulation. In general, in male rats treated for 7 or 14 days plasma T_3 and T_4 concentrations were lower in omeprazole treated animals than in the corresponding controls (data from animals treated for 1 or 3 days is not shown although a similar hormone pattern was seen) but this did not reach statistical significance (Student's *t*-test) at

Table 1. Effect of treatment with omeprazole (500 mg kg^{-1}) for 14 days on plasma thyroid hormones and serum TSH concentrations.

		Males		Females	
Hormone	Treatment	+6 h	+24 h	+6 h	±24 h
Plasma T_3 (ng mL^{-1})	Control	0·92±0·12	0·61±0·11	0·57±0·02	0·50±0·04
	Omeprazole	0·44±0·04**	0·73±0·05	0·58±0·03	0·68±0·04*
Plasma T_4 (μg dL^{-1})	Control	9·1±0·5	6·0±0·4	5·5±0·3	4·3±0·5
	Omeprazole	6·9±0·6	6·2±0·4	4·7±0·5	4·6±0·4
Serum TSH (ng mL^{-1})	Control		1·29±0·21		0·98±0·11
	Omeprazole		1·58±0·13*		1·65±0·22*

Significant difference from controls. * $P<0·05$. ** $P<0·01$. Values are mean ± s.e.m. (n = 5)

Table 2. Effect of treatment with omeprazole (500 mg kg^{-1}) on tissue to blood ratio of ^{125}I. Values are mean ± s.e.m., n = 5.

		Tissue to blood ratio (counts min^{-1} g^{-1}/ counts min^{-1} mL^{-1})	
		Males	Females
Day 2	Control	648±110	325±94
	Omeprazole	565±51	243±132
Day 4	Control	612±70	713±137
	Omeprazole	635±109	682±74
Day 8	Control	603±29	534±97
	Omeprazole	750±90	762±95
Day 15	Control	582±69	835±167
	Omeprazole	806±125	548±40

Table 3. Effect of treatment with omeprazole (414 mg kg^{-1} day^{-1} for 7 days) on 5′-deiodinase activity in rat liver and kidney homogenates. Values are mean ± s.e.m. n = 6.

	5′-Deiodinase activity (pg T_3 formed per mg protein in 10 min)			
	Liver		Kidney	
	Male	Female	Male	Female
Control	147±9	58±8	76±8	479±37
Omeprazole	66±7***	48±9	101±17	401±48

Significant difference from control
*** $P<0·001$

all the timepoints measured (Table 1). There were minimal, or no changes in thyroid hormones in the females with very slight and occasional increases in T_3 concentrations (e.g. day 14; +24 h). Similarly, serum TSH concentrations were higher in omeprazole-treated rats than in controls with 22–68% increases above control values. (Table 1).

Omeprazole treatment did not significantly alter % total ^{125}I-accumulation in the thyroid gland, or tissue to blood ratio (corrected for thyroid gland weight) (Table 2).

Deiodinase measurements. Liver 5′-deiodinase activity was significantly lower in omeprazole treated male rats then in controls but no significant difference was evident in females or in the enzyme activity from kidney tissue (Table 3: Student's *t*-test).

Thyroxine clearance. After a single dose of omeprazole, 500 mg kg^{-1} T_4 clearance was not altered (Results not shown).

Perchlorate discharge test. Omeprazole treatment did not significantly alter $Na^{125}I$ accumulation by the thyroid gland or the amount discharged by $KClO_4$ (i.e. did not alter iodide organification) as compared to control rats (Fig. 1).

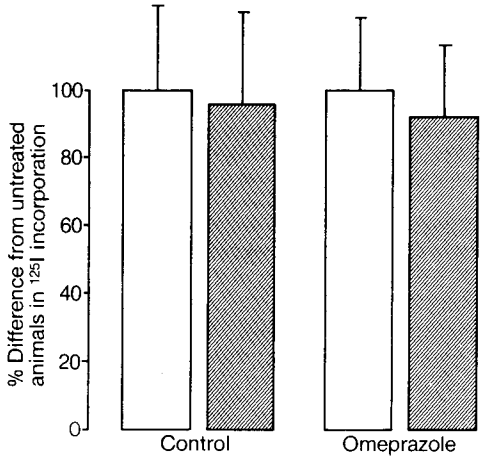

FIG. 1. Effect of treatment with omeprazole (500 mg kg^{-1} day^{-1} for 7 days) on iodide incorporation into the thyroid gland. Open bars, no additional treatment, hatched bars $KClO_4$ (10 mg kg^{-1}). Values are mean ± s.e.m., n = 6.

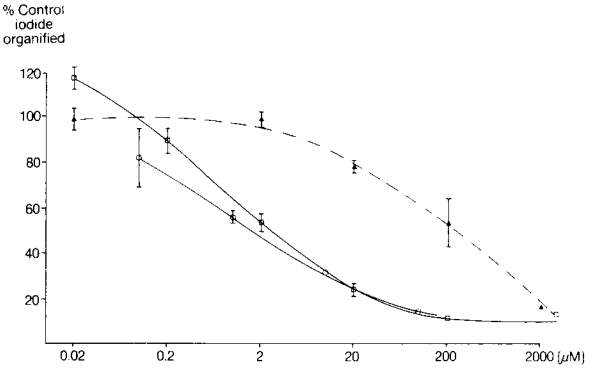

FIG. 2. Effect of omeprazole (▲), methimazole (□) and PTU (○) on TSH-stimulated ^{125}I organification by cultured porcine thyrocytes. Each point represents mean ± s.e.m., n = 4.

In-vitro studies with cultured porcine thyrocytes. Primary cultures of porcine thyroid epithelial cells form three dimensional 'pseudofollicular' structures which are able to accumulate and organify iodide, and concentrate the iodinated thyroglobulin in the lumen of the follicle-like structure.

Omeprazole appeared to be only a weak inhibitor (IC50 = 300 μM) of TSH-stimulated ^{125}I organification in cultured porcine thyrocytes compared with the classic antithyroid drugs PTU and methimazole (IC50 = 1·5 and 2·8 μM, respectively; Fig. 2).

Discussion

Histopathological and functional lesions of the thyroid gland are usually effected either by thyromimetic agents (feedback inhibition of pituitary TSH output leading to thyroid inactivity and follicular cell atrophy) or agents which lower circulating thyroid hormones (feedback stimulation of TSH output leading to thyroid hyperactivity and non-genotoxic promotion of thyroid cell growth; see Zbinden 1987; Atterwill & Brown 1988).

Our data support the findings of Ekman et al (1985) who reported that high doses of omeprazole (138–414 mg $kg^{-1}day^{-1}$) decreased plasma T_3 concentrations, and lowered 5′-deiodinase activity in liver homogenates. We have shown that high doses of omeprazole (up to 500 mg kg^{-1}) appear to inhibit peripheral 5′-deiodinase activity and cause some decreases in plasma T_3 concentrations, particularly in male rats. Little or no reductions in circulating T_4 were found showing that omeprazole probably does not directly inhibit thyroxine biosynthesis (i.e. peroxidase activity). This was supported by only a very weak in-vitro action on iodide organification in cultured porcine thyrocytes. Omeprazole was much less potent in this respect than the directly acting goitrogens PTU and methimazole (100–200 fold more potent than omeprazole). Furthermore, no effect on radioiodide uptake or organification in the rat thyroid gland in-vivo could be detected using the perchlorate discharge test, using high doses of omeprazole which inhibited peripheral deiodinase activity. Additionally, omeprazole did not appear to cause feedback stimulation of TSH output by increasing thyroxine clearance as do many compounds such as phenobarbitone and SK&F 93479 (Brown et al 1987).

These data indicate that the small rises in serum TSH in response to lowered circulating T_3 concentrations induced by subacute omeprazole treatment at toxicological doses are not sufficient to markedly affect functional aspects of thyroid follicular cell function as measured by iodide accumulation and organification in-vivo. In conclusion, therefore, omeprazole appears to have weak effects on the pituitary–thyroid–liver axis, its main action being to inhibit the peripheral deiodination of thyroid hormones. It seems to have only a very weak potential to directly inhibit thyroid hormone synthesis as shown in-vitro using cultured thyroid cells. The net effect of these actions does not appear to enhance TSH stimulation of the thyroid follicles to a level promoting increased functional activity or histopathological lesions.

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The relevance of the presence of certain synthetic steroids in the aquatic environment

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Abstract—Norethisterone and ethinyloestradiol concentrations in sewage effluent, reservoirs, rivers and potable water have been estimated at less than 20 ng L^{-1}, a value unlikely to present a significant risk to human health.

One group of widely used pharmaceutical chemicals to which the public are exposed both in the home and hospital consists of synthetic steroids. An earlier review (Richardson & Bowron 1985) of the Catchment Quality Control (CQC) studies undertaken by the Thames Water Authority (TWA) (Fish & Torrance 1977, 1978; Wood & Richardson 1978, 1980; Nicolson et al 1981; Richardson & Bowron 1983; Bowron & Richardson 1984), highlighted, amongst other groups of pharmaceutical chemicals, the potential public health concern if such chemicals were allowed to enter the aquatic environment and if found to be present in potable water supplies.

The role of immunoassay in the analysis of such microcontaminants in water samples has been described (Aherne 1984, 1987). Immunoassay procedures have been used to determine levels of norethisterone and ethinyloestradiol in various water samples (Aherne et al 1985) and a further set of samples has been analysed in this study. Norethisterone and ethinyloestradiol were chosen as typical synthetic steroids prescribed in significant quantities (Wood & Richardson 1980) and because the specific antisera were available.

Materials and methods

Snap samples of sewage treatment work effluent, rivers, impounding reservoirs and potable water were collected from areas of S.E. England. All sewage works utilized activated sludge processes and were operating at design output. River water

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