THE EFFECT OF INDOMETHACIN ON THE RESPONSE OF THYROID TISSUE TO THYROTROPIN[1]

J. Wolff and Wayne V. Moore

National Institute of Arthritis, Metabolism, and Digestive Diseases

National Institutes of Health

Bethesda, Maryland 20014

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Summary - Indomethacin, in concentrations up to 400 μM, fails to alter the stimulation by thyrotropin in various thyroid preparations of [1-^{14}C]glucose oxidation, hormone and iodine secretion, and adenylate cyclase activity. It is concluded that prostaglandin synthesis is not an obligatory step in the activation of these thyroid functions by thyrotropin.

The prostaglandins mimic a number of effects produced by thyrotropin (TSH) in thyroid tissue. These include glucose oxidation, phosphate incorporation into lipid, organic iodine formation, colloid droplet formation and hormone secretion, and cAMP accumulation (1). Adenylate cyclase can also be stimulated in membrane preparations (2,3). For these reasons it has been proposed that these fatty acids constitute an essential step in the normal response of thyroid tissue to TSH. Initially, it was suggested that TSH and prostaglandin E_1 (PGE_1) share the same membrane receptor because of lack of additivity for adenylate cyclase activation (4). Besides the absence of any chemical similarities between these compounds, various kinds of indirect evidence suggest that this is not a probable explanation (3).

A sequential mechanism, in which TSH → PGE → cAMP, has been proposed on the basis of the similarity of inhibition produced in the TSH and PGE_1 stimulations by 7-oxa-13-prostynoic acid, a structural analogue of PGE_1 (5) and on the basis of TSH-stimulated increases in PGE_1 levels in thyroid tissue (6).

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If the latter effect is a necessary step in the activation of adenylate cyclase by TSH, then inhibition of PGE and PGF synthesis by indomethacin [1-(p-chlorobenzoyl)-5-methoxy-2-methyl indole-3-acetic acid) should abolish the responses of thyroid tissue to TSH. It has been shown that 1-10 μM indomethacin completely inhibits prostaglandin synthesis either as measured by bioassay or from the appropriate labeled precursor polyenoic acid (7-11). We therefore examined the effect of indomethacin on three characteristic responses of thyroid tissue to TSH; namely, oxidation of [1-^{14}C]glucose, secretion of hormone from the prelabeled mouse thyroid gland, and on adenylate cyclase of beef thyroid membrane preparations.

MATERIALS AND METHODS

Indomethacin was generously supplied by Dr. C. A. Stone of Merck, Sharp, Dohme Research Lab, West Point, PA. Bovine TSH was partially purified to 4-5 units/mg protein by the method of Pierce (12). [1-^{14}C]glucose and [α-^{32}P]ATP were obtained from International Chemical and Nuclear Corp.

Dog thyroid slices (30-40 mg) were incubated in 30 ml vials containing 3.0 ml of Earle's medium with 5.5 mM glucose and labeled glucose, TSH where indicated, and indomethacin dissolved in dimethyl sulfoxide, or this solvent alone. The dimethyl sulfoxide concentration was 1%. Vials were equilibrated with CO_2-O_2 (5:95, v/v) and incubated at 37° in a Dubnoff shaker operating at 60 cycles per min for 60 min. $^{14}CO_2$ was collected, processed and counted to 2% accuracy as described by Fain, et al. (13) except that 0.25 ml of phenethylamine was used as the base.

Mouse thyroids were obtained from male NIH white Swiss mice injected 12 hrs before with 2-3 μCi of ^{131}I. Thyroid glands were kept on the trachea and preincubated for two hours in Earle's medium containing the appropriate concentration of drug or dimethyl sulfoxide and 0.1% bovine serum albumin. The thyroids were then transferred to identical media containing, in addition, TSH, where indicated. The media obtained after 5 hours and the thyroid glands were counted as previously described (14).

TABLE I

The Effect of Indomethacin on the Oxidation of [1-^{14}C]Glucose by Dog Thyroid Slices

Indomethacin Concentration (mM)	$^{14}CO_2$ Production from [1-^{14}C]Glucose µmoles/gm/hr	
	Basal ± S.E.	TSH* ± S.E.
Control	0.75 ± 0.10	1.85 ± 0.40
0.01	0.78 ± 0.12	1.98 ± 0.26
0.10	0.81 ± 0.10	1.86 ± 0.14
1.0	0.72 ± 0.10	1.65 ± 0.33

* 10 mU TSH/ml in 0.1% bovine serum albumin. Dimethylsulfoxide 1% was present in all vials. Incubation time = 60 min. Each point represents 4 dogs with triplicate observations on each thyroid.

Beef thyroid membranes were prepared and stored as described previously (3). For assay of adenylate cyclase the final ATP concentration was 1.0 mM, Mg^{++} 2.0 mM, theophylline 10 mM, Tris-HCl buffer pH7.6 8 mM, all in a final volume of 60 µl. The reaction was started by addition of membrane preparation and was allowed to run for 10 min. The rate of labeled cAMP formation was linear for 20 min and over a range of protein concentration up to 5-fold the amount usually employed (3). Each assay point was determined in triplicate.

RESULTS AND DISCUSSION

The effect of increasing concentrations of indomethacin on [1-^{14}C]glucose oxidation to $^{14}CO_2$ by dog thyroid slices is depicted in Table I. It is clear that baseline values are unaffected by up to 1 mM indomethacin and that the response to TSH is not abolished by such concentrations of indomethacin. The decrease to 1.65 of the TSH-stimulated response at 1.0 mM indomethacin was not statistically significant ($p > 0.2$). Similar results were obtained when the

TABLE II

Effect of Indomethacin on Thyroid Secretion

	Control	0.15 mM Indomethacin*
Control	6.4 ± 1.0	4.3 ± 0.6
TSH (10 mU/ml)	15.5 ± 1.1	14.7 ± 0.7

* Percent of ^{131}I released from mouse thyroids in 5 hrs after a 2 hr preincubation with indomethacin or dimethylsulfoxide but without TSH. Five mice were used for each point.

slices were first preincubated with .1 mM indomethacin for 2 hours and were then transferred to the same media containing TSH.

Secretion of ^{131}I from the prelabeled mouse thyroid glands in vitro was also unaffected by indomethacin. Neither the basal secretion nor the TSH-stimulated secretion were affected (Table II).

Very large concentrations of indomethacin do inhibit the ability of adenylate cyclase of beef thyroid plasma membranes to respond to TSH but this occurs at concentrations where the F^--activated enzyme is also inhibited. Moreover, this inhibition occurs at concentrations 100-1000 times those required to inhibit prostaglandin synthesis (7-11) and it seems likely that inhibition, at these concentrations, is nonspecific. At reasonable concentrations indomethacin has no effect on basal, TSH-stimulated, or F^--activated adenylate cyclase activity of beef thyroid membranes (Fig. 1).

It is clear from the above that in three different thyroid systems that respond to TSH, indomethacin, in concentrations known to inhibit prostaglandin synthesis in a variety of tissues (7-11), has no effect on the ability of these systems to respond to TSH. Since completion of these studies it has been shown (15) that concentrations of indomethacin comparable to those used here prevent TSH-induced increases in prostaglandin levels of bovine thyroid tissue. It seems reasonable to conclude, therefore, that the synthesis of prostaglandin

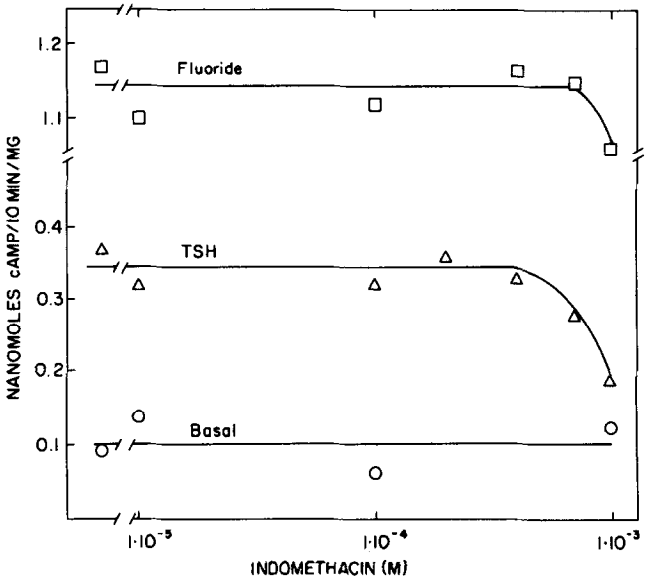

Fig. 1. Effect of indomethacin on adenylate cyclase activity of beef thyroid membranes. 29 µg of membrane protein was added to the start reaction. The TSH concentration was 200 mU/ml and F^- was 10 mM. The concentration of dimethylsulfoxide was 1% throughout. All points are means of triplicates.

does not constitute an obligatory step in the response of thyroid tissue to TSH. This view is strengthened by the fact that relatively purified plasma membranes are perfectly able to respond to TSH (3) although the synthesis of prostaglandin requires the participation of microsomal components (16). What remains to be determined, however, is whether or not the release of preformed prostaglandins from sites within the membrane may be quantitatively important and whether or not this process is affected by indomethacin.

REFERENCES

1. Field, J., Dekker, A., Zor, V., and Kaneko, T., Ann. N. Y. Acad. Sci. 180, 278 (1971).
2. Wolff, J., and Jones, A. B., J. Biol. Chem. 246, 393 (1971).
3. Wolff, J., and Cook, G. H., J. Biol. Chem. 248, 350 (1973.
4. Burke, G., Amer. J. Physiol. 218, 1445 (1970).
5. Sato, S., Szabo, M., Kowalski, K., and Burke, G., Endocrinology 90, 343 (1972).

6. Yu, S. C., Chang, L., and Burke, G., J. Clin. Invest. 51, 1038 (1972).
7. Vane, J. R., Nature New Biol. 231, 232 (1971).
8. Ferreira, S. H., Moncada, S., and Vane, J. R., Nature New Biol. 231, (1971).
9. Tomlinson, R. V., Ringold, H. J., Qureshi, M. C., and Forchielli, E., Biochem. Biophys. Res. Commun. 46, 552 (1972).
10. Levine, L., Hinkle, P. M., Voelkel, E. F., and Tashjian, A. H., Jr., Biochem. Biophys. Res. Commun. 47, 888 (1972).
11. Sorrentino, L., Capasso, F., and Di Rosa, M., Europ. J. Pharmacol. 17, 306 (1972).
12. Pierce, J. G., and Carsten, M. E., in Thyrotropin (Werner, S. C., Ed.) Chas. C. Thomas, Springfield, Illinois (1963), p. 247.
13. Fain, J. N., Scow, R. O., and Chernick, S. S., J. Biol. Chem. 238, 54 (1963).
14. Williams, J. A., and Wolff, J., J. Cell Biol. 54, 157 (1972).
15. Burke, C., Prostaglandins 2, 413, 1972.
16. Samuelson, B., Prog. Biochem. Pharmacol. 5, 109 (1972).