

Inhibitory Effect of Dietary Iodine on the Thyroid Adenylate Cyclase Response to Thyrotropin in the Hypophysectomized Rat

BASIL RAPOPORT, MICHAEL N. WEST, and SIDNEY H. INGBAR

*From the Section on Endocrinology, Veterans Administration Hospital and
Department of Medicine, University of California,
San Francisco, California 94121*

ABSTRACT In hypophysectomized rats given dietary regimens either rich or deficient in iodine, the increase in thyroid cyclic AMP concentration induced acutely by a single dose of TSH was significantly less in iodine-enriched than in iodine-deficient animals. Direct assays revealed that this difference was because the thyroid adenylate cyclase response to TSH was less in the iodine-enriched animals, phosphodiesterase activity being no different in the two groups. This effect may explain the inhibitory action of dietary iodine enrichment on diverse functional and anatomical responses of the thyroid to TSH.

INTRODUCTION

A major unsolved question with respect to the thyroid gland is the mechanism by which iodine inhibits a variety of thyroid functions, particularly their response to thyroid-stimulating hormone (TSH).¹ In animals, for example, dietary iodine enrichment decreases the stimulatory effects of TSH on the activity of the thyroid iodide transport mechanism, the rate of thyroid iodine release, thyroid growth, and several aspects of thyroid intermediary metabolism. In man iodine inhibits hormone release from the normal thyroid, the diffuse toxic goiter, and the toxic adenoma, and causes involution of the hyperplastic thyroid of Graves' disease (see reviews, refs. 1, 2).

This work was presented in part at the annual meeting of the American Federation for Clinical Research, 5 May 1974, Atlantic City, N. J.

Received for publication 17 July 1974 and in revised form 13 April 1975.

¹ *Abbreviations used in this paper:* HID, high-iodine diet; LID, low-iodine diet; TSH, thyrotropin.

Although the mechanism by which iodine exerts these effects is unknown, recent studies indicate that the stimulatory effects of TSH are mediated, at least in large measure, by the adenylate cyclase-adenosine 3'-5'-cyclic monophosphate (cyclic AMP) system (3). Therefore, we have explored the effects of dietary iodine enrichment on the response of the thyroid adenylate cyclase system to TSH in the hypophysectomized rat, this preparation being chosen to eliminate any effects of the variations in endogenous TSH secretion that differences in iodine intake induce.

METHODS

All experiments were conducted in hypophysectomized male Sprague-Dawley rats obtained from commercial sources. Intact rats, weighing 175-199 g, were placed on a Remington low-iodine diet (LID) for 10 days and were then hypophysectomized. Animals were then divided into two groups. One continued on the LID regimen; the other was given, in addition, 0.05% KI in the drinking water (HID group). Experiments were conducted after animals had been on their respective regimens for a period of 4-6 days. Animals were killed by a blow on the head and thyroids were rapidly removed and were processed for analysis according to the methods described below.

Cyclic AMP assay. Thyroid glands were frozen in liquid N₂, rapidly weighed, and then homogenized in 1.0 ml of ice-cold 50% acetic acid. The homogenate was centrifuged at 10,000 *g* for 15 min at 4°C, and the supernate was evaporated at 50°C under a stream of N₂. Samples were resuspended in 0.05 M sodium acetate buffer, pH 6.2, and aliquots were assayed for cyclic AMP by the radio-immunoassay of Steiner, Kipnis, Utiger, and Parker (4), modified in that dioxane (1.5 ml) was used to separate bound from free labeled cyclic AMP after the addition of normal rabbit serum as carrier.² Recovery of added cyclic AMP was

² Animals and materials were obtained from the following sources: hypophysectomized male Sprague-Dawley rats (175-199 g), Horton Laboratories, Oakland, Calif., and

TABLE I
Time Course of Rat Thyroid Cyclic AMP Concentration
after the Injection of 0.5 U TSH i.v.

Time after in- jection	Thyroid cyclic AMP		P
	HID	LID	
min	pmol/mg wet wt		
0	1.04±0.08 (14)	1.09±0.09 (14)	NS
2	1.19±0.08 (15)	1.62±0.15 (16)	<0.025
4	1.25±0.09 (15)	1.62±0.12 (15)	<0.025
7	1.23±0.08 (14)	1.58±0.14 (12)	<0.05
10	1.07±0.10 (13)	1.53±0.11 (14)	<0.01

Values shown represent means ±SE. The figures in parentheses describe the number of animals in each group.

at least 90%. Specificity of the assay was confirmed by demonstrating that cyclic AMP could no longer be measured after treatment of extracts with purified bovine phosphodiesterase.

Adenylate cyclase. Activity of adenylate cyclase was measured according to the principles described by Steiner, Pagliara, Chase, and Kipnis (5). After removal, thyroids were briefly kept at 4°C, pooled in groups of two to three glands, weighed, diced with fine scissors, and homogenized at a concentration of 50 mg/ml in 0.25 M sucrose, 25 mM Tris, pH 7.6, with a Dounce homogenizer. Whole homogenate was used in an assay system containing 25 mM Tris, 6 mM MgCl₂, 10 mM theophylline, 3 mM ATP, 0.15% bovine serum albumin, 10 mM creatine phosphate, and 8 U creatine phosphokinase in a final volume of 0.2 ml. Samples were incubated for 10 min at 30°C, and the reaction was stopped by boiling for 2 min. After centrifugation at 3,000 rpm for 15 min at 4°C, aliquots of supernate were assayed for cyclic AMP by radioimmunoassay. Cyclic AMP present in boiled homogenate, similarly processed and assayed, was subtracted from that present in unboiled samples to yield corrected values for the rate of cyclic AMP generation. Under conditions of the assay, this was linear for 15 min, and the immunoreactive products were destroyed by incubation with partially purified bovine phosphodiesterase.

Cyclic AMP phosphodiesterase. The method employed for phosphodiesterase assay was that of Bastomsky, Zakarija, and McKenzie (6), which utilizes principles described by Krishna, Weiss, and Brodie (7). The final assay mix-

Hormone Assay, Inc., Chicago, Ill.; Remington LID (15–30 µg iodine/kg), General Biochemicals, Div. North American Mogul Products Co., Chagrin Falls, Ohio; aminophylline, theophylline, bovine serum albumin, creatine, phosphate, rabbit muscle creatine phosphokinase, cyclic AMP, Sigma Chemical Co., St. Louis, Mo.; ATP and reagents for cyclic AMP radioimmunoassay, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.; AG50W-X8, 100–200 mesh resin, Bio-Rad Laboratories, Richmond, Calif.; TSH (bovine, 2.54 U/mg protein) was a gift of the National Institute of Arthritis, Metabolism and Digestive Diseases, NIH, Bethesda, Md.

ture contained the equivalent of 5 mg wet wt of thyroid tissue obtained from a pool of two glands and 2.5×10^{-4} M cyclic AMP in a volume of 0.2 ml.

Miscellaneous methods. Protein concentration was determined by the method of Lowry, Rosebrough, Farr, and Randall (8), with bovine serum albumin as standard. Statistical significance of difference between pairs of means was determined by Student's *t* test.

RESULTS

Cyclic AMP response to TSH. Groups of hypophysectomized HID and LID rats were injected with 0.5 U bovine TSH i.v. Animals were killed 2, 4, 7, and 10 min later and their thyroids were assayed for cyclic AMP (Table I). Peak cyclic AMP concentrations after TSH injection in both the HID and LID groups were achieved within 2–4 min. Although basal cyclic AMP concentration (picomoles per milligram wet wt; mean ±SE)³ was essentially the same in the two groups (1.04±0.08 vs. 1.09±0.09), values in the LID group were significantly higher than in the HID group at each of the time points studied after TSH administration.

Additional experiments were performed that provide verification of the foregoing differences in the response of HID and LID animals to TSH. In these and other experiments to be described, a 4-min interval between TSH administration and sacrifice was chosen for study because peak responses of cyclic AMP concentration were achieved within this interval. Three separate experiments were performed in which each of the experimental groups contained five or six hypophysectomized animals, and in which the response to 0.2 U of TSH was tested. In view of the similarity of the results obtained in the three experiments, individual values were pooled for statistical analysis (Fig. 1). Basal cyclic AMP concentrations averaged 0.43±0.04 in the HID group and 0.44±0.05 in the LID group. After 0.2 U TSH i.v., cyclic AMP concentrations were significantly increased above basal values in both the HID and LID groups to values of 0.74±0.09 (*P* < 0.01) and 1.0±0.1 (*P* < 0.001). When compared to one another, the concentrations of cyclic AMP achieved after TSH were significantly lower in the HID than in the LID group (*P* < 0.05).

As differences in phosphodiesterase activity in the HID and LID groups may have conditioned the foregoing results, additional experiments were performed in which rats were given 20 mg aminophylline i.v. 20

³ Values for cyclic AMP concentration were expressed in relation to wet wt of tissue because of the difficulty in measuring the protein content in 50% acetic acid homogenates. However, in thyroids used for the analysis of adenylate cyclase or phosphodiesterase, no difference in protein: wet weight ratios between HID and LID groups was observed.

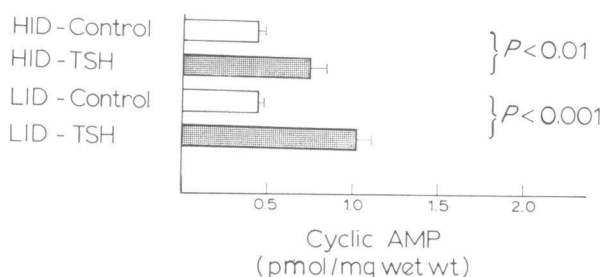


FIGURE 1 The effect of dietary iodine content on the response of thyroid cyclic AMP content to TSH. Experiments were conducted in hypophysectomized rats maintained on LID or LID plus 0.05% KI in the drinking water (HID). TSH-treated animals were given a single injection of bovine TSH (0.2 U i.v.) and killed 4 min later. Data shown represent the pooled results of three separate experiments (total of 15–16 animals per group). Brackets indicate SEM.

min before the injection of TSH. Again, the concordant results of three experiments were pooled (Fig. 2). Basal cyclic AMP concentrations averaged 0.77 ± 0.08 in the HID group and 0.59 ± 0.06 in the LID group (NS). After TSH injection, corresponding values were 0.83 ± 0.08 and 1.5 ± 0.02 , values in the HID group being significantly lower ($P < 0.001$).

Adenylate cyclase. To ascertain whether the foregoing difference between the response to TSH in HID and LID groups could be explained, at least in part, by an effect of iodine intake on adenylate cyclase itself, three experiments were performed in which animals were given 0.5 U TSH i.v. and the activity of the enzyme was assayed in thyroid glands removed 4 min later. Pooled data from three experiments, each of which yielded similar results, are shown in Fig. 3. Basal enzyme activity, in picomoles of cyclic AMP generated per milligram protein per 10 minutes, did not differ significantly in the HID (9.8 ± 1.7) and the LID (11.1 ± 2.5) groups (means \pm SE). Within the HID group, values in TSH-treated animals (15.9 ± 2.0) were somewhat but not significantly higher than in unstimulated animals. Within the LID group, however, values in animals given TSH (30.3 ± 3.1) were much higher than in the animals given no TSH ($P < 0.025$).

Cyclic AMP phosphodiesterase. No significant difference was observed between basal phosphodiesterase activity, in nanomoles cyclic AMP degraded per milligram protein per 10 minutes, in HID (10.8 ± 2.0) and LID (8.8 ± 2.0) groups. Moreover, in accord with the findings of previous workers (6), no stimulation of phosphodiesterase activity could be detected after TSH administration in either the HID (10.0 ± 1.5) or the LID (9.9 ± 0.8) group.

Rat adrenal adenylate cyclase. To test whether the effects of dietary iodine intake on the adenylate cyclase

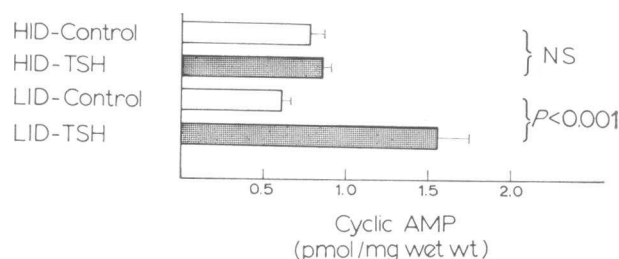


FIGURE 2 The effect of dietary iodine content on the response of thyroid cyclic AMP concentration to TSH in rats pretreated with aminophylline. Conditions of the experiments and data presentation are as described for Fig. 1, except that animals received 20 mg aminophylline i.v. 24 min before sacrifice.

response to trophic stimulation could be demonstrated in a gland other than the thyroid, adrenal glands were removed from hypophysectomized HID and LID rats and were assayed for adenylate cyclase activity as described above, with and without the direct addition of 12.5 mU of ACTH to 0.1 ml of the reaction mixture.

In the LID group, basal adenylate cyclase activity (in picomoles cyclic AMP generated per milligram protein per 10 minutes) was 181.2 ± 41.4 , and this was increased to 365.1 ± 29.3 by addition of ACTH. Corresponding values in the HID group were 210.9 ± 3.1 and 390.8 ± 43.8 . Neither the basal nor the stimulated values differed significantly between the HID and LID group.

DISCUSSION

The present studies have shown that the increase in thyroid cyclic AMP concentration that TSH acutely induces is significantly less in hypophysectomized rats given a diet rich in iodine than in comparable animals given an iodine-deficient diet. This difference could reflect either a lesser rate of cyclic AMP synthesis in the high-iodine group or a greater rate of degradation. We would favor the former mechanism because of these lines of evidence. First, in direct assays, we observed

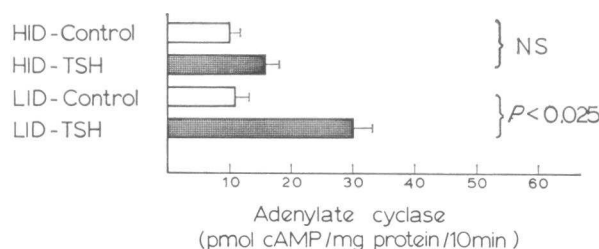


FIGURE 3 The effect of dietary iodine content on the response of thyroid adenylate cyclase activity to TSH. Conditions of the experiments and data presentation are the same as those described for Fig. 1, except that rats received 0.5 U TSH i.v.

no difference in basal thyroid phosphodiesterase activity in the two dietary iodine groups, nor was there any acute response to TSH in either. This line of evidence is not conclusive, however, since the thyroid contains two apparent phosphodiesterases (9), one operative at the approximate concentration of cyclic AMP that we studied, and one operative at lower concentrations. Hence it is possible that an effect of iodine intake on phosphodiesterase activity would have been detected had we studied the latter enzyme.

A second line of evidence was the experiments that revealed pronounced differences between the cyclic AMP responses of HID and LID rats to TSH even when animals were pretreated with huge doses of the phosphodiesterase inhibitor aminophylline. The most compelling evidence, however, was provided by direct assays of thyroid adenylate cyclase, which revealed far smaller increases in enzyme activity after TSH in the HID than in the LID groups.

The differing adenylate cyclase responses to TSH in the two groups may have been due to differences in the relative cellularity of their thyroid glands, since values for enzyme activity and cyclic AMP concentration were related to measurements of wet weight or protein concentration, rather than to DNA or RNA. This possibility is unlikely, however, because during the period of time employed in the present experiments, wide variations in dietary iodine intake in hypophysectomized rats do not significantly affect thyroid weight and mean acinar cell height (10).

It is noteworthy that basal levels of cyclic AMP and adenylate cyclase activity did not differ appreciably in the HID and LID groups, the differences between the two being manifest only in the extent to which these functions increased after administration of TSH. This suggests that iodine does not directly inhibit adenylate cyclase activity, but rather that it inhibits whatever mechanism is responsible for the increase in activity that follows TSH administration. Furthermore, this effect apparently does not represent a general inhibitory effect of iodine on trophic hormone-mediated activation of adenylate cyclase, since no comparable effect was seen in the adrenal stimulated by ACTH.

Agrawal and Furth have reported that the increase in thyroid cyclic AMP concentration that follows the administration of TSH to intact mice is greater in animals receiving a low-iodine than those receiving an iodine-sufficient diet (11). However, because intact animals were used, it was not clear from their experiments whether the differences observed were due primarily to differences in the prevailing level of endogenous TSH secretion or to an effect of dietary iodine

directly on the thyroid. It was for this reason that the present experiments were conducted in hypophysectomized animals, so that the effects of variations in iodine intake per se could be evaluated. The results indicate that iodine diminishes the cyclic AMP response by a direct action of the thyroid. Since many of the effects of TSH on the thyroid probably result from the increased generation of cyclic AMP that TSH induces, the presently described direct effect of iodine on thyroid cyclic nucleotide metabolism would appear to explain at least partially the diverse effects of TSH on thyroid structure and function.

ACKNOWLEDGMENTS

These studies were supported in part by Grant AM-16497 from the National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Md.

REFERENCES

1. Wolff, J. 1969. Iodide goiter and the pharmacologic effects of excess iodide. *47*: 101-124.
2. Ingbar, S. H. 1972. Autoregulation of the thyroid. Response to iodide excess and depletion. *Mayo Clin. Proc.* **47**: 814-823.
3. Dumont, J. E. 1971. The action of thyrotropin on thyroid metabolism. *Vitam. Horm.* **29**: 287-300.
4. Steiner, A. L., D. M. Kipnis, R. Utiger, and C. Parker. 1969. Radioimmunoassay for the measurement of adenosine 3'-5'-cyclic phosphate. *Proc. Natl. Acad. Sci. U. S. A.* **64**: 367-373.
5. Steiner, A. L., A. S. Pagliara, L. R. Chase, and D. M. Kipnis. 1972. Radioimmunoassay for cyclic nucleotides. II. Adenosine 3'-5'-monophosphate and guanosine 3'-5'-monophosphate in mammalian tissues and body fluids. *J. Biol. Chem.* **247**: 1114-1120.
6. Bastomsky, C. H., M. Zakarija, and J. M. McKenzie. 1971. Thyroid hydrolysis of cyclic AMP as influenced by thyroid gland activity. *Biochim. Biophys. Acta.* **230**: 286-295.
7. Krishna, G., B. Weiss, and B. B. Brodie. 1968. A simple, sensitive method for the assay of adenylyl cyclase. *J. Pharmacol. Exp. Ther.* **163**: 379-385.
8. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
9. Szabo, M., and G. Burke. 1972. Adenosine 3'-5'-cyclic phosphate phosphodiesterase from bovine thyroid: isolation and properties of a partially purified soluble fraction. *Biochim. Biophys. Acta.* **284**: 208-219.
10. Halmi, N. S. 1954. Regulation of the rat thyroid in short-term iodine deficiency. *Endocrinology.* **54**: 216-224.
11. Agrawal, R., and E. Furth. 1972. The influence of age, diet and species on in vivo changes in thyroid 3',5' cyclic adenosine monophosphate (cAMP) and 3',5' cyclic guanosine monophosphate (cGMP) following injections of thyroid stimulating hormone (TSH) and long acting thyroid stimulator (LATS). Program of the 48th American Thyroid Association Annual Meeting. 50.