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

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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).

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, Uliger, 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.6 Recovery of added cyclic AMP was

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1 Abbreviations used in this paper: HID, high-iodine diet; LID, low-iodine diet; TSH, thyrotropin.
The results described above were 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 Steinert, 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, Zakariaj, and McKenzie (6), which utilizes principles described by Krishna, Weiss, and Brodie (7). The final assay mix-
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±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 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...
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 adenral 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.

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