Effect of Thyroid-Stimulating Hormone and Norepinephrine on Cyclic AMP Levels in Human Normal Thyroids and Human Thyroid Tumors

YUJI AIYOSHI, KAMEJIRO YAMASHITA, AND YOSHIHIDE FUJIMOTO

Department of Surgery and Department of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, 305, Japan

Abstract

The effect of TSH (100 mU/ml) and norepinephrine (100 µM) on the cyclic AMP levels was studied in 10 human normal tissues, 10 thyroid adenomas and 4 thyroid carcinomas (3 papillary and 1 follicular). Normal tissues responded to TSH with a marked elevation of the cyclic AMP level. Response patterns of 10 thyroid adenomas to TSH were variable; the patterns of 6 cases resembled those of normal tissues, 3 responded mildly, and one had no response to TSH. Thyroid carcinomas had a higher basal level of cyclic AMP than those of normal tissues, although they responded only slightly to TSH. Two among 4 thyroid carcinomas had no response to TSH. Norepinephrine stimulated the accumulation of cyclic AMP in 4 thyroid adenomas and 3 thyroid carcinomas, while it had little effect on normal tissues. Responses to norepinephrine was observed only in thyroid tumors, although they had low response to TSH. It is suggested from these results that tumor cells originating from thyroid follicular cells have a modified response to hormones due to neoplastic alterations.

Many endocrine tumors are shown to produce and secrete hormones. From this point of view, such tumors appear to be suitable material to use in investigating the differences between normal and tumor tissues, and even those between benign and malignant tumor tissues. Recently, several research groups have reported the abnormality in hormone responsiveness of several tumor tissues (adrenal tumor (Schorr et al., 1971) etc.).

Thyroid tumor is one of the most common tumors seen in the endocrine organs. In the present study, we have investigated the hormone response in the cyclic AMP levels of thyroid tumors, and observed their abnormality in response to TSH and norepinephrine.

Materials and Methods

Human thyroid tissues (normal tissues, adenomas, or carcinomas) were obtained from patients at thyroidectomy. A half of each specimen was used for histopathological examinations, and the other half was used for the present experiments. After removal of surrounding vessels, capsules and fibrous tissues from the specimens, the tissues were sliced with a Stadie Riggs microtome, and slices weighing about 30 mg were preincubated at 37°C for 20 min in test tubes containing 1 ml Krebs-Ringer bicarbonate buffer with glucose (1 mg/ml) and theophylline (10 mM). After the addition of TSH (100 mU/ml) or norepinephrine (100 µM), the slices were incubated for 1, 5, 10 or 30 min. In all incubations the gas phase was 95% O₂—5% CO₂.

After incubation the slices were extracted with 0.5 ml hot 50 mM acetate buffer and homogenized with loose fitting Dounce homogenizers. After centrifugation the supernatant was assayed for cyclic
AMP by radioimmunoassay using Yamasa cyclic AMP kit (Chiba, Japan). The cyclic AMP values (nmoles/g wet weight tissue) are means±SEM of triplicate determinations, unless otherwise stated. Bovine TSH and 1-norepinephrine were obtained from Armour Pharmaceutical Co. and Sankyo Pharmaceutical Co., respectively.

Results

We studied using 10 normal thyroid tissues, 10 thyroid adenomas and 4 thyroid carcinomas (3 papillary and 1 follicular). Fig. 1 showed that the addition of TSH (100 mU/ml) in the incubation medium caused a prompt and marked rise in the tissue cyclic AMP levels in ten normal thyroid tissues. The maximum value of approximately 450% of the basal level was obtained after 10 min incubation, and then the level declined gradually.

In contrast, the addition of norepinephrine (100 μM) in the medium caused a prompt but slight rise in the tissue cyclic AMP level as early as in 1 min in five cases, but no significant rise was observed in the mean value for ten cases. These data obtained in five normal tissues of human thyroid glands are consisent with data using dog thyroid slices reported previously (Aiyoshi et al., 1978).

Fig. 2 shows results of experiments obtained with a thyroid adenoma. Though the basal levels of cyclic AMP in the thyroid adenoma were lower than those of normal tissues, the addition of TSH (100 mU/ml) in the medium caused a marked rise in the tissue levels of cyclic AMP. The maximum value of about 700% was obtained after 10 min incubation and the level declined gradually thereafter.

The addition of norepinephrine (100 μM) to the medium in the experiments with thyroid adenoma caused a slight and slow increase in the tissue cyclic AMP level after 5 min incubation, and the levels declined gradually thereafter. Results obtained from this thyroid adenoma were similar to those of normal thyroid tissues. Histopathology of this thyroid adenoma revealed macro- and microfollicular adenoma.

Fig. 3 demonstrates the results obtained from the experiments dealing with the different thyroid adenoma. The basal level of cyclic AMP in thyroid adenoma was much higher than those of normal thyroid tissues (6-fold). The addition of TSH (100 mU/ml) in the medium exerted no significant rise in the tissue cyclic AMP levels, while the addition of norepinephrine (100
Fig. 2. Effect of TSH and norepinephrine on tissue cyclic AMP levels in slices of a thyroid adenoma. Histopathological finding on thyroid adenoma was macro- and microfollicular adenoma.

 Ethanol caused a prompt and slight elevation in the tissue cyclic AMP level, followed by no decline. Time course of the tissue cyclic AMP levels in second thyroid adenoma was quite different from those of normal thyroid tissues. Histopathology of this thyroid adenoma revealed oxyphilic cell adenoma.

Fig. 4 shows the experiments in multiple thyroid adenomas. In thyroid adenoma #1, the addition of TSH (100 mU/ml) to the medium caused a marked rise in the tissue cyclic AMP levels, reaching to the maximum value at 10 min incubation and having a plateau thereafter. Norepinephrine (100 μM) showed a significant increase in cyclic AMP concentration, and the maximum value as much as 250% of cyclic AMP level was obtained at 5 min incubation, followed by a gradual decrease to the basal level after 30 min.

In contrast, the addition of TSH (100 mU/ml) to the incubation medium in the experiment of thyroid adenoma #2 caused a significant elevation of tissue cyclic AMP level, while the addition of norepinephrine (100 μM) caused only slight elevation of tissue cyclic AMP level. These reactions were similar to those of normal thyroid tissues. Histopathology of thyroid adenoma #1 revealed macrofollicular adenoma, and that of thyroid adenoma #2 revealed macr- and microfollicular adenoma. Thus, these two thyroid adenomas derived from the
Fig. 4. Effect of TSH and norepinephrine on tissue cyclic AMP levels in two thyroid adenomas which originated from the same patient. Histopathological finding on thyroid adenoma #1 was macrofollicular adenoma, and that on thyroid adenoma #2 was macro- and microfollicular adenoma.

Fig. 5. Effect of TSH and norepinephrine on tissue cyclic AMP levels in thyroid carcinoma slices. Histopathological finding on thyroid carcinoma was pure papillary carcinoma. The same patient showed different time courses for the tissue cyclic AMP level in response to norepinephrine 100 µM).

Fig. 5 shows that in the thyroid carcinoma the basal level of tissue cyclic AMP was higher than those of normal thyroid tissues. The addition of TSH (100 mU/ml) to the medium revealed a slight elevation of tissue cyclic AMP level, and the addition of norepinephrine (100 µM) also caused a slight increase in tissue cyclic AMP. Histopathology of this thyroid carcinoma revealed a pure papillary carcinoma.

Fig. 6 shows that the basal level of tissue cyclic AMP in another carcinoma of the thyroid was very high in comparison with those of normal thyroid tissues. The stimulation by TSH (100 mU/ml) resulted in a moderate elevation of tissue cyclic AMP level, but the maximum value in the tissue cyclic AMP concentration was only 200% of the basal level. The addition of norepinephrine (100 µM) to the medium caused a significant elevation of the tissue cyclic AMP level, but the maximum level of tissue cyclic AMP was only 150% of the basal level. Histopathology of the latter...
thyroid carcinoma revealed papillary carcinoma with colloidal proliferation.

Discussion

The present results (Table 1) shows that 6 thyroid adenomas had good response to TSH similar to normal thyroid tissues, 3 thyroid adenomas responded mildly, and one thyroid adenoma had no response, which showed histopathologically oxyphilic cell adenoma.

DeRubertis et al. reported that all of 11 cold thyroid adenomas responded well to TSH (50 mU/ml) with marked elevation of tissue cyclic AMP levels (DeRubertis et al., 1972). Thomas-Morvan reported that 7 thyroid adenomas had almost the same basal levels of cyclic AMP as in normal thyroid tissues, and that the response pattern of these thyroid adenomas to TSH (50 mU/ml) was similar to those of normal thyroid tissues. However, he did not report in detail their experimental data on 7 thyroid adenomas (Thomas-Morvan, 1978). Our present results are almost entirely consistent with the reports of DeRubertis et al. and Thomas-Morvan.

There was no report regarding the elevation by norepinephrine of the cyclic AMP level in thyroid adenoma. In our study, four among 10 thyroid adenomas responded to norepinephrine (100 μM) with mild cyclic AMP elevations. The other 6 thyroid adenomas had no response to norepinephrine, similar to normal thyroid tissues.

The basal levels of tissue cyclic AMP in 3 thyroid carcinomas in our study were higher than those of normal thyroid tissues. TSH (100 mU/ml) caused a mild increase in the level of cyclic AMP on two cases of thyroid carcinomas, while two thyroid carcinomas showed no response to TSH. Elevation of cyclic AMP levels by norepinephrine was observed in three among 4 thyroid carcinomas.

Field et al. reported that 8 thyroid carcinoma (7 papillary and 1 Hürthle cell) demonstrated greater basal cyclic AMP levels but significantly diminished response to TSH (100 mU/ml), and that one thyroid carcinoma had no response to TSH (Field et al., 1975). Thomas-Morvan also reported

| Table 1. Hormone responsiveness in normal human thyroid tissues and human thyroid tumors |
|---------------------------------|---------|-----------------|
|                                | Normal  | Adenoma         |
| No.                            | TSH     | Norepinephrine  |
| Normal                         | 6       | ++              |
| Adenoma                        | 10      | 3               |
|                                | 1       | −               |
| Carcinoma                      | 4       | 1               |
|                                | 1       | −               |

Fig. 6. Effect of TSH and norepinephrine on tissue cyclic AMP levels in thyroid carcinoma slices. Histopathological finding on thyroid carcinoma was papillary carcinoma with colloidal proliferation.
that in 8 differentiated thyroid carcinomas, the basal cyclic AMP levels were higher than those of normal thyroid tissues, but that four among 8 thyroid carcinomas had no response to TSH (50 mU/ml). (Thomas-Morvan, 1978).

There was a report that 2 medullary thyroid carcinomas had no TSH response (DeRubertis \textit{et al.}, 1972), but it seems to be a pertinent result since medullary thyroid carcinoma derives from parafollicular cell in the thyroid gland.

To summarize the present data, thyroid adenomas were divided into three groups by means of TSH and norepinephrine responses. The first group showed good responses to TSH and no response to norepinephrine. The second group showed slight responses to TSH and norepinephrine, and the third group showed no response to TSH and a slight response to norepinephrine.

Response patterns to TSH and norepinephrine in 4 thyroid carcinomas were divided into three types; 2 carcinomas had a slight response to TSH and norepinephrine, 1 carcinoma had a slight response to norepinephrine but no response to TSH, and 1 carcinoma had no response to TSH and norepinephrine. Although the relation of norepinephrine response to the formation and release of thyroid hormones from thyroid tumors is not elucidated yet, it is interesting to note that thyroid tumors responding to norepinephrine were observed only in tumors which had no response or only slight responses to TSH.

It is suggested from these results that tumor cells which originate from thyroid follicular cells have a modified response to hormones due to neoplastic changes.

\textbf{References}