INTRODUCTION

This study is the third in a series devoted to analyzing secretory phenomena in the thyroid follicular cell. The first and second dealt, respectively, with the fine structure of the cell under normal conditions and after acute stimulation with TSH (1, 2). In this study, we report biochemical data on the effect of acute stimulation with TSH on protein synthesis in the thyroid gland and correlate these data with the results of the earlier morphological studies.

Chronically elevated or depressed circulating levels of TSH affect the amount of thyroglobulin, the secretory product of follicular cells, stored in the gland as follicular colloid: elevated levels lead to its depletion, whereas depressed levels lead to its accumulation. These changes may be interpreted to indicate that the turnover of thyroglobulin is regulated by means of TSH. Although there is little reason to question this interpretation, the effects of TSH on individual steps in the turnover cycle, i.e., the secretion, storage, and resorption of thyroglobulin, have not been thoroughly analyzed.

In the past, the effect of TSH on thyroglobulin secretion has been evaluated on the basis of morphological data. The appearance of numerous large colloid droplets in the apical cytoplasm of follicular cells minutes after the thyroid is stimulated with TSH was interpreted, until recently, to denote accelerated synthesis of thyroglobulin. However, on the basis of evidence from radioautographic and histochemical studies (3–5), the droplets are now considered to contain thyroglobulin phagocytized by follicular cells from the follicular lumen, as the initial step in its breakdown. Under these circumstances, the numbers of intraepithelial colloid droplets should no longer be considered indicators of increased protein synthetic activity in follicular cells.

Several aspects of protein synthesis in the thyroid gland have been examined with biochemical techniques. Debons and Pitman (6) observed that incubation with TSH resulted in increased uptake of amino acid by bovine and canine thyroid slices, but Raghupathy et al. (7) did not confirm this finding in similar experiments with ovine thyroid slices. Raghupathy et al. (8) observed that thyroid slices from guinea pigs pretreated with TSH showed increased incorporation of amino acid into protein. The increase was first detected 10 to 15 hr after treatment with the hormone began. In a preliminary account of a similar study, Schneider and Goldberg (26) report increased incorporation of amino acid by thyroid slices from hypophysectomized rats pretreated with TSH. However, in their study the response to stimulation was more rapid, reaching a peak 8 hr following a single injection of TSH. Exposure of ovine thyroid slices to TSH in vitro does not stimulate incorporation of amino acid into protein (7).

MATERIALS AND METHODS

The experiments were carried out on 44 male C3H mice 9 to 13 wk old and weighing 19 to 25 g. They were placed on a low-iodine diet 10 days prior to the experiment, and their diet was removed the evening before labeled amino acid was injected. The mice were anesthetized by an intraperitoneal injection of pentobarbital. Experimental mice received an intravenous injection via the external jugular vein of 1.0 USP unit of TSH in 0.05 ml of normal saline at either 30 min, 4 hr, or 20 hr before sacrifice; control mice were injected with only saline. Thirty min prior to sacrifice, the mice were injected via the same route with 25 to 100 μc of carrier-free L-leucine-1,4-3H in 0.05 ml of normal saline.

The mice were sacrificed by exsanguination. Their thyroids were quickly removed, stripped of adherent adipose and connective tissue, and homogenized in a ground-glass tissue homogenizer in 4 ml of cold 0.05 M
carrier leucine solution. Total protein in the gland was determined with 0.2 ml aliquots of the homogenate according to the method of Lowry (9). The total DL-leucine-1,4\(^3\)H taken up during the 30-min labeling period was determined from measurement of the total radioactivity in 1.0-ml aliquots of the homogenate. The amount of radioactive amino acid incorporated into protein was measured in protein precipitated from the remainder of the homogenate by the modification of the method of Wool and Krahi (10). These measurements were corrected for variations in body weight and amount of radioactive amino acid injected. Radioactivity was measured with a Packard Tri-Carb liquid scintillation counter in which correction for quenching was made with internal standards.

A small number of measurements of total protein as well as total uptake and incorporation into protein of DL-leucine-1,4\(^3\)H was also carried out on specimens of diaphragm removed from some mice in each experimental and control group.

RESULTS

Uptake of Amino Acid

The total amount of radioactivity in each thyroid gland at the conclusion of the 30-min labeling period is given in the fourth column of Table I. In order to correct for variation in size of the glands, these data are expressed as dpm/mg thyroid protein. The findings from an earlier study concerned with the fate of labeled amino acid in the pancreas, a gland which, like the thyroid, secretes protein, indicate that amino acid which is taken up is retained for short intervals and not degraded or released (11). Therefore, the total radioactivity in the gland can serve as a measure of the total amount of radioactive amino acid that has been taken up during the labeling period and is present in the gland at the time of sacrifice either in free form or incorporated into protein.

The uptake of amino acid by glands of the experimental and control mice did not differ significantly in any of the time periods, nor from one time period to another. Therefore, this study does not furnish any evidence indicating that a single intravenous injection of TSH stimulates the thyroid gland to increase its uptake of amino acid from the circulation.

Incorporation of Amino Acid into Protein

The proportion of total radioactivity incorporated into protein by the thyroid gland during the labeling period is shown in the sixth column of Table I. This parameter was used in this study as a measure of the rate of protein synthesis by the gland. It was selected for several reasons: (1) it is relatively unaffected by variation in the total uptake of amino acids that might result from the

<table>
<thead>
<tr>
<th>Time after injection of TSH</th>
<th>No. of mice</th>
<th>Total thyroid protein*</th>
<th>Total uptake</th>
<th>Protein precipitate</th>
<th>Incorporation*:§</th>
<th>Control§</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-Min</td>
<td>C</td>
<td>5</td>
<td>0.297</td>
<td>37.4 ± 11.4</td>
<td>37.4 ± 11.4</td>
<td>21.8 ± 3.8</td>
</tr>
<tr>
<td>[TSH]</td>
<td></td>
<td>7</td>
<td>0.350</td>
<td>35.1 ± 6.9</td>
<td>35.1 ± 6.9</td>
<td>29.6 ± 6.1</td>
</tr>
<tr>
<td>4-Hr</td>
<td>C</td>
<td>5</td>
<td>0.386</td>
<td>39.0 ± 7.8</td>
<td>39.0 ± 7.8</td>
<td>15.9 ± 6.7</td>
</tr>
<tr>
<td>[TSH]</td>
<td></td>
<td>9</td>
<td>0.369</td>
<td>39.3 ± 9.2</td>
<td>39.3 ± 9.2</td>
<td>26.7 ± 10.2*</td>
</tr>
<tr>
<td>20-Hr</td>
<td>C</td>
<td>8</td>
<td>0.362</td>
<td>40.0 ± 13.2</td>
<td>40.0 ± 13.2</td>
<td>25.3 ± 9.4</td>
</tr>
<tr>
<td>[TSH]</td>
<td></td>
<td>9</td>
<td>0.342</td>
<td>44.7 ± 13.2</td>
<td>44.7 ± 13.2</td>
<td>28.9 ± 7.4</td>
</tr>
</tbody>
</table>

* Group means; standard deviation indicated in some cases.
‡ % of total uptake of radioactivity incorporated into protein precipitate.
§ Ratio of incorporation of experimental group to control group.
|| P < 0.10.
** P < 0.05.
action of TSH; and (2) unlike measurements of the specific activity of thyroid protein, it is unaffected by changes in the total protein in the gland. Concerning the latter point, morphological and physiological data indicate that TSH accelerates the depletion of thyroglobulin from the gland. It causes follicular cells to phagocytize greater quantities of colloid (3, 5), and it also causes the gland to release more hormone into the circulation (12), which can be accomplished only by hydrolyzing additional amounts of thyroglobulin, the molecule in which the hormone is stored. Since an accelerated depletion of protein from the gland could, even if the rate of protein synthesis remained unchanged, result in an increase in specific activity of the gland’s protein, changes in specific activity should not be used as an index of synthetic activity.

When examined by analysis of variance and the t test, the increase in % incorporation of amino acid into protein induced by TSH was statistically significant in the 30-min (P < 0.10) and 4-hr (P < 0.05) groups but not in the 20-hour group. The ratio of the % incorporation of the experimental group to that of the control group at each interval is shown in the last column of Table I. These figures indicate that intravenous injection of TSH caused a sharp and very rapid increase in protein synthesis by the thyroid gland. The increase was substantial within 30 min of the injection and persisted at a high rate for at least 4 hr. By 20 hr the stimulating effect of the tropic hormone had subsided.

It should be noted from the data in Table I that the % incorporation of amino acid in the 4-hr control group was lower than in the 30-min and 20-hr control groups. The depression may have been a side effect of anesthesia. In the 4-hr group, an initial injection of anesthetic was needed to inject TSH and a supplemental injection of anesthetic was needed subsequently to inject radioactive amino acid 31/2 hr later. Consequently, the mice in this group were under anesthesia for an extended period preceding the 30-min labeling period.

The amount of radioactive amino acid incorporated into protein by the diaphragm was much less than by the thyroid, and was quite variable. The findings gave no indication that the rate of protein synthesis by the diaphragm was influenced in a consistent fashion by TSH.

**DISCUSSION**

Heretofore, the effect of TSH upon the uptake of amino acids by thyroid tissue has been evaluated only in two studies and both were carried out under in vitro conditions. Their results are not in agreement. In the current study, the total amount of labeled leucine taken up from the circulation by the thyroid gland of intact mice at 1/2, 4, and 20 hr following a single intravenous injection of TSH was the same as in control untreated mice. The data obtained under in vivo conditions do not, therefore, furnish evidence that a single injection of TSH can enhance amino acid uptake by the thyroid.

Two previous in vitro studies have demonstrated that thyroid slices removed from guinea pigs and hypophysectomized rats pretreated with TSH incorporate increased amounts of labeled amino acid into protein, and the effect was first detected 10 to 15 and 8 hr, respectively, after treatment with TSH began (8, 26). The present study shows that, as early as 30 min after intravenous injection of TSH, the fraction of the total labeled amino acid in the gland that has been incorporated into protein is appreciably greater than in saline-injected mice. These observations are interpreted to indicate that under in vivo conditions TSH can enhance protein synthesis by the thyroid gland, and the onset of enhancement occurs rapidly after the gland is exposed to increased levels of the trophic hormone. It is presumed, although not yet established, that a portion of the increase can be attributed to increased synthesis of thyroglobulin.

TSH is known to stimulate several aspects of thyroid function, such as glucose oxidation (13–16), O2 consumption (16), phospholipid synthesis (17–19), release of hormone (12), formation of colloid droplets (2–5), etc., and stimulation of these activities can be detected shortly after the trophic hormone is administered. On the basis of findings from the present study, protein synthesis by the gland should be added to this list. One function of thyroid tissue, the trapping of iodide from the circulation, is unique in that, although it is enhanced by TSH, the effect of the tropic hormone becomes detectable only after a period of several hours (27). The reason for the prolonged period before stimulation takes effect is not known. Latency of action may itself be of some assistance in the analysis of the mode of action of hormones on their target organs, for a lengthy latent period.

**BRIEF NOTES**

435
could indicate that the chain of events at a molecular and cellular level linking the hormone and the process it affects is relatively complex.

Knowledge of the site of action of TSH within thyroid follicular cells, which would facilitate our understanding how it exerts its effects, has not yet been clearly established. The solution has been approached by attempts to localize the trophic hormone within the thyroid parenchyma by means of fluorescent labeling techniques, but there is little agreement in the findings from several studies. In these, TSH has been localized in the follicular basement membrane (20), at the apex of the follicular cell (21), and in nuclei of follicular cells (22). The nuclear localization of TSH is of particular interest because biochemical studies have shown that TSH stimulates the synthesis of purines (23) and RNA (24) by follicular cells.

Apart from inducing the appearance of apical pseudopodia and large numbers of colloid droplets in follicular cells, acutely administered TSH also seems to induce enlargement of their Golgi apparatus (2). Since it was recently pointed out that this organelle can give rise to lysosomes as well as secretory droplets (25), its hypertrophy in response to TSH could be correlated with either the synthesis or breakdown of thyroglobulin, or both.

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