Effect of Thyroid-stimulating Hormone and Prostaglandins on Thyroid Adenyl Cyclase Activation and Cyclic Adenosine 3',5'-Monophosphate*

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SUMMARY

Thyroid-stimulating hormone (TSH) increased within 1 min the adenyl cyclase activity of bovine and canine thyroid homogenate. A similar effect was also found when adenyl cyclase activity was measured in thyroid slices. In experiments in vitro, adenyl cyclase activity was augmented within 3 min after TSH injection, but this effect did not persist for 60 min after the injection. Despite the transient stimulation of adenyl cyclase activity, increased 14C-1-glucose oxidation was still present 60 min after TSH injection. Stimulation in vitro of adenyl cyclase activity in thyroid homogenate was obtained using as little as 1 milliunit of TSH while adrenocorticotropic hormone and proactin were without effect. TSH did not increase adenyl cyclase activity in adrenal or testis homogenates. TSH stimulation could still be obtained in the presence of 10^-2 M NaF. TSH did not modify thyroid phosphodiesterase activity under conditions in which it stimulated adenyl cyclase activity. TSH rapidly increased cyclic adenosine 3',5'-monophosphate (cyclic AMP) concentration in canine thyroid slices while luteinizing hormone, adrenocorticotropic hormone, and proactin were ineffective. Although NaF stimulated adenyl cyclase activity and 14C-1-glucose oxidation in canine thyroid slices, it did not increase cyclic AMP concentrations. During 15-min incubations, 10^-2 M theophylline had very little effect on cyclic AMP levels, but significantly increased them during 2-hour incubations. At both times, the effect of TSH on cyclic AMP was markedly potentiated by theophylline. Prostaglandin E$_2$ reproduced effects of TSH on both 14C-1-glucose oxidation and cyclic AMP concentration in canine thyroid slices. Prostaglandin F$_2 alpha$ stimulated 14C-1-glucose oxidation, but did not increase cyclic AMP levels. Prostaglandin B$_2$ did not modify either cyclic AMP concentrations or 14C-1-glucose oxidation.

Much recent evidence indicates that cyclic adenosine 3',5'-monophosphate mediates hormonal effects on target tissues (1).

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Thyroid slices were incubated in 2 ml of Krebs-Ringer bicarbonate buffer, containing 1 mg per ml of glucose for 30 min. The gas phase was 5% CO2-95% O2. The slices were then transferred to fresh medium containing TSH or other substances in the appropriate flasks and the incubations continued for variable times as indicated in the tables. The slices were then removed and immediately homogenized in 0.5 ml of 5% trichloracetic acid. The trichloracetic acid was removed by ether extraction and the cyclic AMP was purified by Ba(OH)2-ZnSO4 precipitation and Dowex-50 column chromatography as mentioned for the adenyl cyclase assay, except that no carrier adenine nucleotides were added. 3H-cyclic AMP (20 μl of 0.6 μCi per ml, 4800 cpm, 5 μmoles) was added to the trichloracetic acid homogenate to correct for losses during the procedure. The eluate from the Dowex column containing cyclic AMP was lyophilized and then dissolved in 0.1 ml 0.05 M Tris-HCl buffer (pH 7.5). The cyclic AMP was converted to ATP by incubation with phosphodiesterase, myokinase, and pyruvate kinase. A similar tube which lacked phosphodiesterase was included as a blank. The ATP formed was then assayed as previously described (14).

Phosphodiesterase was assayed using thyroid homogenate. Canine thyroid slices (120 mg) were homogenized in 1 ml of 0.04 M Tris-HCl buffer (pH 7.8), containing 3.5 × 10^{-3} M Mg++, 6 × 10^{-5} M theophylline, 6 × 10^{-4} M ATP, 0.5 to 3 μCi 32P- or 3H-phosphoenolpyruvate, 250 μg per ml of pyruvate kinase, and 1 mg per ml of albumin. The tubes were incubated in a Dubnoff metabolic shaker at 37°C for variable times as indicated in the tables. The reaction was terminated by placing the tubes in a boiling water bath for 3 min. Then 0.1 ml of a mixture of ATP, ADP, AMP, and cyclic AMP (2 mg per ml of each) was added to each tube. After the addition of 0.5 ml of 0.1 N HCl, the slices were homogenized, placed in a boiling water bath for 15 min, and then neutralized with 0.5 N NaOH. 3H-cyclic AMP (20 μl of 0.6 μCi per ml, 4800 cpm) was added to monitor recovery during the subsequent purification. The cyclic AMP was isolated and counted as outlined above for the determination of adenyl cyclase in homogenates. A boiled slice was included in the experiment as a control.

The effect of TSH in vivo on adenyl cyclase and glucose oxidation was measured as follows. One lobe of the thyroid gland from an anesthetized dog (pentobarbital 45 mg per kg) was removed, and then TSH was injected over a 30-sec period into the carotid artery below the level of the inferior thyroid artery. At variable times after this injection, the remaining thyroid lobe was removed. Both the control lobe, which had been kept in iced 0.85% sodium chloride solution, and the lobe removed after TSH injection were then sliced. Homogenate for adenyl cyclase was made from some of the slices from each lobe. TSH (2 units per ml) was added to an aliquot of each homogenate to determine the response in vitro of adenyl cyclase to the hormone. The remainder of the slices were used for glucose oxidation, which was measured during a 45-min incubation, as previously described (10). TSH (0.1 unit per flask) was also added to some of the slices to assess the responsiveness of glucose oxidation to the hormone in vivo.

The concentration of cyclic AMP in thyroid slices was measured by a modification (12) of the method of Breckenridge (13). Thyroid slices were incubated in 2 ml of Krebs-Ringer bicarbonate buffer, containing 1 mg per ml of glucose for 30 min. The gas phase was 5% CO2-95% O2. The slices were then transferred to fresh medium containing TSH or other substances in the appropriate flasks and the incubations continued for variable times as indicated in the tables. The slices were then removed and immediately homogenized in 0.5 ml of 5% trichloracetic acid. The trichloracetic acid was removed by ether extraction and the cyclic AMP was purified by Ba(OH)2-ZnSO4 precipitation and Dowex-50 column chromatography as mentioned for the adenyl cyclase assay, except that no carrier adenine nucleotides were added. 3H-cyclic AMP (20 μl of 0.6 μCi per ml, 4800 cpm, 5 μmoles) was added to the trichloracetic acid homogenate to correct for losses during the procedure. The eluate from the Dowex column containing cyclic AMP was lyophilized and then dissolved in 0.1 ml 0.05 M Tris-HCl buffer (pH 7.5). The cyclic AMP was converted to ATP by incubation with phosphodiesterase, myokinase, and pyruvate kinase. A similar tube which lacked phosphodiesterase was included as a blank. The ATP formed was then assayed as previously described (14).

Bovine TSH (2 units per mg) and prolactin (20 units per mg) were generous gifts from the Endocrinology Study Section, National Institutes of Health. Adrenocorticotropic hormone (100 units per mg) was purchased from Parke-Davis and Company. 3H-C ATP (25 μCi per mmole) and 3H-AMP (700 to 2000 μCi per mmole) were purchased from Amersham-Searle Corporation and International Chemical and Nuclear Corporation, respectively. Some ATP was prepared for chromatography on Dowex-50 and eluted with water to reduce the background counts in the adenyl cyclase assay. 3H-cyclic AMP (2.35 Ci per mmole) was obtained from Schwarz BioResearch. 3H-glucose (2.4 μCi per mmole) was a product of Amersham-Searle Corporation. Dowex 50X-4 was purchased from Calbiochem. Phosphoenolpyruvate and pyruvate kinase were obtained from Sigma. Prostaglandin A1, B1, E1, and F1α were kindly provided by Dr. John Pike, of Upjohn.

**RESULTS**

The data in Table I show that TSH increased adenyl cyclase activity in bovine thyroid homogenate within 1 min of its addition. In general, enzyme activity, both in the presence and
absence of TSH, was proportional to the time of incubation up to 4 min, but not for longer periods. Enzyme activity also tended to be proportional to the amount of homogenate used. As little as 1 milliunit of TSH increased adenyl cyclase activity, and further stimulation was obtained with larger amounts (Table II). ACTH and prolactin did not reproduce the effect of TSH on the thyroid, and TSH did not stimulate adenyl cyclase activity in either testis or adrenal tissue (Table III). Similar specificity was observed when the cyclic AMP level in thyroid was measured (Table IV). The TSH stimulation of cyclic AMP in thyroid was not reproduced by luteinizing hormone, ACTH or prolactin. The magnitude of the TSH effect was much greater when cyclic AMP concentration was measured than when adenyl cyclase was assayed.

The data in Table V indicate that, although TSH augmented adenyl cyclase in thyroid slices, the stimulation was somewhat less than that obtained using homogenate from the same gland. NaF, a potent stimulator of adenyl cyclase in many different tissues including thyroid (15–18), also increased adenyl cyclase activity.

### Table I

**Time response curve of adenyl cyclase activity in bovine thyroid homogenate**

The results are the average of duplicate determinations. Thyroid homogenate equivalent to 3 mg of tissue, wet weight, was used for each determination. 200 milliunits of TSH were added to the appropriate tube. Two separate experiments are shown.

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Adenyl cyclase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Experiment 1</td>
</tr>
<tr>
<td>1</td>
<td>47</td>
</tr>
<tr>
<td>2</td>
<td>94</td>
</tr>
<tr>
<td>4</td>
<td>167</td>
</tr>
<tr>
<td>6</td>
<td>238</td>
</tr>
</tbody>
</table>

### Table II

**Effect of graded doses of TSH on adenyl cyclase activity in bovine thyroid homogenate**

The results are the average of duplicate determinations. Thyroid homogenate equivalent to 3 mg of tissue, wet weight, was used for each determination. Incubation time in each experiment was 5 min. Two separate experiments are shown.

<table>
<thead>
<tr>
<th>Adenyl cyclase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>cpm</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

### Table III

**Specificity of TSH stimulation of canine thyroid adenyl cyclase activity**

The results are the average ±S.E.M. of quadruplicate determinations. Homogenate equivalent to 10 mg of tissue, wet weight, was used for each determination. Incubation for the adenyl cyclase assay was 10 min. Two separate experiments were done using thyroid and testis.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Adenyl cyclase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>TSH (1 unit)</td>
</tr>
<tr>
<td>cpm</td>
<td>Thyroid</td>
</tr>
<tr>
<td></td>
<td>372 ± 13</td>
</tr>
<tr>
<td></td>
<td>316 ± 11</td>
</tr>
<tr>
<td></td>
<td>1071 ± 17</td>
</tr>
<tr>
<td></td>
<td>1363 ± 43</td>
</tr>
<tr>
<td></td>
<td>1527 ± 138</td>
</tr>
</tbody>
</table>

### Table IV

**Effects of anterior pituitary hormones on cyclic AMP accumulation in canine thyroid slices**

Two separate experiments are shown. The results are the averages of duplicate determinations. The slices were incubated with the appropriate hormone for 10 min.

<table>
<thead>
<tr>
<th>Cyclic AMP</th>
<th>Control</th>
<th>TSH (10 milliunits)</th>
<th>Luteinizing hormone (10 milliunits)</th>
<th>ACTH (200 milliunits)</th>
<th>Prolactin (200 milliunits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>μmoles/g, wet weight</td>
<td>0.6</td>
<td>19.1</td>
<td>0.5</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>3.9</td>
<td>1.3</td>
<td>1.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>

### Table V

**Comparison of TSH and NaF stimulation of adenyl cyclase activity in bovine thyroid slices and homogenate**

Three separate experiments are shown. In each experiment, the same thyroid gland was used for both the slices and homogenate. The results are either the averages of duplicate or quadruplicate ±S.E.M. determinations. In the first experiment, the results are given as cycles per min per 6 mg of thyroid slice during a 4-min incubation. In the second and third experiments, the results are expressed based on 10 mg of tissue incubated for 30 min in the slice experiments and 8 min in the homogenate ones.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Slices (Control)</th>
<th>TSH (200 milliunits)</th>
<th>NaF (10⁻² M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krebs-Ringer bicarbonate</td>
<td>91</td>
<td>162</td>
<td>408</td>
</tr>
<tr>
<td>Tris-HCl (0.04 M)</td>
<td>224 ± 31</td>
<td>338 ± 36</td>
<td>1410 ± 151</td>
</tr>
<tr>
<td>Tris-HCl (0.04 M)</td>
<td>102 ± 12</td>
<td>200 ± 5</td>
<td>350</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Homogenate (Control)</th>
<th>TSH (200 milliunits)</th>
<th>NaF (10⁻³ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>219</td>
<td>773</td>
<td>1659</td>
</tr>
<tr>
<td></td>
<td>242 ± 11</td>
<td>886 ± 14</td>
<td>1326</td>
</tr>
</tbody>
</table>
activity in thyroid slices. In some tissues hormones did not increase adenyl cyclase activity when the assay was done in the presence of NaF (16, 19). However, in the thyroid, TSH still stimulated adenyl cyclase activity even in the presence of $10^{-2}$ M NaF (Table VI). Despite the fact that NaF was a potent stimulator of adenyl cyclase activity and augmented glucose oxidation (15, 20), it did not increase cyclic AMP in canine thyroid slices (Table VII).

Although cyclic AMP could accumulate in the thyroid as a consequence of inhibition of its degradation, TSH did not inhibit phosphodiesterase, whether a relatively large (180 mmoles) or small (55 mmoles) amount of cyclic AMP was used as substrate. Only about 20% of the larger amount of cyclic AMP disappeared during a 4 min incubation, whether TSH was present or not. Addition of theophylline resulted in some decrease in cyclic AMP degradation, but this was not modified by TSH. Nonetheless, under similar conditions of assay and using homogenate from the same thyroid, TSH still increased adenyl cyclase activity. Using 55 mmoles of cyclic AMP, only about 3% of it remained after 8 min of incubation in the absence of theophylline. Even under these conditions, TSH still doubled adenyl cyclase activity, even though it did not modify cyclic AMP degradation. Inhibition of phosphodiesterase by theophylline was more impressive when small amounts of cyclic AMP were used, but again TSH did not modify this even though it almost tripled adenyl cyclase activity. Theophylline, by itself, did not increase cyclic AMP levels in canine thyroid slices during a 15-min incubation period, but did during longer incubations (Table VIII). The effects of TSH on cyclic AMP were potentiated by theophylline during both shorter and longer incubations. TSH also increased adenyl cyclase activity in vitro (Table IX). In these experiments effects of TSH on glucose oxidation in vivo and in vitro were also measured. Although injection of 0.5 unit of TSH did not stimulate adenyl cyclase activity in 3 min, it did increase glucose oxidation in slices during a subsequent 45-min incubation. In this experiment TSH in vitro augmented adenyl cyclase activity in homogenate obtained from both the control and treated lobe. Ten units of TSH increased adenyl cyclase activity when the thyroid lobe was removed 3 min later. Addition of the hormone in vitro caused further stimulation of enzyme activity. The effect in vitro was approximately equivalent in both the control lobe and the one removed following TSH administration. Glucose oxidation was increased in slices from the lobe removed after TSH injection. When the thyroid lobe was removed 20 min after TSH

### Table VI

**TSH stimulation of adenyl cyclase activity in the presence of NaF in thyroid homogenate**

The results are the average ±S.E.M. of quadruplicate determinations. Homogenate equivalent to 10 mg of tissue, wet weight, was used for each determination. The incubation time was 10 min. NaF ($10^{-2}$ M) was present during the assay. Canine thyroid homogenate was used for the first three experiments and bovine thyroid homogenate for the last two.

<table>
<thead>
<tr>
<th>Adenyl cyclase activity</th>
<th>Control</th>
<th>TSH (1 unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm</td>
<td></td>
</tr>
<tr>
<td>271 ± 33</td>
<td>546 ± 32*</td>
<td></td>
</tr>
<tr>
<td>268 ± 17</td>
<td>456 ± 30*</td>
<td></td>
</tr>
<tr>
<td>306 ± 19</td>
<td>500 ± 19*</td>
<td></td>
</tr>
<tr>
<td>330 ± 10</td>
<td>470 ± 14*</td>
<td></td>
</tr>
<tr>
<td>375 ± 17</td>
<td>647 ± 44*</td>
<td></td>
</tr>
</tbody>
</table>

* $p < 0.01$.

### Table VII

**Effect of NaF on adenyl cyclase activity, cyclic AMP concentration, and glucose oxidation in canine thyroid slices**

When cyclic AMP was measured, slices were incubated with the appropriate substance for 4 min. Adenyl cyclase activity and glucose oxidation were measured in triplicate. The cyclic AMP results are either duplicate or quadruplicate ±S.E.M. determinations.

<table>
<thead>
<tr>
<th>Adenyl cyclase activity</th>
<th>Cyclic AMP</th>
<th>14CO2 produced by glucose oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>NaF ($10^{-2}$ M)</td>
</tr>
<tr>
<td></td>
<td>cpm</td>
<td>20 mmol/g, wet weight</td>
</tr>
<tr>
<td>38 ± 12</td>
<td>1,130 ± 292</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* 3 milliunits.
* 2 milliunits.
* 20 milliunits.
* 10 milliunits.
TABLE IX

Effect in vivo of TSH on adenyl cyclase activity and glucose-14C oxidation in canine thyroid

The results for the adenyl cyclase assay represent averages of duplicate determinations. Thyroid homogenate equivalent to 3 mg, wet weight, of tissue was used for each determination. A 4-min incubation was used for adenyl cyclase assay. Glucose-14C oxidation was determined in triplicate during a 45-min incubation.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Amount of TSH injected</th>
<th>Minutes</th>
<th>Lobe</th>
<th>Adenyl cyclase activity</th>
<th>14CO2 produced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units</td>
<td></td>
<td>control</td>
<td>TSH in vitro (0.2 unit)</td>
<td>cpm</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>3</td>
<td>control</td>
<td>104 580</td>
<td>34,300 ± 750</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>treated</td>
<td>190 500</td>
<td>60,000 ± 85a</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>3</td>
<td>control</td>
<td>138 677</td>
<td>26,100 ± 818</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>treated</td>
<td>422 622</td>
<td>57,900 ± 185a</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>3</td>
<td>control</td>
<td>203 517</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>treated</td>
<td>370 494</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>20</td>
<td>control</td>
<td>242 548</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>treated</td>
<td>400 713</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>20</td>
<td>control</td>
<td>330 670</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>treated</td>
<td>327 530</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>60</td>
<td>control</td>
<td>345 588</td>
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<td>322 615</td>
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<td>7</td>
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<td>60</td>
<td>control</td>
<td>130 263</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>treated</td>
<td>125 250</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>60</td>
<td>control</td>
<td>23,000 ± 715</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>treated</td>
<td>33,100 ± 3220a</td>
<td></td>
</tr>
</tbody>
</table>

a p < 0.01 when treated lobe is compared to the control lobe.

b p < 0.01 when effect in vitro of TSH on glucose oxidation is compared with slices from the same thyroid lobe.

c Time interval between injection of TSH and removal of the second lobe.

Injection, stimulation of adenyl cyclase was variable, although TSH in vitro consistently augmented enzyme activity. The transient nature of the TSH stimulation of adenyl cyclase was evident when the thyroid lobe was removed 60 min after hormone injection. Under these conditions, no effect in vivo of TSH on adenyl cyclase activity was indicated. This did not represent tissue unresponsiveness since the addition in vitro of TSH to homogenates still stimulated the enzyme. Despite the absence of an increase in adenyl cyclase in vivo, glucose oxidation was clearly greater in slices from the lobe removed 60 min after TSH injection. The addition in vitro of relatively large amounts of TSH to slices from both the control and treated lobe increased glucose oxidation even more.

Prostaglandin E1 reproduces the TSH stimulation of glucose oxidation (21) and cyclic AMP (4) in canine thyroid slices. However, these effects are not shared by all of the prostaglandins (Table X). Prostaglandin E2 also stimulated glucose oxidation and cyclic AMP accumulation in canine thyroid slices. In contrast, prostaglandin F12, which increased 14CO2 production as much as prostaglandin E2, did not augment cyclic AMP. Prostaglandin B1, at an equivalent dose, failed to stimulate either glucose oxidation or cyclic AMP levels. In one of two experiments, prostaglandin A1 increased glucose oxidation and cyclic AMP, but to a lesser extent than did prostaglandin E2.
This demonstration that TSH increases adenyl cyclase activity in thyroid confirms and extends the previous observations of Kleinert et al. (2) and Pastan and Katzen (9). The results are consistent with those of Gilman and Rall (3) who found that 10^{-4} M theophylline increased cyclic AMP about 40% in bovine thyroid slices during a 10-min incubation. Both in their experiments and in ours, the TSH-mediated increase in cyclic AMP was potentiated by theophylline. If TSH were acting only by inhibition of phosphodiesterase, such potentiation would not be expected if theophylline, by itself, markedly inhibited cyclic AMP degradation. The theophylline stimulation of cyclic AMP during longer incubations probably reflects the cumulative effect of its inhibition of phosphodiesterase activity. Despite the fact that theophylline augments cyclic AMP concentration and potentiates effects of TSH, similar concentrations of theophylline decreased both glucose oxidation and 32P incorporation into phospholipid (21). Although these concentrations of theophylline were reported to mimics effects of low doses of TSH on glucose oxidation by bovine thyroid slices (22), it is important to stress that in this species, low doses of TSH decrease and high doses of TSH increase glucose oxidation (23). It would be difficult to distinguish in the experiments of Gilman and Rall (22) whether the decreased glucose oxidation caused by theophylline represented the inhibitory effect we have reported (21), or actual reproduction of the action of low doses of TSH.

The rapidity, specificity, and sensitivity of the TSH stimulation of thyroid adenyl cyclase activity and cyclic AMP accumulation are similar to those qualities reported by Pastan and Katzen (9) and Gilman and Rall (3), and would certainly be consistent with the concept that TSH control of thyroid function may reflect this effect (24). TSH stimulated adenyl cyclase in vitro, as well as in vivo, in both thyroid homogenates and slices. The effect on slices could not be attributed to TSH carried over to the homogenate, since the formation of 32P-cyclic AMP took place in the intact slice which was then homogenized in HCl. Although ATP-P probably does not enter intact slices, adenyl cyclase is a membrane enzyme which may be in close proximity to the extracellular space. Stimulation of adenyl cyclase in intact cells was demonstrated by Oye and Sutherland (25) with turkey red blood cells, and Williams et al. (26) with isolated fat cells. The stimulation in vitro of thyroid adenyl cyclase was also very rapid when 10 units TSH were administered. The variable effect of TSH on adenyl cyclase after 20 min and the absence of stimulation after 60 min indicate that this event is rather short-lived. The absence of an effect of TSH on adenyl cyclase activity after 10 min is a result of the unresponsive-ness of the enzyme, since addition of TSH to homogenates in vitro increased enzyme activity. Nonetheless, the augmented glucose oxidation, which is thought to be dependent upon increased cyclic AMP, continues to be present even at a time when the more primary event is no longer demonstrable. As a result of such a succession of events, it is possible that an early effect of TSH to stimulate adenyl cyclase can thus set into motion the diverse biochemical and morphological changes which have been attributed to the hormone.

NaF was a potent stimulator of adenyl cyclase activity in both thyroid slices and homogenates. It also stimulates this enzyme in many other tissues (15-18). O'Malley and Field (20) and Pastan et al. (15) reported that NaF increased glucose oxidation in thyroid slices, and the latter workers postulated that this reflected augmented adenyl cyclase activity. However, the fact that NaF did not increase cyclic AMP, suggests that NaF augments glucose oxidation by a mechanism other than cyclic AMP accumulation. Furthermore, Pastan et al. compared the effects of NaF to those of 100 millimolar/ml of TSH using bovine thyroid slices. In this species low doses of TSH or N6-2'-O-dibutyl cyclic AMP actually decrease 32P-glucose oxidation, and it is only high doses which increase glucose oxidation (5, 23). Thus, NaF did not reproduce the effects of either N6-2'-O-dibutyl cyclic AMP (15), or low doses of TSH on glucose oxidation by bovine thyroid slices. In addition, NaF did not mimic the effects of TSH or N6-2'-O-dibutyl cyclic AMP on colloid droplet formation (15). These observations only emphasize the caution which should be exercised in attributing biological effects of hormones and substances to the adenyl cyclase and cyclic AMP systems in the absence of direct measurements of both of these parameters. It is not apparent why the NaF stimulation of adenyl cyclase is not accompanied by cyclic AMP accumulation. This dissociation of effects of NaF on adenyl cyclase and cyclic AMP has been commented on previously (4). Perhaps TSH and NaF increase adenyl cyclase activity by different mechanisms which might account for the ability of TSH to stimulate adenyl cyclase even in the presence of NaF. Weiss (27) presented evidence that epinephrine and NaF stimulated pineal adenyl cyclase activity by different mechanisms. In addition, glicine increased heart adenyl cyclase activity even in the presence of NaF (28). This is in contrast to results which have been reported in several other tissues where hormonal stimulation was not observed in the presence of NaF (16, 19).

Although TSH, prostaglandin E (4, 21), and E increased glucose oxidation and cyclic AMP levels in canine thyroid slices, prostaglandin E (4, 21) does not reproduce the effects of TSH on 32P incorporation into phospholipid. The relationship between the prostaglandin stimulation of glucose oxidation and elevation of cyclic AMP levels appears to be quite complex, since prostaglandin A stimulated glucose oxidation, yet had no effect on cyclic AMP. This may be analogous to NaF and other substances which also augmented glucose oxidation but had no effect on cyclic AMP concentration. Prostaglandin B was completely inactive, whereas in one experiment prostaglandin A stimulated glucose oxidation and cyclic AMP levels, although to a lesser extent than prostaglandin E. The previous results of Butcher and Baird (29) indicate the complexity of prostaglandin action. Although prostaglandin E inhibited the elevation of cyclic AMP induced by various lipolytic agents in isolated adipose tissue, by itself it stimulated cyclic AMP levels
in other tissues such as lung, spleen, diaphragm, kidney, and adipose tissue pieces. In adipose tissue, differences were also apparent when various prostaglandins were tested. Prostaglandin $E_1$ was considerably more effective than prostaglandin $F_2$, and prostaglandin $F_2a$ was completely inactive. Furthermore, prostaglandin $E_1$ markedly raised cyclic AMP in fat pads, but did not increase glycerol release. This contrasts with the present results with prostaglandin $F_2$, which did not change cyclic AMP but did augment glucose oxidation. Differences have also been reported between prostaglandin $E_1$, $E_2$, and $F_2a$ in respect to their ability to stimulate adrenal steroidogenesis in vitro (30). We have not been able to demonstrate that prostaglandin $E_1$ or $E_2$ in similar amounts increases rat adrenal cyclic AMP concentrations in vitro. Thus, these diverse results obtained with the prostaglandins clearly dictate caution in attributing their actions to any one specific mechanism of action.

Although the present results support the concept that TSH controls thyroid function as a consequence of adenyl cyclase activation, the mechanism of this stimulation and how cyclic AMP mediates its effects remains to be elucidated.

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Effect of Thyroid-stimulating Hormone and Prostaglandins on Thyroid Adenyl Cyclase Activation and Cyclic Adenosine 3',5'-Monophosphate
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