Changes in adenylate cyclase activity in rat pituitary after TRH and T₃ injection in vivo

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Abstract. Increased activity of adenylate cyclase in whole anterior pituitary was repeatedly found in groups of 3–6 adult male rats 5 min after iv injection of 10, 100 and 200 nmol of TRH kg⁻¹. After the administration of 100 nmol TRH kg⁻¹ the activity of adenylate cyclase was significantly increased at 2, 5, 10 and 30 min with a peak level at 5 min, while at 60 min the values did not differ from controls. The level of TSH in serum was significantly increased in all TRH injected groups with a peak value at 10 min, while no dose-response relationship was found at 5 min. Finally, the activity of adenylate cyclase was significantly decreased in animals injected with 20 nmol of 1-triiodothyronine kg⁻¹ 180 min before sacrifice, the decrease being not overcome by 100 nmol TRH kg⁻¹ injected 5 min before sacrifice.

It has been repeatedly demonstrated that thyrotrophin releasing hormone (TRH) may increase the concentration of cyclic adenosine monophosphate (cAMP) in anterior pituitary tissue or in incubation medium in vitro (Bowers 1971; Bowers et al. 1975). The same was found in isolated pituitary GH cells (Dannies et al. 1976; Gautvik et al. 1977) or in normal anterior pituitary cells enriched in thyrotrophs or mammatrophs (Barnes et al. 1978) which represent a less complex system consisting namely in a case of GH cells of clonal cells synthesizing and secreting only growth hormone and prolactin, but not thyrotrophin (TSH).

Moreover, TSH was found to be released from the pituitary tissue in vitro by cyclic nucleotide analogues (Wilber et al. 1969; Bowers 1971) and, finally, the phosphodiesterase inhibitor theophylline has been found to stimulate the accumulation of cAMP in anterior pituitary incubated with TRH (Bowers 1971) or the release of TSH into the incubation medium under the same conditions (Wilber et al. 1969; Steiner et al. 1970).

In this investigation it was attempted to demonstrate changes in adenylate cyclase activity in whole anterior pituitary and changes in serum TSH level in rats injected with various doses of TRH at various time intervals before sacrifice. Moreover, a possible preventive effect of triiodothyronine on the effect of TRH on adenylate cyclase activity in the pituitary and on the increase of TSH in serum was tested.

Materials and Methods

Animals
Male Wistar rats of specific pathogen free colony bred in VELAZ (Prague) weighing about 200 g were used. The animals were fed standard pelleted diet (VELAZ, Prague) and housed in stainless steel cages in temperature, moisture and light (6.00 to 18.00 h) controlled room.

Experiments
A total of 169 animals were used in 11 experiments. In each experiment (consisting of 3–5 groups of 3–6 animals each) the rats were anaesthetized with pentobarbiturate (Pentobarbital Spofa, 40 mg kg⁻¹ ip; about a
half of this dose being added every 60–90 min in some long lasting experiments – see below). Control animals (used in each experiment but No. 5) were decapitated about 20 min after pentobarbiturate injection, each of them being injected with 0.5 ml of saline into the jugular vein 5 min before sacrifice. The other groups were injected in a similar way with either TRH alone (10, 100 or 200 nmol kg⁻¹), L-3,5,3'-triiodothyronine (T₃) alone (20 nmol kg⁻¹) or pre-injected the same dose of T₃ and then injected with 100 nmol TRH kg⁻¹ 180 min later. The anaesthetized animals were decapitated at various time intervals (2 to 6 min) after TRH injection. Special attention was paid to keep the time interval between the decapitation and immersion of dissected pituitary into ice cold fluid (for details see below) less than 2 min.

The above indicated doses of TRH per kg were dissolved in 1 ml saline. To obtain a dose of T₃ indicated above, 1 μg of this compound was dissolved in 0.4 ml ammonium hydroxide and 9.6 ml propylene glycol in saline (1:5, v/v) was added. Moreover, to 1 ml of this solution 8.23 ml of the above solution was added and 0.12 ml of this cocktail per 100 g b.w. was injected into the jugular vein. As observed previously (Langer et al. 1977), the injected amount of propylene glycol may cause a transitory haemolysis which was, in these experiments, neglected.

**Estimation of adenylate cyclase**

The pituitaries were homogenized immediately after dissection in ice cold 100 mmol l⁻¹ Tris-HCl buffer (pH 7.4) containing 1 mmol l⁻¹ EDTA and 5 mmol l⁻¹ MgCl₂ in all-glass homogenizers, the final dilution being made up to 2.5 per cent homogenate. All estimations were made on the day of the experiment. Adenylate cyclase was estimated essentially according to Krishna et al. (1972) with minor modifications. The reaction was initiated by the addition of 20 μl 2.5 per cent homogenate (containing 40–60 μg protein) to the assay system containing 0.5 mmol l⁻¹ ³²P-ATP (Radiochemical Centre, Amersham, S.A. 30 Ci mmol⁻¹) 1 mmol l⁻¹ cAMP, 500 mmol l⁻¹ KCl, 0.08 per cent bovine serum albumin and an ATP regenerating system consisting of pyruvate kinase (100 μg ml⁻¹) and sodium phosphoenol-pyruvate (16 mmol l⁻¹) in a total volume of 50 μl. The tubes were incubated for 20 min at 37°C. The blank was treated the same way, but did not contain the pituitary homogenate. After incubation, the reaction was stopped by 4 min boiling after the addition of 50 μl 330 mmol l⁻¹ hydrochloric acid (Counis & Mongondu 1978). The formed [³²P]cAMP was separated on microcolumns of neutral aluminium trioxide, washed with 3 ml 100 mmol l⁻¹ Tris-HCl buffer (pH 7.4) (Ramachandran 1971). One ml of the eluate was counted in 9 ml Bray's liquid in a Packard Tricarb liquid scintillation counter. The protein content in the homogenate was measured according to Lowry et al. (1951).

**Estimation of TSH in serum**

A kit for radioimmunoassay of rat TSH (Hormone Distribution Program, NIH, Bethesda, Md) was used in a

![Fig. 1.](https://example.com/fig1.png)

Activity of adenylate cyclase in anterior pituitary at 5 min after the administration of various doses of TRH and T₃ in individual animals (points) of experiments No. 1–10. Horizontal lines indicate means and SE of pooled groups.

For details see Material and Methods.
double antibody system, the second antibody (goat anti-rabbit gamma globulin) being prepared and supplied by Dr. K. Hruska (Research Institute of Veterinary Medicine, Brno).

Results

Effect of increasing doses of TRH on adenylate cyclase activity

First, it should be pointed out that in a total of 9 experiments, a total of 39 control animals was used (Fig. 1). In 20 of them the values of adenylate cyclase activity were between 5.5 and 7.5 pmol cAMP mg⁻¹ protein min⁻¹, the average of all values being 6.44 ± 0.27 pmol cAMP mg⁻¹ protein min⁻¹ (mean and se). It was concluded that the values obtained in controls were highly reproducible in spite of various batches of labelled ATP and various lots of animals and that it is justified to pool all the control values obtained. The same approach was also used for other groups injected with the same doses of hormones at the same time intervals. A significant effect of 10 (P < 0.05), 100 (P < 0.001) and 200 nmol TRH kg⁻¹ (P < 0.001) on adenylate cyclase activity at 5 min after the injection could be demonstrated (Fig. 1).

Time-dependent effect of 100 nmol of TRH kg⁻¹ on adenylate cyclase activity

After the injection of 100 nmol TRH kg⁻¹ a significant increase of adenylate cyclase activity was found at 2 min (P < 0.05), 5 min (P < 0.001), 10 min (P < 0.05) and 30 min (P < 0.01), while the values obtained at 60 min did not differ from controls (Fig. 2). As demonstrated, the maximum activity was found 5 min after TRH injection.

Preventive effect of T₃ on the increase of adenylate cyclase activity after TRH injection

When examined at 180 min after iv injection of 20 nmol of T₃ kg⁻¹, the activity of adenylate cyclase was found to be consistently less than in controls (Fig. 1). There was no change of this depressed activity even at 5 min after the injection of 100 nmol TRH kg⁻¹ (Fig. 1), a condition which caused a definite increase of adenylate cyclase activity in other experiments where no T₃ pre-injection had been used. The difference between each of the above indicated groups and controls was significant (P < 0.05), which supports the conclusion on the prevailing effect of T₃.

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**Table 1.**

Level of TSH in serum in groups of animals from various experiments administered various doses of TRH and T₃ (means and se).

<table>
<thead>
<tr>
<th>No. of group</th>
<th>TRH nmol kg⁻¹</th>
<th>T₃ nmol kg⁻¹</th>
<th>min after TRH</th>
<th>No. of animals</th>
<th>No. of experiment</th>
<th>TSH in serum µU ml⁻¹</th>
<th>P against group No.</th>
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<tr>
<td>1*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>24</td>
<td>7,8,9,10</td>
<td>83.5 ± 6.62</td>
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<tr>
<td>2</td>
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<td>-</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>221.5 ± 9.12</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>-</td>
<td>11</td>
<td>7,11</td>
<td>292.9 ± 22.49</td>
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<td></td>
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<tr>
<td>4</td>
<td>200</td>
<td>-</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>270.3 ± 17.37</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>-</td>
<td>2</td>
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<tr>
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<td>100</td>
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<td>10</td>
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<td>401.0 ± 38.55</td>
<td>1,5</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>-</td>
<td>50</td>
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<td>307.0 ± 27.22</td>
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<tr>
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<td>-</td>
<td>60</td>
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<td>9**</td>
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<td>-</td>
<td>9</td>
<td>8,11</td>
<td>48.7 ± 7.02</td>
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<tr>
<td>10**</td>
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<td>5</td>
<td>10</td>
<td>8,11</td>
<td>89.7 ± 5.67</td>
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</table>

* Control group. ** 180 min after T₃ injection.

**Effect of increasing doses of TRH on TSH level in blood**

As shown in Table 1, the level of TSH in serum was significantly increased in all time intervals (i.e. 2 to 60 min) after the administration of 10, 100 and 200 nmol TRH kg⁻¹. The maximum level was found at 10 min after TRH injection, while no apparent dose-response relationship could be demonstrated at the 5 min interval. Furthermore, significantly decreased TSH levels were found 180 min after the injection of 20 nmol T₃ kg⁻¹, but TSH levels in a group injected with 100 nmol TRH kg⁻¹ in addition to the above dose of T₃ did not differ from controls.

**Discussion**

Although several reports show that the action of TRH on the release of TSH from the pituitary may be mediated by the adenylate cyclase — cyclic AMP system (see Introduction), all criteria supporting this assumption have not yet been confirmed by definite experimental evidence. Thus, Dannies et al. (1976) stated that the activation of adenylate cyclase by TRH, which is considered as one of Sutherland's criteria, has not yet been demonstrated and Bowers (1978) concluded that his data are strongly against the concept that cAMP may act as intracellular mediators of pituitary hormonal release. Similar conclusions were reported by Eto & Fleischer (1976).

Hence, these findings may be considered as a definite demonstration of an increase of adenylate cyclase activity in vivo after the administration of TRH. This observation appeared to be consistently reproducible in spite of the use of whole anterior pituitary which is considered to be less favourable than the use of clonal strains of pituitary cells. This is because of the presence of several functionally different cell types in the anterior pituitary of which only a minority appears to be TRH-sensitive. Thus, any change resulting from TRH action in separate and limited pools may not result in detectable changes in the whole pituitary. In contrast, however, in these experiments, it appears that the experiments using a whole pituitary in vivo may be advantageous compared to any in vitro system because of the perfusion of the tissues through the blood vessels network which presumably results in a rapid and appropriate contact of TRH with the membrane receptors. Moreover, it should be pointed out that in our experiments the increase in adenylate cyclase activity was found even without the use of any phosphodiesterase inhibitor (e.g. theophylline), which, in contrast, was used in most of the studies made so far to facilitate the demonstration of changes of adenylate cyclase — cyclic AMP system. In fact, the increase of cAMP after TRH in vitro was observed under a simultaneous action of theophylline (Dannies et al. 1976) or other xanthine analogues (Barnes et al. 1978) which at least may potentiate the effect of TRH on
cAMP accumulation (Gautvik et al. 1977) and on TSH release (Bowers 1971).

One of the questions to be discussed is a possible interfering effect of pentobarbiturate anaesthesia on the observed effects of TRH. Even though pentobarbiturate is known to induce a rapid growth hormone release from the pituitary (Martin 1972), to prevent the spontaneous or induced gonadotrophin release (Chappel & Barraclough 1976) and to enhance TSH response to TRH in female rats in vivo (Männistö et al. 1976), it should be presumed that in these experiments such an effect of pentobarbiturate on TSH release might take place, but it should be stressed that the interference of the drug should be the same in controls and in TRH injected groups. Moreover, no interfering effect of pentobarbiturate on adenylate cyclase or cAMP after TRH administration could be demonstrated so far.

As observed previously, a dose of 5 nmol T₃ kg⁻¹ can completely prevent the effect of 25 μg TRH (per cent) on TSH release in vivo, if injected 180 min before TRH (Kokesová et al. 1977). Even though a higher dose of T₃ was used in these experiments (i.e. 20 nmol kg⁻¹), the finding of a decrease of adenylate cyclase activity in the pituitary 180 min later may perhaps still be considered of some importance in the physiological regulation of TSH release. Moreover, this should not be overwhelmed by the administration of a relatively large dose of TRH which normally increases TSH release. This finding seems to be in accordance with that by Bowers et al. (1975) who described a significant decrease of cAMP in pituitaries of hypothyroid rats injected with T₃ 16 h before sacrifice. However, some additional evidence seems to be necessary before some further conclusions about this phenomenon can be reached.

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References


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