Factors influencing the release of cyclic AMP from mouse thyroid tissue stimulated by TSH in vitro

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Abstract. The accumulation of cyclic AMP (cAMP) in mouse thyroid tissue in response to TSH in the presence of 1 mm theophylline was accompanied by a release of the nucleotide from the tissue into the incubation medium. This cAMP release was almost rectilinearly related to the time of exposure to TSH, and rectilinearly related to the log concentration of TSH in the range 0.1-5 mU/ml. The cAMP release proved to be independent of the pre-incubation time up to 4 h, and took place also in the absence of methylxanthines when the cAMP level was low. The total cAMP accumulation in response to TSH was augmented by different inhibitors of protein synthesis but the fraction of the nucleotide that was retained intracellularly was increased only by puromycin. Depolarization or treatment with ouabain did not change the distribution of cAMP between tissue and medium.

It is concluded that the release of cAMP from thyroid tissue stimulated by TSH may take place under physiological conditions, that it seems to be regulated by the actual concentration of TSH, and that it may be of significance for the regulation of the intracellular cAMP level.

When the metabolism of cyclic AMP (cAMP) is studied in in vitro systems methylxanthines are practically always included to minimize breakdown of the nucleotide. In this way the accumulated cAMP synthesis in response to a hormone stimulation may be studied selectively but the cAMP concentrations will be unphysiologically high. Under these conditions thyroid cells stimulated by TSH have been found to respond with an increased synthesis of cAMP that levels off after about 30 min (Rapoport 1976; Ahrén et al. 1978). In spite of this the content of cAMP in the cells showed a decreasing tendency from 30 min of stimulation and onwards, principally due to the fact that the nucleotide left the cells (Ahrén et al. 1978). The mechanism for this cAMP release and its possible significance in the regulation of the intracellular cAMP level is not known.

The present experiments were designed to investigate whether the release of cAMP from thyroid tissue takes place also under more physiological conditions, and to study some factors of significance for its regulation.

Materials and Methods

Female mice of the NMRI strain, weighing 15—25 g, were used. In each experiment 8 animals were killed by neck elongation and the thyroid lobes were dissected out. They were distributed into two sets of 4 tubes each in a randomized manner so that each tube in the one set had a corresponding tube in the other with regard to animal material. When the effect of drugs or other changes in the experimental conditions were investigated one of the tube sets was used as control. The tubes contained 1 ml of Krebs Ringer bicarbonate buffer with 10 mm glucose, bubbled with carbogen (95% oxygen plus 5% carbon dioxide). This buffer was used for pre-incubation and incubation as well. To obtain depolarizing medium the sodium in the Krebs Ringer bicarbonate buffer was substituted by potassium.

The standard pre-incubation time was 30 min. The incubation medium was always supplied with 1 mm theophylline unless otherwise stated.

The incubation was terminated by replacing the incubation medium by ice-cold 10% TCA in which the tissue was immediately homogenized. The purification of the
homogenate prior to cAMP assay was made by chromatography on a Dowex 50 column followed by lyophilization according to Birnbaumer et al. (1976) with slight modifications (Nilsson et al. 1981).

Cyclic AMP was determined by the protein binding method of Gilman (1970) with modifications developed by Tovey et al. (1974) using charcoal for separation of bound from free cAMP. The protein content of each tube was determined according to Lowry et al. (1951), and cAMP values in tissue and medium as well are given as pmole cAMP per mg tissue protein. Bovine TSH was a gift from Ferring AB, Malmö, Sweden. [3H]cAMP was purchased from NEN, and cAMP-depending protein kinase and drugs from Sigma Chemical Company.

Results

Fig. 1 shows the total accumulation of cAMP (sum of intra- and extracellular cAMP) as well as the tissue fraction after stimulation with TSH, 5 mU/ml, for 2 h. As can be seen the major part of the TSH-stimulated cAMP accumulation took place during the first 25 min of incubation. The contribution to the total amount of cAMP in the system then occurred at a fairly constant but comparatively low rate. During the first 25 min the total amount of cAMP increased from the control level of 33.4 ± 8.0 pmole/mg protein (SEM) to 360.6 ± 44.8 pmole/mg protein, and during the following 35 min the increase continued to 460.7 ± 43.0 pmole/mg protein. During the second hour of incubation the total amount of cAMP increased further to 499.2 ± 38.7 pmole/mg protein which is significantly higher than the 25 min value (P < 0.05) but not significantly different from the 60 min value.

Fig. 2.
Content of cAMP in the incubation medium after the addition of TSH, 5 mU/ml, at 0 min. The bars indicate SEM. Total number of observations: 120. The interrupted line indicates controls (no TSH given).
The release of cAMP from the tissue with time is shown in Fig. 2. The rate of the release seemed to be almost constant during the observation period of 2 h. After 6 h of incubation only 10 ± 4% of the nucleotide was intracellular, and in spite of the continuous accumulation of total cAMP shown in Fig. 1 it is evident from the same figure that the cAMP concentration in the tissue was continuously decreasing from 25 min and onwards, the 2 h value of 200.3 ± 23.7 pmole/mg protein being significantly lower ($P < 0.05$) compared to the 25 min value of 315.8 ± 40.6 pmole/mg protein.

In a series of experiments where only the medium cAMP was measured it was found that the cAMP concentration in the incubation medium was related to the concentration of TSH in the range 0.1—5 mU/ml. The log concentration-response curve was rectilinear for observation times 15—60 min (Fig. 3). For each TSH concentration level the medium cAMP concentration increased almost rectilinearly with time. Addition of TSH after 4 h of pre-incubation induced a total cAMP accumulation response that was not significantly different from that observed after 30 min of pre-incubation. This was true for all times of stimulation with TSH, 5 mU/ml, up to 2 h. The distribution of cAMP between intracellular and extracellular spaces was not significantly different after 4 h of pre-incubation compared to 30 min of pre-incubation when followed for 2 h in both instances.

In five experiments the cAMP response to 5 mU/ml of TSH was registered 0, 25, 60 and
Total (sum of tissue and medium) cAMP accumulation (upper curves) in response to TSH, 5 mU/ml, added at 0 min without (left panel) and with (right panel) puromycin, 500 μg/ml. The curves over the hatched areas delineate the tissue cAMP content. The bars indicate SEM. Total number of observations: 78.

120 min after hormone administration with and without 1 mM theophylline, respectively. As might be expected the cAMP response to TSH was lower \( (P < 0.01) \) without theophylline. However, the patterns of the curves were similar whether theophylline was present or not \( (P < 0.01) \), with a maximum value in the tissue after 25 min of incubation with TSH, and thereafter a decreasing tendency, while the medium cAMP concentration showed a continuous increase with time \( (P < 0.01) \).

Puromycin, 500 μg/ml, caused an increased total accumulation of cAMP in response to TSH over an observation period of 2 h as reported elsewhere \( (\text{Nilsson et al. 1981}) \). It was associated with the retention of a significantly \( (P < 0.05) \) higher proportion of the nucleotide within the cells \( (\text{Fig. 4}) \). On the other hand, cycloheximide, 5 μg/ml, and actinomycin D, 1 μg/ml, that increased the total accumulation of cAMP in response to TSH to about the same extent as did puromycin \( (\text{Nilsson et al. 1981}) \) had no effect on the distribution of cAMP between intra- and extracellular compartments, respectively.

Diprydiamole, 10 μM, increased the tissue cAMP response to 5 mU TSH/ml \( (P < 0.025) \) and the proportion of the nucleotide retained intracellularly as well \( (P < 0.01) \).

Addition of ouabain, 1 mM, slightly reduced the total cAMP response to TSH 5 mU/ml but did not significantly affect the distribution of cAMP between intra- and extracellular compartments.

The exchange of the standard incubation medium to depolarizing medium did not significantly change the total cAMP production in response to TSH, 5 mU/ml, nor its distribution between tissue and medium.

Discussion

Apparently the membrane of the thyroid cell is relatively impermeable to cAMP. In an in vitro system like ours the intracellular concentration of cAMP during TSH stimulation is mostly more than 1000 times that of the incubation medium. Cyclic AMP is believed not to be confined to any cell organelles but seems to reside in the cytoplasm \( (\text{Whitley et al. 1975}) \). Therefore the cell membrane should be the structure that maintains the concentration gradient. It is well known that the thyroid cell can be fragmented in several ways, but the adenylyl cyclase can still operate to a certain extent as evident from the synthesis of cAMP in broken cell preparations \( (\text{Yamashita & Field 1970}) \). Thus a possible explanation for the continuous release of cAMP from TSH stimulated cells in vitro with time might be that the cell membrane deteriorates faster than the adenylyl cyclase. However, we found the same production of cAMP and the same pattern of distribution between intra- and extracellular cAMP when the pre-incubation time was extended to 4 h instead of the usual 30 min. This indicates that the...
cell membrane retains its competence to inhibit the outflow tendency of cAMP caused by the concentration gradient to a considerable degree during the 2 h that most of our experiments cover. The fact that a continuous release of cAMP took place also from tissue stimulated by TSH without the presence of theophylline suggests that the release is not confined to the unphysiologically high cAMP levels obtained in the presence of methylxanthines but may be of significance also under physiological conditions. The results of the experiments without theophylline and those with long pre-incubation thus indicate that cAMP release is not an artefact caused by the experimental conditions but should be a factor of significance for the intracellular cAMP level under physiological conditions.

During the later part of a TSH stimulation there seems to be a reduced activity of adenyl cyclase. Part of this reduction seems to depend on new protein synthesis (Nilsson et al. 1981), and such a mechanism might add to the reduction in cAMP accumulation by increasing its outflow. If this be the case, inhibitors of protein synthesis should be expected to cause an increased total cAMP accumulation and an increased retention of the nucleotide intracellularly as well. This proved to be the case for puromycin while cycloheximide and actinomycin D affected the total cAMP accumulation in the expected way but lacked effect on the distribution between the intra- and extracellular compartments, respectively. Thus inhibition of protein synthesis seems to augment the TSH-induced synthesis of cAMP but not change its distribution between tissue and medium. It is not known why puromycin differed from the other inhibitors of protein synthesis in increasing also the proportion of cAMP retained intracellularly but it is possible that puromycin may also influence mechanisms other than protein synthesis. Its effect was similar to that of dipyridamole, a substance known to inhibit the transport of nucleotides like adenosine into cells (Hopkins & Goldie 1971), but also able to inhibit phosphodiesterase (Hamilton 1972). The mechanism of action of puromycin thus remains unclear but the increased intracellular cAMP level combined with a decreased release that was induced by the drug (Fig. 5) suggests that the cAMP release may not be merely a passive leakage governed by the intracellular cAMP level but a process that may be influenced selectively.

Attempts to more directly interfere with different functions of the cell membrane like depolarization or disturbing the Na-K pump by ouabain did not affect the release of cAMP.

We found the release of cAMP in response to TSH to increase almost rectilinearly with time up to 2 h with a TSH concentration of 5 mU/ml (Fig. 2). The same proved to be true also with 0.1 and 0.5 mU/ml followed for 60 min. In agreement with results from human thyroid tissue in vitro (Bidey et al. 1980) there was an increasing release of cAMP with increasing concentrations of TSH (Fig. 3). This pattern suggests that the cAMP release is not related to the activity of adenyl cyclase that different from the cAMP release seems to be low after the first 30 min of stimulation by TSH (Rapoport 1976; Ahrén et al. 1978). Instead, the cAMP release from thyroid tissue seems to be related to the actual concentration of TSH. We do not know whether this effect of TSH is a direct one or mediated via the mobilisation of iodothyronines, or mediated via intracellular cAMP. Nor do we know whether cAMP release is attained by interference with a mechanism that actively keeps cAMP within the cell, or by inducing an active secretion of cAMP from the thyroid cell. The latter possibility may, however, gain support by the finding that cAMP can be released against a concentration gradient in other cell systems (Davoren & Sutherland 1963; Clark et al. 1975).

References


