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# Forskolin stimulation of thyroid secretion of $T_4$ and $T_3$

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Forskolin is a potent activator of adenylate cyclase in many tissues including the thyroid gland. We compared the effects of $10^{-5}$ M forskolin and 100 $\mu$units/ml TSH on the dynamics of $T_4$ and $T_3$ secretion from perfused dog thyroid lobes. Both agents induced pronounced increases in $T_4$ and $T_3$ secretion. The increase in secretion was significantly steeper during forskolin than during TSH stimulation. This may suggest that early processes such as TSH–receptor interaction and the subsequent activation of the catalytic unit of adenylate cyclase are of importance for the pattern of very gradual increase in hormone secretion during TSH stimulation of the thyroid. In other respects forskolin seems to induce absolute and relative secretion of $T_3$ and $T_4$ very similar to those obtained by cAMP and TSH.

*Forskolin* *Thyroid secretion* *Thyroxine* *Triiodothyronine* *cAMP* *Thyroid perfusion*

## 1. INTRODUCTION

Forskolin is a potent activator of the adenylate cyclase in many tissues [1,2] including the thyroid gland [3–6].

Here we have compared the effects of $10^{-5}$ M forskolin and 100 $\mu$units/ml TSH on the dynamics of $T_4$ and $T_3$ secretion from perfused dog thyroid lobes. One hundred $\mu$units/ml TSH induce a maximal $T_4$ and $T_3$ secretion in our preparation [7] and $10^{-5}$ M forskolin is known to induce rapid and profound activation of the thyroidal adenylate cyclase [3–6]. This was done to evaluate the degree to which processes before cAMP are involved in the delayed and sluggish thyroid hormone response to TSH. To ascertain that forskolin actually activated the cAMP system in our model we measured cAMP in thyroid effluent. cAMP in effluent is a good indicator of thyroidal cAMP [8,9].

## 2. MATERIALS AND METHODS

Once-through thyroid perfusions were performed in four mongrel dogs weighing 20–27 kg. In each dog both of the two separate lobes were perfused simultaneously and independently using a buffer medium. The method has been described in [7]. Forskolin was infused in the left lobe in two experiments and in the right lobe in two experiments. The perfusion flow was 0.63 ml/min per lobe ~0.5–1.0 ml/min per g tissue. The perfusion pressure was constant in every perfusion and in the range 30–40 mm Hg. Forskolin or TSH were infused (13.5 $\mu$l/min) in the afferent catheters via a canula penetrating a rubber membrane giving the final concentrations indicated [10]. Forskolin was dissolved in ethanol, and diluted in perfusion medium. The final ethanol concentration in the medium during forskolin infusion was 0.2%. To the TSH containing medium was added a similar amount of ethanol. The tubes sampling 5 min effluents contained EDTA to give a final concentration of 40 mM. Samples were kept at −20°C until analysis.

$T_4$ and $T_3$ were measured by radioimmunoassays [11,12]; cAMP was measured as in [13]. In [9] it was demonstrated that cAMP was stable in the effluent and that the total cAMP activity disappeared after incubation with phosphodiesterase. The sensitivity of the cAMP assay was 0.4 nmol/l and inter- and intra-assay coefficients of variations

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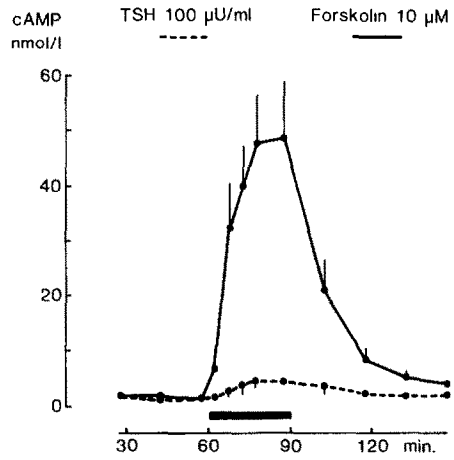


Fig.1. cAMP in effluent from perfused dog thyroid lobes. One of the two lobes in a dog received $10^{-5}$ M forskolin during the 60–90 min period of perfusion, while the other lobe received 100 μunits/ml TSH during the same period. Mean ± SE, $n = 4$.

9.2 and 6.0%, respectively. TSH and forskolin did not interfere in any of the assays.

Bovine TSH was from Ferring (Malmö, Sweden) and forskolin from Calbiochem (La Jolla, CA).

Paired *t*-tests were employed for statistical evaluation using a 5% limit of statistical significance.

## 3. RESULTS

Fig.1 shows the effect of $10^{-5}$ M forskolin and 100 μunits/ml TSH on the concentration of cAMP in effluent from perfused thyroid lobes. Both agents induced increases in cAMP which were high and nearly stable during the last 10 min of the 30-min infusion period. Cessation of infusion of the stimulant was followed by a gradual decrease in cAMP release. The response to $10^{-5}$ M forskolin was much higher than that to 100 μunits/ml TSH at all points.

The increase in cAMP was accompanied after approximately 20 min by increases in $T_4$ and $T_3$ secretion (fig.2). The initial phase of the hormone secretion was significantly steeper after stimulation with forskolin than after TSH. However, the response to forskolin began to decrease earlier after cessation of stimulation than that to TSH.

During forskolin infusion, the initial increase in $T_3$ secretion was slightly steeper than that in $T_4$

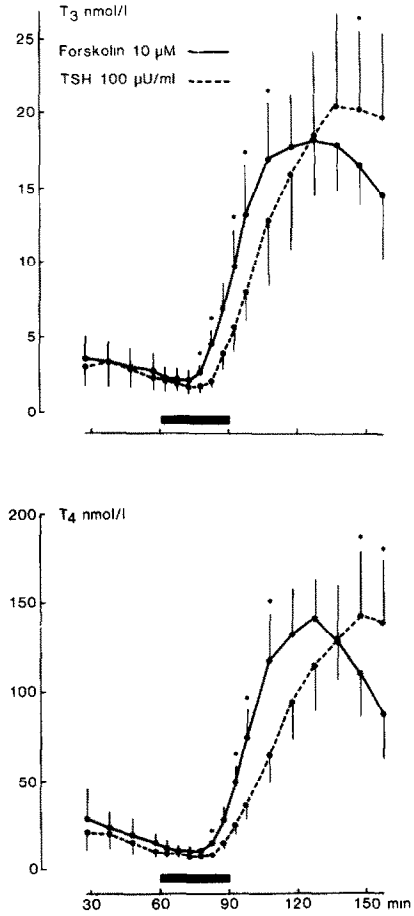


Fig.2. $T_4$ and $T_3$ in effluent from perfused thyroid lobes stimulated with $10^{-5}$ M forskolin or 100 μunits/ml TSH during the 60–90 min period. * $p < 0.05$, comparing values from the two lobes. Mean ± SE, $n = 4$.

secretion. This is most easily depicted by the ratio $T_4/T_3$ in effluent showing a temporary fall during the early period of increase in secretion (fig.3).

## 4. DISCUSSION

Forskolin induced a considerable increase in cAMP which began already in the first 5-min sample after the start of forskolin infusion. A considerably smaller increase in cAMP was seen during infusion of TSH. There was a lag period from the increase in cAMP until an increase was obtained in $T_4$ and $T_3$ secretion both during forskolin and TSH infusion. However, the increase in hormone secretion was steeper after forskolin than

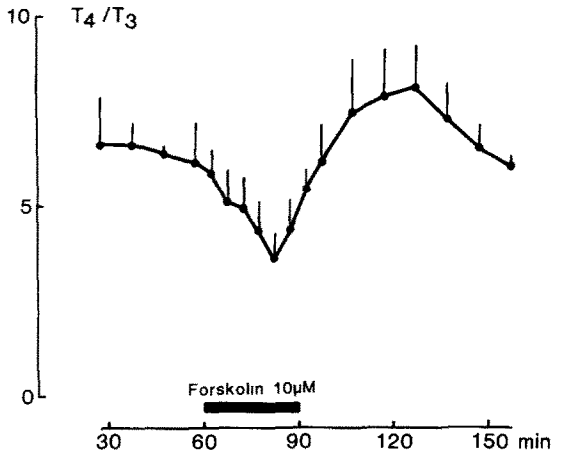


Fig.3. $T_4/T_3$ ratio in effluent from perfused thyroid lobes stimulated with $10^{-5}$ M forskolin during the 60–90 min period. Average values from the 75–90 min period were significantly lower than average values from the 35–60 min period ($p < 0.05$). Mean ± SE, $n = 4$.

after TSH. This difference is not due to the more profound activation of the cAMP system by $10^{-5}$ M forskolin than by 100 μunits/ml TSH. If higher doses of TSH which induce larger increases in cAMP [9], are employed the increase in $T_4$ and $T_3$ secretion becomes less steep than after 100 μunits/ml [9,14]. Hence 100 μunits/ml TSH induce the fastest rate of increase obtainable with TSH.

This difference in effects may suggest that the early processes involved in the TSH induced increase in thyroid hormone secretion such as TSH/receptor interaction and the subsequent activation of the catalytic unit of the adenylate cyclase are relatively time-consuming. Forskolin activates cAMP generation more directly by interaction with the regulatory protein or the catalytic unit of the adenylate cyclase [3–6]. Hence these early processes may be of significant importance for the pattern of a slow increase in thyroid hormone secretion after TSH stimulation. Both TSH and forskolin are administered via the vascular bed but still there might be differences in the diffusion time to reach the cells. Considering that even extremely high concentrations of TSH do not make the increase in hormone release more steep, such a difference in diffusion time is probably of little importance. Another possibility is that TSH, besides activating the secretory processes, also activates a non-cAMP dependent mechanism which tends to give a slower and lower increase in hormone secretion. The presence of such a mechanism is suggested by our previous studies showing that the secretory response is dampened during infusion of very high concentrations of TSH despite increasing cAMP formation [9,14]. Such a mechanism could be operative already at low TSH concentrations, albeit to a lesser degree.

In [6], a study on the effects of forskolin on various intrathyroidal processes known to be stimulated by TSH, it was concluded that forskolin could not be used to reproduce other intracellular effects of TSH than the activation of the cAMP system. The present study demonstrates that forskolin, except for some minor differences, one of which is commented upon above, reproduces the effect of TSH on the process sequence leading to thyroid hormone secretion very closely. In this context, forskolin gave a similar temporary fall in the $T_4/T_3$ ratio in thyroid secretion as found previously when cAMP [15] or TSH were used as stimulants [14,16].

The results are in accordance with the concept that most, but not necessarily all effects of TSH on thyroid hormone secretion in the dog [17] are mediated via the cAMP system.

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