Forskolin stimulates adenylate cyclase and idodine metabolism in thyroid

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Forskolin is a potent activator of the cyclic AMP-generating system in many tissues. In dog thyroid slices, the enhancement of cyclic AMP level was rapid, sustained in the presence of forskolin, but easily reversible after its withdrawal. Contrary to TSH, forskolin induced little apparent desensitization. Forskolin potentiated the effects of TSH, PGE_1 and cholera toxin. However, the forskolin-induced cyclic AMP accumulation was still sensitive to inhibitors of dog thyroid adenylate cyclase such as iodide, norepinephrine and adenosine. As fluoride, but contrary to TSH and PGE_1, forskolin stimulated adenylate cyclase in a medium where Mg^{2+} was replaced by Mn^{2+}. This suggests that in thyroid, as in other tissues, forskolin acts beyond the receptor level but, as it potentiates hormone action and does not impair modulation by inhibitors, it may interact with the nucleotide-binding regulatory proteins. Forskolin mimicked the effect of TSH on iodide organification and secretion.

Forskolin *Dog thyroid* *Cyclic AMP* *Adenylate cyclase* *Iodine metabolism*

1. INTRODUCTION

Forskolin is an extremely powerful activator of the cyclic AMP-generating system in both intact cells and plasma membranes [1]. These studies were designed to determine whether forskolin activates adenylate cyclase in dog thyroid and consequently reproduces the effect of TSH ascribed to intracellular cyclic AMP rise.

2. MATERIALS AND METHODS

2.1. *Slices incubation*

Thyroid slices were prepared with a Stadie Riggs microtome (Arthur Thomas, Philadelphia, PA) from thyroids of dog pretreated with thyroid extract daily for 1–3 days (100 mg/10 kg, Thyranon, Organon, Oss). Within 30 min after resectioning, the slices were incubated at 37°C in Krebs-Ringer bicarbonate buffer (pH 7.4), saturated with 95% O_2–5% CO_2 and enriched with 8 mM glucose and 0.5 mg bovine serum albumin/ml. The slices were always preincubated 60 min before the test incubation in fresh medium.

2.2. *Cyclic AMP assay*

At the end of the incubation, the slices were dropped in 1.5 ml of boiling deionized water for 5 min. Cyclic AMP was measured as in [2,3].

2.3. *Iodine metabolism*

To measure thyroid hormone secretion, dogs were injected with 150 μCi ^{131}I before the administration of thyroid extract and the incubation medium supplemented with $NaClO_4$ (1 mM) and methimazole (2 mM). Secretion was estimated by the ratio between the butanol-extractable radioactivity in the medium at the end of the incubation, and the total radioactivity of the slices before the incubation (% BEI) [4].

Abbreviations: TSH, thyroid stimulating hormone; PGE_1, prostaglandin E_1; cyclic AMP, 3′,5′-adenosine monophosphate

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For the measurement of iodide incorporation into iodoproteins, slices were incubated for 45 min in medium supplemented with KI (40 μM) and ^{131}I (0.5 μCi/ml), and homogenized in a methimazole solution (2 mM). The proteins were precipitated with 5% trichloroacetic acid [5]. Results were expressed as the ratio between trichloroacetic acid-precipitable radioactivity and the total radioactivity in the slices.

2.4. *Membrane preparation*

The thyroids were sliced, chopped and homogenized at 4°C in a Dounce tissue grinder, containing 50 mM Tris–HCl buffer with sucrose 250 mM, EDTA 1 mM and EGTA 1 mM. The homogenate was centrifuged 5 min at 500 × g and the pellet discarded. A 10000 × g pellet obtained after 20 min centrifugation was washed once and then resuspended in a 50 mM Tris–HCl (pH 7.4) buffer at 0.5–1.5 mg protein/ml and assayed immediately.

2.5. *Adenylate cyclase assay*

Adenylate cyclase assay was performed as in [6]. The membranes were incubated 10 min at 30°C in the following medium: 50 mM Tris–HCl buffer (pH 7.4); NaCl 30 mM; phosphocreatine (disodium salt) 5 mM; creatine kinase 10 U/ml; $MgCl_2$ 5 mM; ATP 0.1 mM; EGTA 0.1 mM; GTP 0.1 mM; isobutylmethylxanthine 0.8 mM; sucrose 250 mM; bovine serum albumin 0.04% and 1–2 × 10^6 cpm [α-^{32}P]ATP. Medium (50 μl) was pre-warmed 5 min before the addition of 50 μl membrane preparation. Under the conditions used cyclic AMP generation was linear both with time up to 20 min and with up to 120 μg protein/tube.

Forskolin was dissolved in ethanol and used with the appropriate controls. Protein concentration was estimated by the adapted method of Lowry [7] using bovine serum albumin as standard. Results are expressed as mean ± SEM of triplicate sets of slices in one typical experiment.

2.6. *Materials*

TSH as thytropar was obtained from Armour Pharmaceuticals (Phoenix AZ), forskolin from Hoechst Pharmaceuticals (Bombay), norepinephrine from Calbiochem (Lucerne), cholera toxin (Dr R.A. Finkelstein preparation) from Schwarzman (Becton Dickinson, Orangeburg NY) and PGE_1 from Upjohn Company (Kalamazoo MI). The phosphodiesterase inhibitor RO20-1724 was a gift from Hoffman-La Roche (Nutley NY). Radioactive materials were provided by the Radiochemical Centre (Amersham). All of the other reagents were of the highest purity commercially available.

3. RESULTS AND DISCUSSION

3.1. *Characterization of forskolin action on cyclic AMP level in dog thyroid slices*

Forskolin rapidly and markedly enhanced cyclic AMP accumulation. The effect was already significant (50%) at 25 nM. At 10 μM the effect was marked (500%) at 3 min and maximal at 30 min (1200%). The effect of forskolin is easily reversible, contrary to TSH action (table 1).

Table 1

Effect of washing on forskolin or TSH-induced cyclic AMP accumulation

	Washing time (min)					
	0	2	5	10	30	60
A	100	79		59		36
B	100	52	29	17	5.5	5.1

After 1 h incubation in the presence of TSH (1 mU/ml) (A) or forskolin (10 μM) (B) and RO20-1724 (0.1 mM) as phosphodiesterase inhibitor, the slices were dropped successively in 2 vials of 100 ml fresh medium and then reincubated for 2–60 min in fresh medium without any addition to follow cyclic AMP disappearance in the slices. The results are expressed as percent of the value obtained before the washing–basal value: 92 ± 25 pmol/100 mg wet wt tissue; TSH stimulated tissue, 1325 ± 92; forskolin-stimulated tissue, 1891 ± 110

Thyroid slices are sensitive to TSH desensitization. Indeed a second TSH challenge increased the cyclic AMP level to only 40–50% of the level reached in control slices stimulated for the first time [8,9]. Under the same conditions a second forskolin challenge enhanced cyclic AMP levels almost to the same levels as in control slices (80–90%) (fig. 1).

The characteristics of the forskolin effect on cyclic AMP accumulation in thyroid are thus

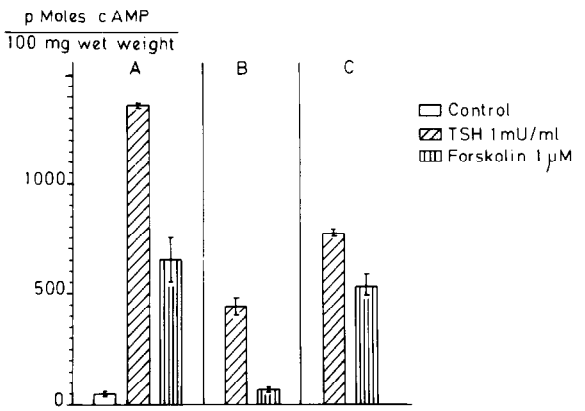
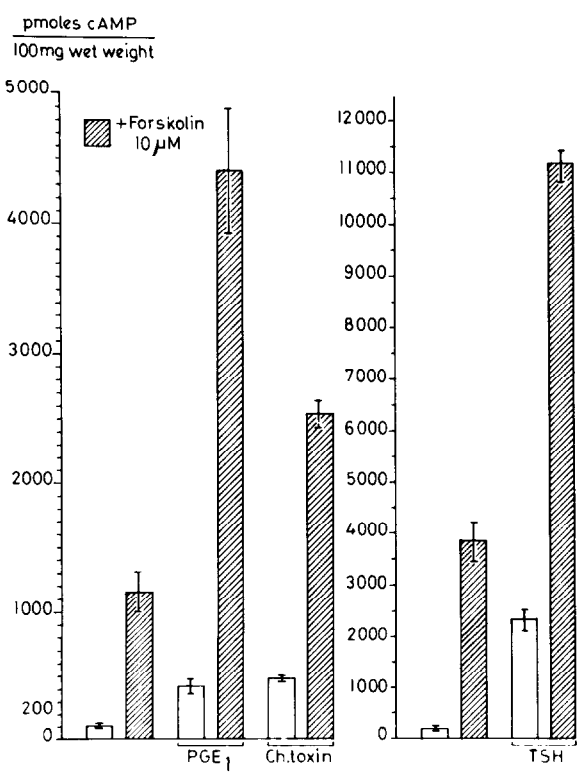


Fig. 1. TSH- or forskolin-induced desensitization. The experimental protocol involved 3 consecutive incubations (I) 2 h ± the stimulators and caffeine 10 mM; (II) 1 h in medium with no addition; (III) 21 min ± stimulator and caffeine 10 mM. A rinse in 100 ml medium at 37°C was performed between incubations I–II, and II–III: (A) stimulation in incubation III only = one challenge; (B) stimulation in incubation I only and cyclic AMP measured at the end of incubation III = residual cyclic AMP; (C) stimulation in incubation I and restimulation in incubation III = two challenges. Results are expressed as pmol cyclic AMP ± SEM/100 mg wet wt tissue.



similar to those reported for brain [10]. Its action is rapid and steady for several hours. It is already active at 10^{-7} M and is easily washed off. There is little apparent desensitization. This will allow one to test the effects of pulses of increased cyclic AMP levels.

The effects of TSH, PGE_1 and cholera toxin on cyclic AMP accumulation are greatly potentiated by forskolin (fig. 2). The response to TSH was increased by forskolin even at maximal concentration of the hormone, but the tissue sensitivity to TSH was not increased (fig. 3). Forskolin-induced cyclic AMP accumulation was inhibited by iodide, norepinephrine and adenosine, known as adenylate cyclase inhibitors in dog thyroid (fig. 4) [11–13]. The fact that forskolin potentiates the effects of stimulators such as TSH and PGE_1 on cyclic AMP

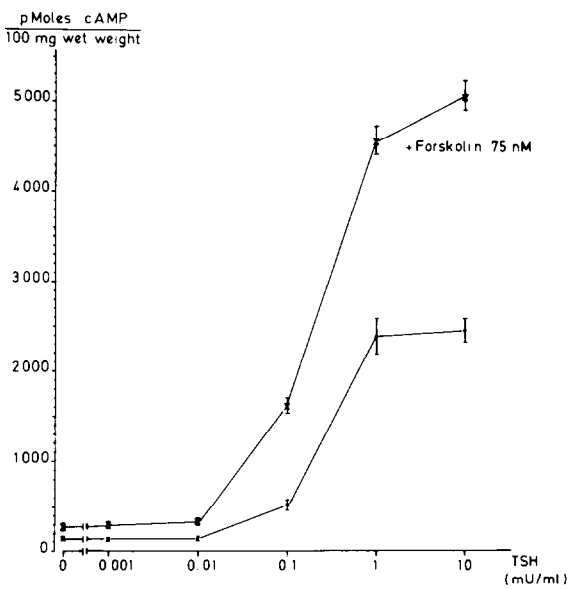


Fig. 3. Effect of forskolin on the cyclic AMP response to increasing [TSH]. The test incubation in the presence of forskolin (75 nM), TSH, and RO20-1724 (0.1 mM) as phosphodiesterase inhibitor lasted 30 min.

Fig. 2. Effect of forskolin on known stimulators of cyclic AMP accumulation in dog thyroid. The test incubation in the presence of the agent and a phosphodiesterase inhibitor lasted 30 min. Cholera toxin (ch. Toxin) was already present in the 60 min preincubation: PGE_1, 2.8 μM; ch. toxin, 1 μg/ml; TSH, 1 mU/ml.

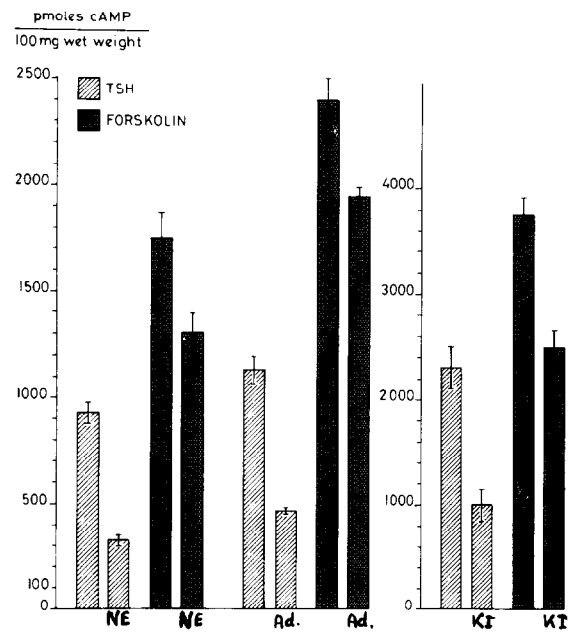


Fig. 4. Effect of known inhibitors of the TSH stimulation on forskolin stimulation of cyclic AMP accumulation in dog thyroid slices. The test incubation in the presence of RO20-1724 (0.1 mM), forskolin (10 μM), TSH (1 mU/ml), and the inhibitors lasted 30 min. KI was already present in the 1 h preincubation: NE, norepinephrine 10 μM; Ad., adenosine 200 μM; KI, potassium iodide 100 μM. The basal value in the experiments presented are 73–120 pmol cyclic AMP/100 mg wet wt tissue.

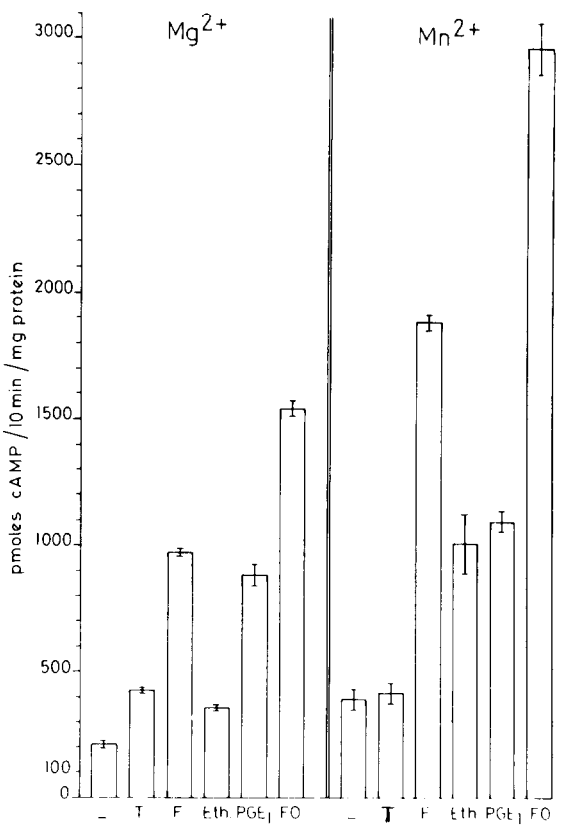


Fig. 5. Effect of Mn^{2+} on thyroid adenylate cyclase activation. Forskolin and PGE_1 were dissolved in ethanol (5% final conc.). The reaction was started with 35 μg membrane protein added/tube: T, TSH 10 mU/ml; F, fluoride 10 mM; Eth., ethanol 5%; PGE_1, prostaglandin E_1 56 μM; FO, forskolin 10 μM; Mg^{2+} and Mn^{2+}, 5 mM.

accumulation and that the forskolin-enhanced cyclic AMP levels are still submitted to the different negative controls operating at various levels of the cyclase system suggest that forskolin-activated cyclase is still coupled to its stimulatory and/or inhibitory regulatory proteins.

3.2. *Effects on adenylate cyclase activity*

Forskolin stimulates adenylate cyclase activity with app. K_a from 1–10 μM. The activation was already observed at 2 min, the shortest time studied, and was constant for up to 20 min (not shown). These properties are compatible with the effect on cyclic AMP levels in intact cells.

The replacement of Mg^{2+} by Mn^{2+} in the cyclase incubation medium uncouples receptor from cyclase in rat erythrocytes [14]. In this study we show a similar uncoupling of TSH and PGE_1 action on thyroid cyclase. The replacement of Mg^{2+} (5 mM) by Mn^{2+} (5 mM) in the incubation mixture enhanced the basal adenylate cyclase activity but blocked the TSH (10 mU/ml) and PGE_1 (28 μM) response. On the contrary, the forskolin (10 μM) and fluoride (10 mM) stimulations were preserved in the same experiment (fig. 5). Thus under such conditions, forskolin and fluoride, which acts on G/F, still stimulate the cyclase. This suggests that in thyroid, as in other tissues, this drug acts beyond the receptor level but, as it potentiates hormone action and does not impair the modulation by inhibitors, it may interract with the nucleotide binding regulatory proteins as proposed in platelets [15].

Table 2

Effect of forskolin on iodine metabolism (see section 2)

–	Forskolin (1 μM)	Parameter
4.69 ± 0.9	9.34 ± 1	% PB ^{131}I[a]
1.07 ± 0.05	3.1 ± 0.16	% BE ^{131}I[b]

[a] % PB ^{131}I = evaluation of iodide organification; i.e., hormone synthesis

[b] % BE ^{131}I = evaluation of hormone secretion

3.3. *Effect on iodine metabolism*

TSH stimulates iodide organification and thyroid hormone secretion. It has been proposed that the reproduction of a hormonal effect by forskolin could constitute a new criterion of the validity of Sutherland's model in a given system [10]. As shown in table 2 for iodide organification and secretion, forskolin mimicks the effects of TSH. These data therefore further confirm that these effects of TSH are mediated by cyclic AMP [16].

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