SPECIFIC INHIBITION BY Na^+ OF TSH-STIMULATED THYROID CELL ADENYLATE CYCLASE

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1. Introduction

Thyrotropin (TSH), isoproterenol and prostaglandin E_2 (PGE_2) increase acutely intracellular cAMP levels in cultured porcine thyroid cells [1,2] whereas muscarinic agonists decrease this stimulation [3].

Responsiveness to the various stimulators can be modified by culture conditions and by the chronic treatment by the agonists themselves [2,4].

In membrane preparations derived from these cells, TSH, isoproterenol and PGE_2 stimulate adenylate cyclase activity [5]. This stimulation is increased (2–3-fold) in the presence of GTP (0.1 mM). The negative effect of carbachol can also be observed in cell-free system provided GTP (0.1 mM) and Na^+ (70 mM) are present in the assay mixture [6].

Aside from its effect on the negative modulation of adenylate cyclase by α-adrenergic agonists, carbachol and opiates [7,9,10,14,15], Na^+ appears to have complex effects on the activity of adenylate cyclase [8–10]. We report here that Na^+ at low concentrations (0–40 mM) is a potent inhibitor of TSH-stimulated adenylate cyclase activity of membrane preparations derived from cultured thyroid cells. In contrast no effect of Na^+ was observed on isoproterenol or PGE_2-stimulated activities.

2. Materials and methods

2.1. *Cell culture and membrane preparation*

Thyroid epithelial cells were obtained from porcine thyroid glands by a discontinuous trypsin–EGTA treatment [11]. Freshly isolated cells were suspended at 2×10^6 cells/ml in Eagle minimum essential medium supplemented with newborn calf serum (10%, v/v), gelatin (0.25%) and TSH (0.1 mU/ml) unless otherwise mentioned. Cells were incubated for 4 days as unstirred suspension in untreated polystyrene dishes (10 ml cell suspension/100 mm dishes) in 95% air/5% CO_2 water-saturated atmosphere at 37°C. Under these conditions cells reorganized into follicles and displayed maximal responsiveness to acute TSH stimulation [1,11].

At the end of the incubation period cells were collected into a centrifuge tube, diluted with 2 vol. Earle solution buffered with 20 mM Hepes at pH 7.2 (Earle–Hepes) and washed twice ($250 \times g$ for 7 min) in Earle–Hepes. Homogenization was performed in Tris–HCl (10 mM, pH 7.0 at 4°C, 250 μl/dish), with 30 strokes of a hand-driven Teflon Potter homogenizer. The homogenate was rapidly frozen and stored in liquid nitrogen. Before adenylate cyclase assay the homogenate was thawed and centrifuged ($10^4 \times g$ for 10 min). The particulate fraction was washed once with Tris–HCl buffer (washed membrane fraction).

2.2. *Adenylate cyclase assay*

Assays were performed at 30°C in 50 μl final vol. and contained 50 μg protein of washed membrane preparation with 4.5 mM $MgCl_2$, 0.2 mM ATP, 0.6–0.8 μCi[α-^{32}P]ATP, 0.5 mM EGTA, 0.1 mM (3-isobutyl-1-methyl xanthine (IBMX)), 0.1 mM GTP and 0.1% bovine serum albumin in 20 mM Tris–HCl (pH 7.8) [5]. When the ATP regenerating system was omitted to obtain low Na^+ and low ionic strength, incubation was limited to 5 min. When creatine kinase (8 mU/ml) and creatine phosphate (Tris-salt form,

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15 mM) were present, 10 min incubations were used. Cyclic AMP produced was measured as in [12]. Results reported here are representative of ⩾3 expt. performed with differents membrane preparations. Values are given as a mean of duplicate determinations differing by <5%. Adenylate cyclase activity was reported with respect to the protein content of the corresponding homogenate.

2.3. *Materials*

Bovine TSH was supplied by Armour (Chicago IL), isoproterenol creatine phosphate (Tris-form) and bovine serum albumin by Sigma (St Louis MO), [α-^{32}P]-ATP (30 Ci/mmol) by New England Nuclear (Dreieich) and trypsin, new born calf serum and Eagle minimum essential medium by Grand Island Biological (Grand Island NY). Other biochemicals and nucleotides were from Boehringer (Mannheim). IBMX was from Aldrich (Milwaukee WI). Other chemicals were of the highest purity commercially available. Prostaglandin E_2 was a gift of Dr J. Pike (Upjohn Company MI).

3. Results

3.1. *Na^+ inhibition of TSH-stimulated adenylate cyclase activity*

When measured in the absence of an ATP-regenerating system and in the presence of GTP (0.1 mM), the TSH-stimulated adenylate cyclase activity of washed membrane fractions derived from cultured thyroid cells was strongly inhibited by NaCl (fig.1). This inhibition occurred over 0–50 mM and was not complete (60–70%).

Inhibition was Na^+ specific. Below 50 mM, Li, Cs and Rb were without effect whereas K^+ was stimulatory. Above 100 mM all monovalent cations tested decreased TSH-stimulated adenyl cyclase activity (fig.1).

In the condition of the assay adenosine was a poor inhibitor ($D_{0.5}$ = 1 mM) of both basal or TSH- and PGE_2-stimulated adenylate cyclase. This inhibition was independent of the presence of Na^+ (20 mM) in the medium (not shown). Morever the addition to the assay of an ATP-regenerating system (section 2), or myokinase (0.7 U/assay) or of adenosine deaminase (0.25 U/assay) did not impair the inhibitory effect of Na^+ (not shown). Therefore inhibition by Na^+ does not result from ATP depletion or from the accumulation of an ATP metabolite (ADP, AMP or adenosine).

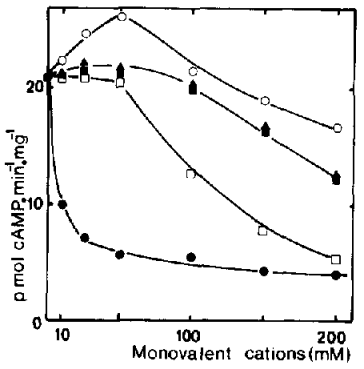

Fig.1. Effect of monovalent cations on TSH-stimulated adenylate cyclase activity. Incubation of washed membrane preparations derived from cultured thyroid cells was performed in the absence of an ATP generating system for 5 min at 30°C. GTP (0.1 mM), TSH (2.5 mU/ml) and monovalent cations in the chloride form were added simultaneously at the onset of incubation: NaCl (•); LiCl (□); KCl (○); CsCl (■); RbCl (▲).

The TSH dose–response curve was not modified by the presence of 10 mM Na^+ (fig.2). Only maximal stimulation was decreased. At higher concentration (40 mM) Na^+ increased the TSH concentration required for half-maximal stimulation (0.3–2 mU/ml).

The inhibition induced by low Na^+ concentrations occurred mainly when GTP was present together with TSH (fig.3).

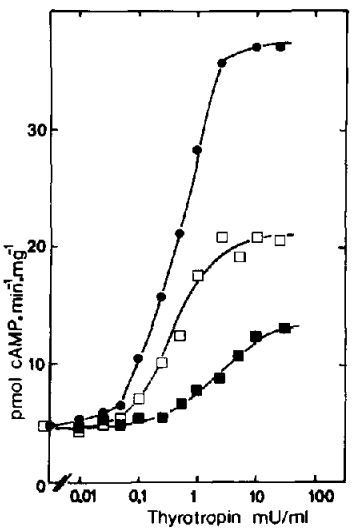

Fig.2. Effect of Na^+ on TSH dose–response curves. Incubation was performed as in fig.1. Na^+ and TSH were added simultaneously at the onset of incubation. Na^+ concentration: none (•); 10 mM (□); 40 mM (■).

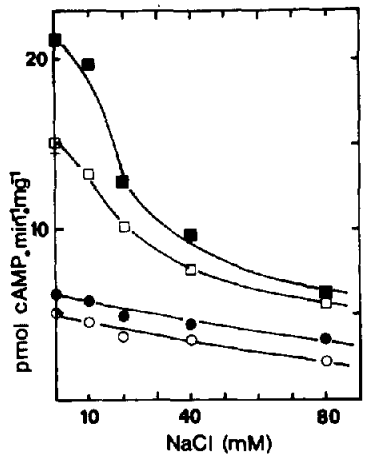

Fig.3. Effect of GTP on Na^+-dependent inhibition of TSH-stimulated adenylate cyclase activity. Washed membrane fractions were incubated in the presence of TSH (2.5 mU/ml), various concentrations of GTP (none (○); 0.1 μM (●); 1 μM (□); 10 μM (■)) and graded concentrations of Na^+. The incubation medium contained an Na^+-free ATP regenerating system and incubation time was 10 min.

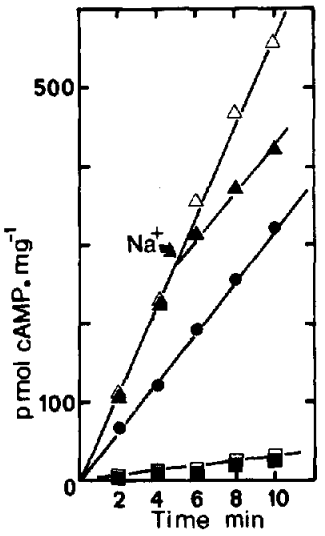

Fig.4. Effect of Na^+ on the rate of cAMP synthesis. Washed membrane fractions were incubated for given times in the presence of GTP (0.1 mM) and with (△,▲,●) or without (□,■) TSH (2.5 mU/ml). An Na^+-free ATP regenerating system was present. NaCl (40 mM) was added to the incubation mixture at zero time (△,●,□,■) or after 5 min incubation in Na^+-free medium (▲).

When GTP, Na^+ and TSH were present together from the onset of incubation the rate of cAMP synthesis was constant over 10 min. When Na^+ was added after a 5 min incubation in Na^+-free medium containing TSH and GTP an immediate decrease in the rate of cAMP synthesis was observed (fig.4).

If membranes were preincubated for 10 min in the presence of GTP (0.1 mM) and Na^+ (10, 20 or 40 mM) in the complete assay medium (labelled ATP omitted) and then washed twice in Tris–HCl (20 mM, pH 7.8) by centrifugation and resuspension, no permanent inhibition of subsequent stimulation by TSH in the absence of Na^+ was observed (not shown). The continuous presence of sodium was therefore required for inhibition.

3.2. *Effect of Na^+ on PGE_2 and isoproterenol stimulation*

We had shown that culture conditions modified the responsiveness of thyroid cell adenylate cyclase to TSH, PGE_2 and isoproterenol. High stimulation by isoproterenol was observed when cells were cultured in the absence of TSH [2,5]. We compared the effects of Na^+ on basal and stimulated adenylate cyclase activities of membrane preparations derived from thyroid cells cultured in the presence or absence of TSH (0.1 mU/ml). Na^+ inhibited TSH-stimulated activities in both membrane preparations with the same $D_{0.5}$ (20 mM) (fig.5). In contrast no effect of Na^+ was observed on basal and on PGE_2- or isoproterenol-stimulated cAMP synthesis.

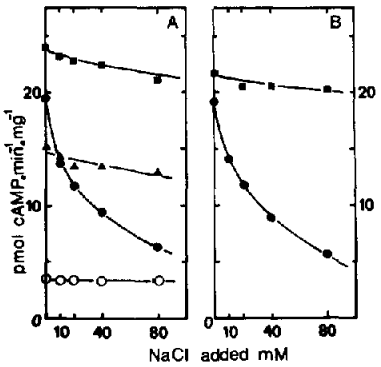

Fig.5. Effect of Na^+ on basal and TSH, PGE_2 or isoproterenol stimulated adenylate cyclase activities. Washed membrane fractions derived from thyroid cells cultured in the absence (A) or in the presence of 0.1 mU/ml TSH (B) were incubated for 10 min in a complete assay mixture containing graded concentrations of Na^+. Basal level (○) was identical for both membrane preprations. cAMP synthesis was stimulated with: TSH, 2.5 mU/ml (●); PGE_2, 30 μM (■); or isoproterenol, 50 μM (▲).

4. Discussion

Our results show that in the presence of GTP, Na^+ inhibited TSH-stimulated thyroid cell adenyl cyclase activity. This inhibition was Na^+ specific below 50 mM and TSH specific since neither basal nor PGE_2- or isoproterenol-stimulated cAMP synthesis were modified by Na^+. Inhibition was immediate and readily reversible.

In addition to its action on the inhibition of cAMP synthesis by adrenergic [9], muscarinic cholinergic agonists [6–13] and opiates [6,14] Na^+ appears to have divergent effects on adenylate cyclase activities depending on the biological system studied. In the porcine kidney medulla, Na^+ was required for full expression of stimulation by vasopressin [8]. In hamster adipocytes more complex interactions were reported, including increase of basal level and decrease of ACTH and isoproterenol stimulation [9]. In human platelets monovalent cations decreased both basal and PGE_1-stimulated activities [10]. Inhibition by lithium of bovine thyroid adenylate cyclase has been reported [13].

Studies on various hormone- and neurotransmitter-modulated systems have shown that the adenylate cyclase complex has ⩾3 distinct components: the hormone receptor, the catalytic unit and a guanyl nucleotide binding component (review [16]). Na^+ may act at any of them. We have shown [17] that monovalent cations reduced TSH binding to porcine thyroid membranes. This effect was not salt specific, occurred in the absence of GTP and required higher Na^+ concentrations than those needed to observe the inhibition of TSH stimulation. Na^+ having no effect on basal activity, an interaction of the salt with the catalytic subunit or with the TSH receptor cannot explain the observed inhibition.

The requirement for GTP suggests that Na^+ may uncouple the nucleotide binding component from the catalytic unit as proposed in [10]. Preliminary results show that stimulation induced by optimal concentrations of TSH and PGE_2 are not additive and indicate that the specific receptors of the 2 hormones can activate the same enzyme pool. All hormonal responses would then be affected by Na^+. The specificity of salt inhibition for TSH-induced stimulation suggest that some differences may exist in the mode of coupling of TSH or PGE_2 receptors with adenylate cyclase in the thyroid cells.

The consequence of these observations on the interpretation of results obtained with cellular systems might be important. The Na^+ concentration range in which inhibition was observed (0–40 mM) is close to actual intracellular Na^+ concentration (0–10 mM) [18]. Fluctuations of intracellular Na^+ concentration might therefore influence the cellular cAMP response to TSH stimulation.

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