Effects of prostaglandin E_2 and cholera toxin on apical sodium uptake in thyroid epithelial cells: role of cAMP

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When cultured in tissue culture polystyrene dishes, porcine thyroid cells formed polarized monolayers Their apical pole was oriented towards the culture medium Influx of sodium 22 (5 min) through the apical surface was partially inhibited by amiloride Amiloride-sensitive Na uptake was increased about 3-fold by prostaglandin E_2 (1 μM, 30 min) and by cholera toxin (0 01 μg/ml, 1 h) This increase, which was also obtained with forskolin and 8-(4-chlorophenylthio)adenosine-3′ 5′-monophosphate, cyclic (8-chloro-cAMP), is probably a consequence of the increase of the intracellular cAMP level by prostaglandin and cholera toxin

Sodium uptake *Thyroid cell* *Amiloride* *cAMP* *Cholera toxin* *Prostaglandin*

1. INTRODUCTION

When cultured on adhesive substrates, such as tissue culture-treated polystyrene, porcine thyroid cells organized into polarized monolayers. The basal pole of the cells was in contact with the substrate whereas the apical surface was oriented towards the culture medium [1]. A transepithelial potential difference of about 20 mV (apical negative) has been measured in such cultures [2]. We have previously observed that the sodium transport properties of the apical and basolateral domains of the thyroid cell plasma membrane were different: the (Na^+,K^+)-ATPase was localized on the basolateral membrane whereas an amiloride-sensitive Na^+ uptake occurred at the apical pole (unpublished). We report here, that the amiloride-sensitive apical uptake of Na^+ was stimulated by prostaglandin E_2 and cholera toxin; both enhanced adenylate cyclase activity in thyroid cell monolayers. Stimulation was also obtained with 8-chloro-cAMP and forskolin.

2. MATERIALS AND METHODS

2.1. *Cell preparation and culture*

Thyroid cells were obtained from porcine thyroid glands by a discontinuous trypsin-EGTA treatment [1]. Freshly isolated cells were suspended at 2×10^6 cells/ml in Eagle's minimum essential medium supplemented with newborn calf serum (10%, v/v), penicillin (200 units/ml) and streptomycin sulfate (50 μg/ml). Cells were seeded (2 ml cell suspension) in 35 mm Corning tissue culture dishes, and incubated for 6 days at 36°C in 95% air/5% CO_2 water saturated atmosphere. The culture medium was changed at day 4. Under these conditions cells formed a confluent monolayer.

2.2. *Cell stimulation and apical sodium uptake*

The different effectors (prostaglandin, cholera toxin, 8-chloro-cAMP and forskolin) were solubilized in Eagle's medium and added to the culture medium. After appropriate incubation the monolayer was washed once with modified Eagle's salt solution: NaCl, 20 mM; KCl, 5.4 mM; Na_2HPO_4, 0.4 mM; $MgSO_4$, 0.8 mM; $CaCl_2$, 1.8 mM; choline chloride, 120 mM; glucose, 5.5 mM; bovine serum albumin, 0.1%; Hepes, 20 mM; Tris, 5 mM (pH 7.2). Cells were incubated for 10 min at 37°C in washing buffer in the presence or absence of 0.1 mM amiloride; 22 Na^+ (0.5 μCi, 1 μCi/ml) was then added. After 5 min incubation the medium was removed by aspiration

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and the monolayer was rapidly washed (3 times within 20 s) with ice-cold Tris–HCl, 0.1 M (pH 7.4). The cell layer was digested with 1 ml NaOH, 0.1 N. The radioactivity incorporated in the cells was determined using a gamma counter. Zero time samples were routinely subtracted from the experimental values (about 15% of cellular uptake). Each value ($nmol \cdot cm^{-2} \cdot min^{-1}$) was the means of triplicate determinations (± SD). Experiments were reproduced at least 3 times with similar results. The statistical differences were calculated using Student's *t*-test.

2.3. *Chemicals*

Prostaglandins were a gift from Dr J. Pike (Upjohn Co.). Forskolin was from Calbiochem. Stock solutions (30 mM) of prostaglandins and forskolin in ethanol were stored at −20°C. Cholera toxin was from Sigma, 8-chloro-cAMP from Boehringer, 22 Na^+ (5 mCi/ml) from NEN. Amiloride was a gift from Merck Sharp and Dohme.

3. RESULTS

3.1. *Time-dependent effect of prostaglandin E_2 and cholera toxin*

In cells organized into monolayers, sodium uptake was linear up to 5 min when incubated in the presence or absence of stimulator (prostaglandin E_2) (fig.1). Maximal inhibition of Na uptake was obtained after 10 min preincubation in the presence of 0.1 mM amiloride. Na uptake was determined following various times of treatment with prostaglandin E_2 (1 μM) or cholera toxin (0.1 μg/ml). These concentrations of effectors enhance 5-fold cellular cAMP content within an hour (not shown). A marked increase of total Na uptake was observed with a maximum for 30 min (fig.2,3). This stimulated level persisted at least 120 min. In contrast, the effects of prostaglandin E_2 and cholera toxin on Na uptake were not significant ($p > 0.05$) if incubation was done in the presence of 0.1 mM amiloride. So, when measured in the presence of 20 mM Na, only amiloride-sensitive Na uptake was stimulated (3.8 and 2.2-fold, respectively, for cholera toxin and prostaglandin E_2). During the period of incubation the level of Na uptake remained constant in the unstimulated cells either in the presence of amiloride or in its absence. Equivalent results were

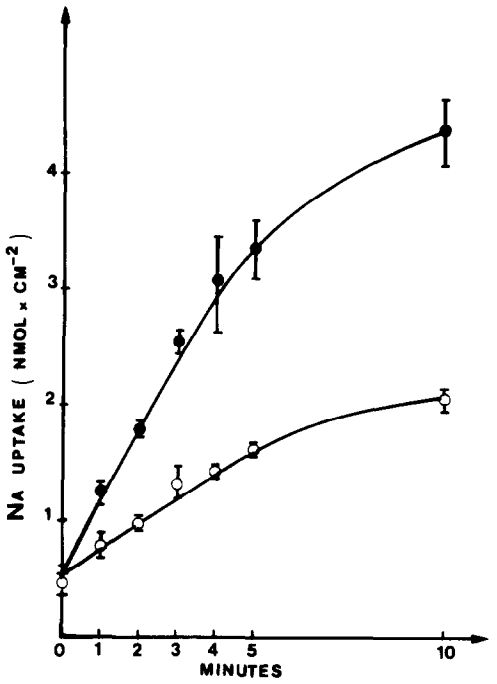

Fig.1. Time course of Na^+ uptake Monolayers were preincubated 30 min in the presence (upper curve) or absence (lower curve) of prostaglandin E_2 (1 μM), washed and incubated for 10 min in Eagle's salt solution, Na^+ uptake was then measured up to 10 min

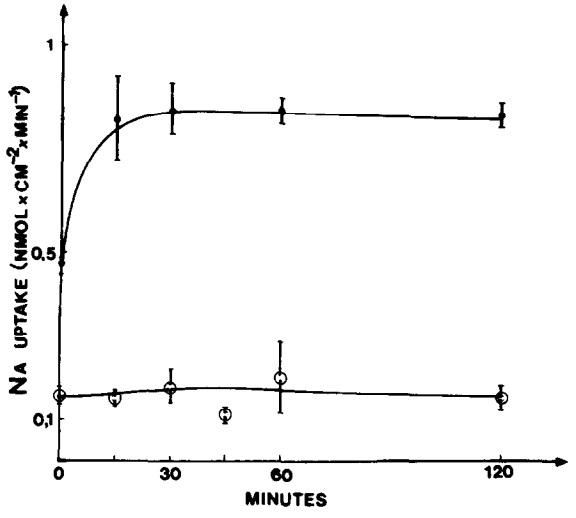

Fig 2 Kinetics of prostaglandin effect. Monolayers were first incubated with prostaglandin E_2 (1 μM), then washed and Na uptake was measured with (lower curve) or without (upper curve) amiloride (0.1 mM) as described in section 2

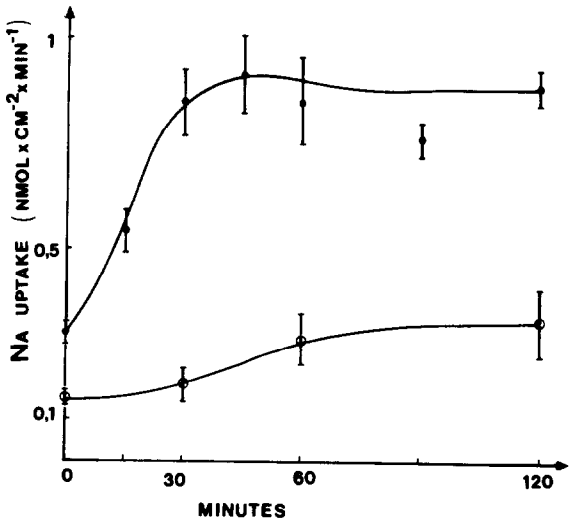

Fig.3 Kinetics of cholera toxin effect. Same legend as in fig.2 except that cholera toxin (0.1 μg/ml) was used instead of prostaglandin.

obtained when Na uptake was measured in the presence of 140 mM Na.

3.2. *Effect of graded concentrations of prostaglandin E_2 and cholera toxin*

We have determined Na influx as a function of prostaglandin E_2 and cholera toxin concentrations in the presence or absence of amiloride. When cells were stimulated for 30 min with increasing concentrations of prostaglandin E_2, a dose-dependent stimulation of amiloride-sensitive uptake was observed (fig.4). Half maximal stimulation was obtained with 0.08 μM prostaglandin E_2. Prostaglandin E_1 (1 μM) gave a similar stimulation whereas prostaglandin $F_{2\alpha}$ (1 μM) was without effect (table 1).

A dose-dependent stimulation of amiloride-sensitive sodium uptake was also observed with cholera toxin (fig.5). After 60 min, maximal effect was obtained in the presence of 10 ng/ml cholera toxin (0.1 nM). Half maximal stimulation occurred for 0.8 pM.

3.3. *Effect of forskolin and 8-chloro-cAMP*

Our results suggested a modulation of apical sodium uptake via a change of intracellular cAMP. We have evaluated the effect of other drugs which increased cAMP content (table 1). Forskolin (1 μM) stimulated 6.5-fold basal adenylate cyclase

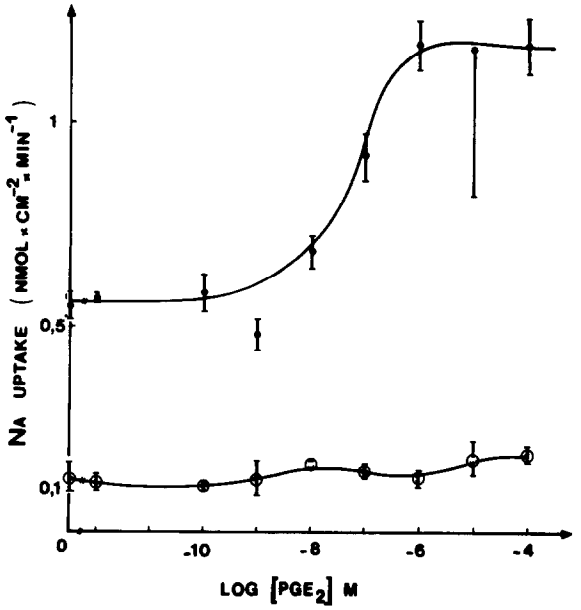

Fig.4. Effect of prostaglandin on Na uptake. dose-response curve. Monolayers were incubated for 30 min with increasing concentrations of prostaglandin E_2. Na uptake was then measured in the presence (lower curve) or absence (upper curve) of amiloride (0.1 mM).

Table 1

Effect of prostaglandins, 8-chloro-cAMP and forskolin on apical Na uptake (nmol·cm^{-2}·min^{-1})

Effector	Amiloride (0)	Amiloride (100 μM)
Control	0.18 ± 0.01	0 06 ± 0 02
PG E_2 (1 μM)	0.67 ± 0.02	0 12 ± 0 03[a]
PG $F_{2\alpha}$ (1 μM)	0.18 ± 0.01	0.07 ± 0.00[a]
PG E_1 (1 μM)	0.54 ± 0.05	0.12 ± 0 02[a]
8-Chloro-cAMP (500 μM)	0.41 ± 0.02	0.12 ± 0 02[a]
Forskolin (1 μM)	0.40 ± 0.07	0.15 ± 0 03[a]

[a] Difference not significant *vs* amiloride control

Monolayers were incubated for 1 h with the effector and Na uptake was measured in the presence or absence of amiloride (0.1 mM). Means ± SD

of thyroid cells (not shown) and enhanced 2-fold amiloride sensitive Na uptake. Equivalent increase of adenylate cyclase activity was obtained with

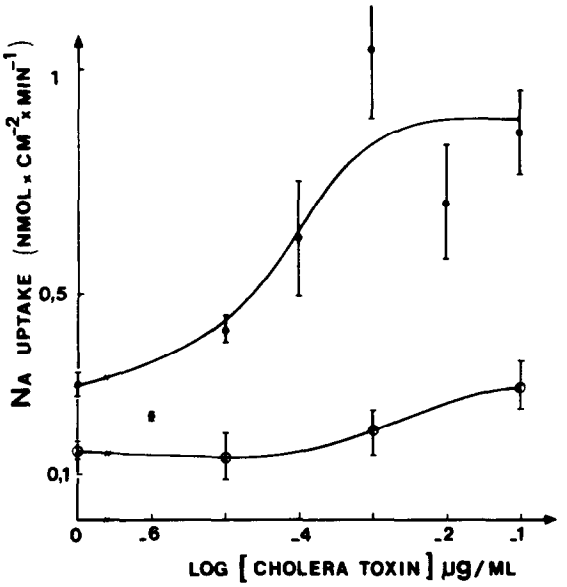

Fig.5. Effect of cholera toxin: dose-response curve. Cells were incubated for 1 h with cholera toxin and Na uptake was determined in the presence (lower curve) or absence (upper curve) of amiloride (0.1 mM)

1 μM prostaglandin E_2 (5.9-fold). 8-Chloro-cAMP (0.5 mM) stimulated amiloride sensitive Na uptake as the other effectors tested.

4. DISCUSSION

Thyroid cells in culture formed a polarized monolayer. The thyrotropin (TSH) receptor adenylate cyclase complex has been located on the basal cell membrane [3]. In this system TSH had no effect on the cAMP level whereas prostaglandin E_2 and cholera toxin could act on the two domains of the membrane. So we have routinely used prostaglandin E_2 or cholera toxin and not TSH as effectors of the system. Previous experiments have shown that apical sodium uptake was inhibited by amiloride. On the contrary, in our experimental conditions ouabain has no effect on apical Na influx and was always omitted from incubation medium. We show in this paper that an increase of apical sodium uptake was provoked by prostaglandins E (E_1, E_2), cholera toxin and forskolin. These effectors can enhance intracellular cAMP concentration by stimulating adenyl cyclase activity through different pathways: prostaglandin binds to membrane receptors [4], G subunits are ADP-ribosylated by cholera toxin [5] and forskolin interacts directly with C catalytic subunit [6]. Moreover, the stimulation of Na influx is also obtained with 8-chloro-cAMP. It seems, therefore, that enhancement of cellular cAMP content can induce an increase of apical Na uptake. In addition, we show that this increase is restricted to the amiloride sensitive part of Na influx. Our results are in agreement with those obtained by other groups working on amphibian tight epithelia (skin, urinary bladder, kidney). For example, in the cell line A6 derived from the kidney of *Xenopus laevis* short circuit current was enlarged by the addition of exogenous cAMP and fell rapidly after the addition of amiloride [7,8]. Effects of applied prostaglandin E and of endogenous prostaglandin E on sodium transport in frog skin are mediated by cAMP [9]. Moreover, prostaglandin, vasopressin and theophylline appeared to exert their influence on the short circuit current via changes primarily of the resistance of the apical barrier of the skin epithelial cells without modification of the basal resistance [10].

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