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RAT ENTEROCYTE Na^+ TRANSPORT IN VITRO

ACTION OF PARATHYROID HORMONE AND CALCITONIN

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Parathyroid hormone (PTH) and calcitonin exert well known effects on the renal tubule which are thought to involve specific hormone receptors and adenyl cyclase. In the intestine, it is not clear whether the action of PTH and calcitonin is only indirect or also direct, and their mechanisms of action are much less well established. In the present study, possibly direct effects of PTH and calcitonin on Na^+ transport in isolated intestinal epithelial cells of rats were investigated. In the presence of bovine PTH (1.2 I.U./ml) in the incubation medium, the Na^+ efflux rate constant ($^oK_{Na}$) of isolated enterocytes was significantly reduced when compared to that in control experiments with the hormone vehicle only. The mean depression of $^oK_{Na}$ induced by bovine PTH was 26% as compared to the control (100%) and to that induced by ouabain (4.0 mM) which was 44%. No depressant effect of bovine PTH on $^oK_{Na}$ was observed when the isolated enterocytes were incubated with ouabain (4.0 mM). Thus, bovine PTH appeared to inhibit the ouabain-sensitive Na^+ pump. When incubating the isolated epithelial cells in an EGTA-containing Ca^{2+}-free medium, bovine PTH lost its capacity to inhibit $^oK_{Na}$. Thus, the presence of extracellular Ca^{2+} appeared necessary for the inhibitory effect of bovine PTH. In contrast to its effect on $^oK_{Na}$, bovine PTH induced no change in net Na^+ uptake by isolated enterocytes. Moreover, no significant effect on enterocyte Na^+ transport could be demonstrated for salmon or porcine calcitonin at two different concentrations in the incubation medium. Neither bovine PTH nor salmon calcitonin induced significant changes in enterocyte cyclic AMP or cyclic GMP concentrations. It was concluded that bovine PTH, but not calcitonin, exerted a direct inhibitory effect on the ouabain-sensitive $^oK_{Na}$ of isolated rat enterocytes. The effect of bovine PTH occurred without measurable activation of the cyclic nucleotide system but needed the presence of Ca^{2+} in the incubation medium to be operative.

Introduction

The existence of hormonal regulation of cellular Na^+ transport has been recently claimed in some reports [1,2]. Hormones such as aldosterone, vasopressin, catecholamines, insulin and thyroid hormone appear to influence the Na^+ pump either by modulating the rate at which the pump units cycle, or by varying the absolute number of pump units involved in the transport of Na^+ and K^+ [1,2].

Parathyroid hormone (PTH) and calcitonin influence the renal [3–8] and intestinal [9–14] handling of Na^+ and H_2O. The precise mechanism of action by which PTH inhibits the transport of Na^+ in renal proximal tubule [6] and in the jejunum [9–11] remains unknown. This is also true for the inhibitory action of calcitonin on Na^+ transport by the renal tubule [7,8] and intestinal mucosa [10,12,14]. It is not well established, at least for the intestine, whether the observed effects are due to direct action

Abbreviations: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; M.C.R. U, Medical Research Council units.

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of the hormones on these epithelia, or whether their action is only indirect via other mediators. In at least one recent work using rat ileum in vitro, however, calcitonin appeared to exert a direct inhibitory effect on intestinal Na^+ absorption [13]. In the renal tubule, specific receptors for PTH [15] and calcitonin [16, 17] have been described, and the cellular action of both hormones is thought to be mediated by adenylate cyclase [18]. However, in the intestine no such mediation by cyclic nucleotides has so far been demonstrated for the effects of PTH and calcitonin [11,19].

The purpose of the present study using isolated intestinal cells of the rat was (1) to investigate whether PTH and calcitonin exert direct effects on enterocyte Na^+ transport, and if so (2) to obtain insight into the mechanism by which such effects might occur.

Materials and Methods

Animals. Non-fasted male Wistar AF rats weighing 100–150 g were used in all experiments. Before experiments, animals were fed a standard laboratory diet containing 24% protein, 6.5% cellulose, 4.5% lipids, 45% nonprotein extract, 8% mineral salts, 200 I.U. vitamin D and 12% water. Calcium and phosphorus contents were, respectively, 1.5 and 0.95%. Animals were housed at 20°C with lighting from 0900 to 1700 h. All animals had free access to tap water.

Preparation of isolated intestinal cells. On the day of experiment animals were killed by a blow to the head. After midline incision, a duodeno-jejunal segment of approx. 20 cm length was entirely filled with ice-cold isotonic saline before excision. Then, the excised intestine was equilibrated during 20 min in a shaking water bath (shaking bath, SB-4, Osi, Paris, France) at 37°C in a working medium of the following composition (in mM): NaCl, 120; Tris, 20; K_2HP04, 3.0; $MgCl_2$, 1.0; $CaCl_2$, 1.0; glucose, 10 ; containing also 1 mg/ml bovine serum albumin (pH 7.4 ± 0.1) with an osmolality of 295–305 mosmol/kg. Epithelial cells were isolated from the everted jejunum by a mechanical vibration procedure (Vibro-Mischer El, Chemap AG, Switzerland) similar to that described by Levine and Weintraub [20] with the jejunum immersed in the working medium (60 vibrations/s, 1.0 mm amplitude during 20 min). No enzymatic digestion was performed.

Viability and electron microscopy. The cells were incubated with a 0.2% trypan blue solution [21] for testing of viability by evaluating the fraction of cells which were able to exclude the dye.

In addition, the enterocytes were studied by electron microscopy. After isolation of cells from the intestine either before or at the end of the incubation period with hormone (vide infra), the cells were washed and fixed during 1 h with a 2% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.4. Then they were postfixed in 1% OsO_4 during 1 h, and dehydrated in a series of alcohol solutions from 70 to 100% and lastly in propylene oxide. Finally, they were embedded in Epon. At each step, cells were centrifuged at $600 \times g$ to obtain the pellet and remove the supernatant. The polymerized blocks were sliced at 900 Å using a diamond knife, collected on a grid covered with a Formvar film, impregnated with uranyl acetate and lead citrate using the method of Reynolds [22] and observed by electron microscopy (EM 200, Philips). Four observations were made from samples of different mucosal specimens. Thus, 160 cells were observed.

Determination of the Na^+ efflux rate constant ($^oK_{Na}$). Measurement of Na^+ efflux was performed using the method described by Gall et al. [23]. Briefly, the isolated enterocytes were incubated at 37°C in the working incubation medium containing 10 μCi $^{22}Na^+$ (New England Nuclear, Dreieick, F.R.G.) for 20 min ('loading period'). The cell suspension was then centrifuged at $1000 \times g$ and the cells rapidly washed twice with ice-cold $MgCl_2$/Tris buffer (110 and 2.0 mM, respectively, pH 7.4) at 4°C. The cells were finally returned to fresh tracer-free working medium at a final dilution of 1/30 by volume and $^{22}Na^+$ efflux was determined ('efflux period'). $^oK_{Na}$ was calculated as reported by Gall et al. [23]. Hormones and their respective vehicle solutions ('vehicle') were added to the incubation medium during the loading and efflux periods, at identical concentrations.

Na^+ uptake studies. The cell pellet obtained after mechanical vibration was divided into two parts. Each part was suspended in 6 ml of working buffer and maintained in constant agitation during 20 min at 37°C in a shaking water bath. Then the hormone studied was added to one cell suspension and its

vehicle to the other. Two samples of 1000 μl from each suspension were pipetted into a vial containing 5.0 ml ice-cold washing solution. Then 80 μl of a $^{22}NaCl$ solution were added (corresponding to 8 μCi ^{22}Na, spec. act. 0.087 mCi/mg, New England Nuclear, Dreieick, F.R.G.). Aliquots of 500 μl each were obtained from both suspensions maintained and stirred at 37°C, at the following time points: 30, 60, 90 and 120 s, 3, 4, 5 and 15 min after $^{22}Na^+$ addition. Each sample was immediately distributed into its respective vial containing 5.0 ml ice-cold washing solution. 10 μl of the $^{22}NaCl$ solution (1 μCi ^{22}Na) were added at the end of the experiment to the two samples obtained before $^{22}Na^+$ addition. These two samples were used to determine the value for non-specific retention of radioactivity. All vials were thus centrifuged at 1000 × *g* for 1 min at 4°C. The supernatant was discarded and the pellets washed twice with 10 ml ice-cold washing buffer. Finally, 1.0 ml of 10% trichloroacetic acid solution was added to the last pellet, the pellet was vibrated with a vortex mixer (Bioblock, Paris, France) during 2 min, and the vials were kept at 4°C during 15 min. After centrifugation, a 500 μl aliquot of the supernatant was measured for its radioactivity using a liquid scintillation counter (S.L. 30, Intertechnique, Plaisir, France). The other 500 μl containing the disrupted cells were added to 5.0 ml of a 1.0 M NaOH solution to solubilize the proteins, which were determined using the method described by Lowry et al. [24] using bovine serum albumin as standard.

Study of cyclic nucleotide formation during parathyroid hormone or calcitonin action on isolated intestinal cells. These studies were performed using isolated cells after two washings. The pellet was resuspended in 12 ml of Krebs-Ringer-Henseleit medium containing 2.5 mM $CaCl_2$ and 2.0 g/l bovine serum albumin. The pH of this medium was kept constant at 7.4 using continuous bubbling with a gas mixture of 5% CO_2 and 95% O_2.

A 1.0 ml aliquot of the cell suspension maintained and stirred at 37°C was sampled before addition of hormone or vehicle. After this addition, two other 1.0 ml samples were drawn at 2 and 10 min for each experiment. Each sample was immediately transferred to a vial containing 1.0 ml of 4.5 M trichloroacetic acid. The cyclic nucleotides of the supernatant (cyclic AMP and cyclic GMP), which are nucleotides contained in the cells, and also that secreted into the medium, were determined using radioimmunoassay [25]. Each pellet was analyzed for DNA content using the method described by Burton [26].

Hormones and other substances. (a) For the studies concerning Na^+ uptake and efflux, the concentration of bovine PTH used in the incubation medium was 1.2 I.U./ml. Prior to experiments, bovine PTH (highly purified bovine PTH, Wilson Laboratories, Flobio, Courbevoie, France) was maintained in a frozen vehicle stock solution (6.0 I.U./5.0 μl total volume) containing 1.0 mM cysteine chloride and 5.0 mM acetic acid in 154 mM NaCl. The concentration of salmon calcitonin used in the incubation medium was 2.0 M.R.C. U/ml. Prior to experiments, salmon CT (100 M.R.C. U/ml, corresponding to 25 μg, graciously provided by Sandoz Laboratories, Rueil-Malmaison, France) was maintained at 4°C at a concentration of 1000 M.R.C. U/ml in a 5.0 mM acetic acid vehicle solution.

The effect of porcine calcitonin (Armour-Montagu, Paris, France) was tested at two concentrations: 2.0 and 20.0 M.R.C. U/ml, always in the working medium.

The effect of ouabain (Boehringer, Mannheim, F.R.G.) on $^{o}K_{Na}$ was tested at a final concentration of 1.0 or 4.0 mM. Addition took place just at the start of the $^{22}Na^+$ loading period. When the effect of bovine PTH on enterocytes was studied in the presence of ouabain, ouabain was added to the incubation medium at a concentration of 4.0 mM during loading and efflux periods.

The effect of bovine PTH on epithelial cells was also studied when incubated in the working medium in the absence of calcium and in the presence of 0.1 mM EGTA (Merck, Darmstadt, F.R.G.) during the efflux period.

(b) For the studies concerning cyclic nucleotide formation the hormones or vehicle studied were bovine PTH at a final concentration of 1.0 I.U./ml, salmon calcitonin at final concentrations of 2.0 and 10.0 M.R.C. U/ml and prostaglandin E_1 (Upjohn Company, Kalamazoo, MI, U.S.A.) at a final concentration of 10 μg/ml. The stock solution of prostaglandin E_1 was prepared in 80% (v/v) alcohol and stored at −20°C. This solution was diluted at 1 : 100 in cell suspension solution to 10 μg/ml. Thus, the alcohol concentration in the incubation medium was

0.8%. In the control experiment for prostaglandin E_1, alcohol without prostaglandin E_1 was added to the medium to obtain a final concentration of 0.8%.

Theophylline (anhydrous crystal, Sigma Chemical Co., St. Louis, U.S.A.) at a concentration of 0.01 M was also added in some experiments done with bovine PTH or salmon calcitonin.

Statistical analysis of results. Results of enterocyte flux and nucleotide formation studies were expressed as means ± S.E. Student's paired *t*-test was performed when comparing values obtained with the hormones to that obtained with their respective vehicle, since in each experiment the suspension of cells was divided into two parts, one treated with the respective hormone and the other with its vehicle only.

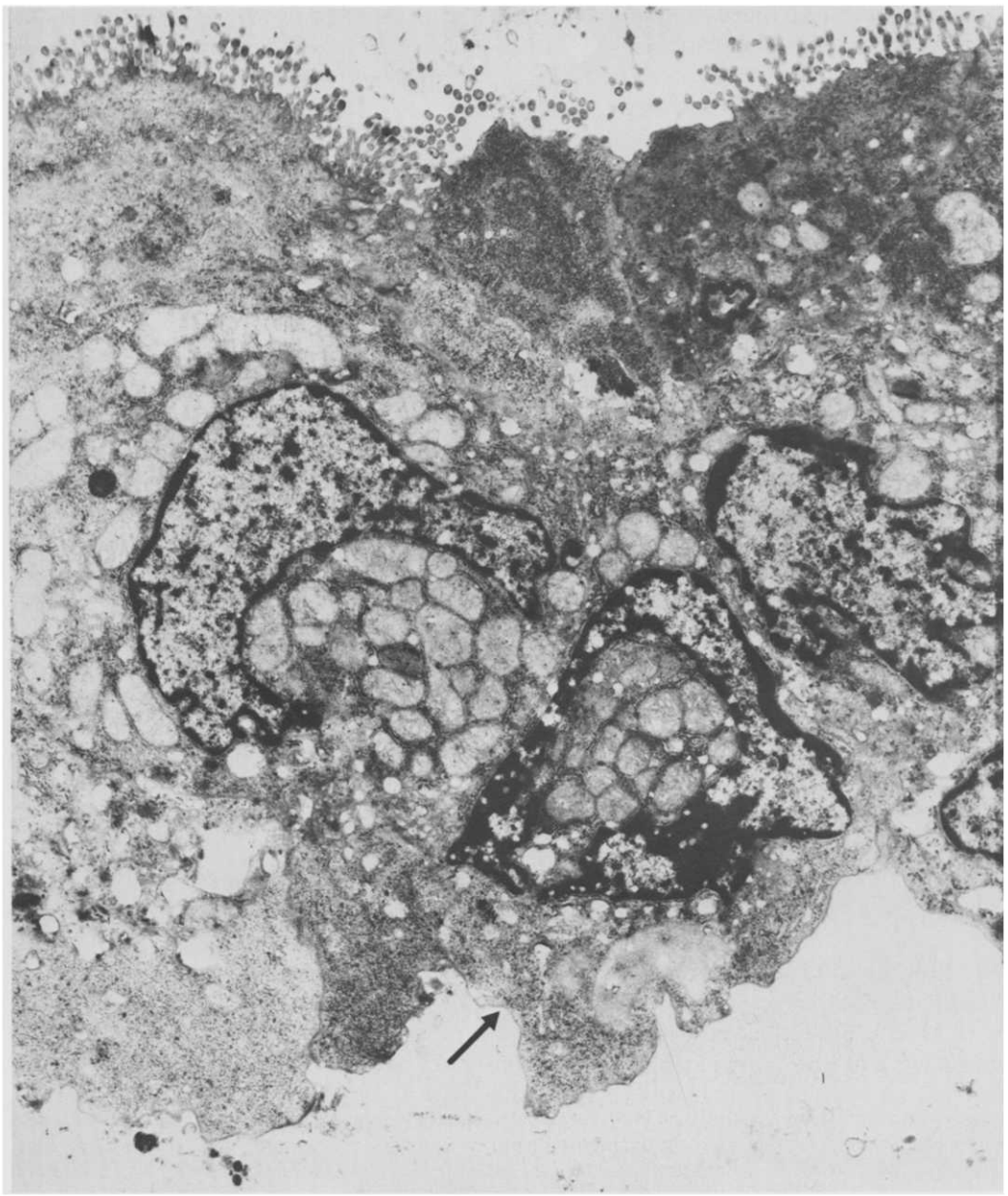

Fig. 1a.

Results

Viability and electron microscopy of isolated intestinal cells

The study of viability of isolated intestinal cells using trypan blue coloration showed that 80–90% of the cells were able to exclude the dye. This proportion was comparable at the end of accumulation or efflux periods. When performing electron microscopy, the cells which were presented either as entirely isolated elements or in groups of well structured epithelia had an aspect that appeared identical to that of normal whole mucosa. Generally, the basal membrane remained intact, even though it was dilacerated in some instances. Two types of enterocytes were encountered, i.e., absorptive cells and mucus-containing cells. No endocrine cells were observed in these preparations. The majority of cells (88%) had a viable aspect of active, protein-synthesizing enterocytes with their characteristic ribosomes and ergastoplasm. The apical zone contained a preserved brush border with well developed microvilli and their fibrillar

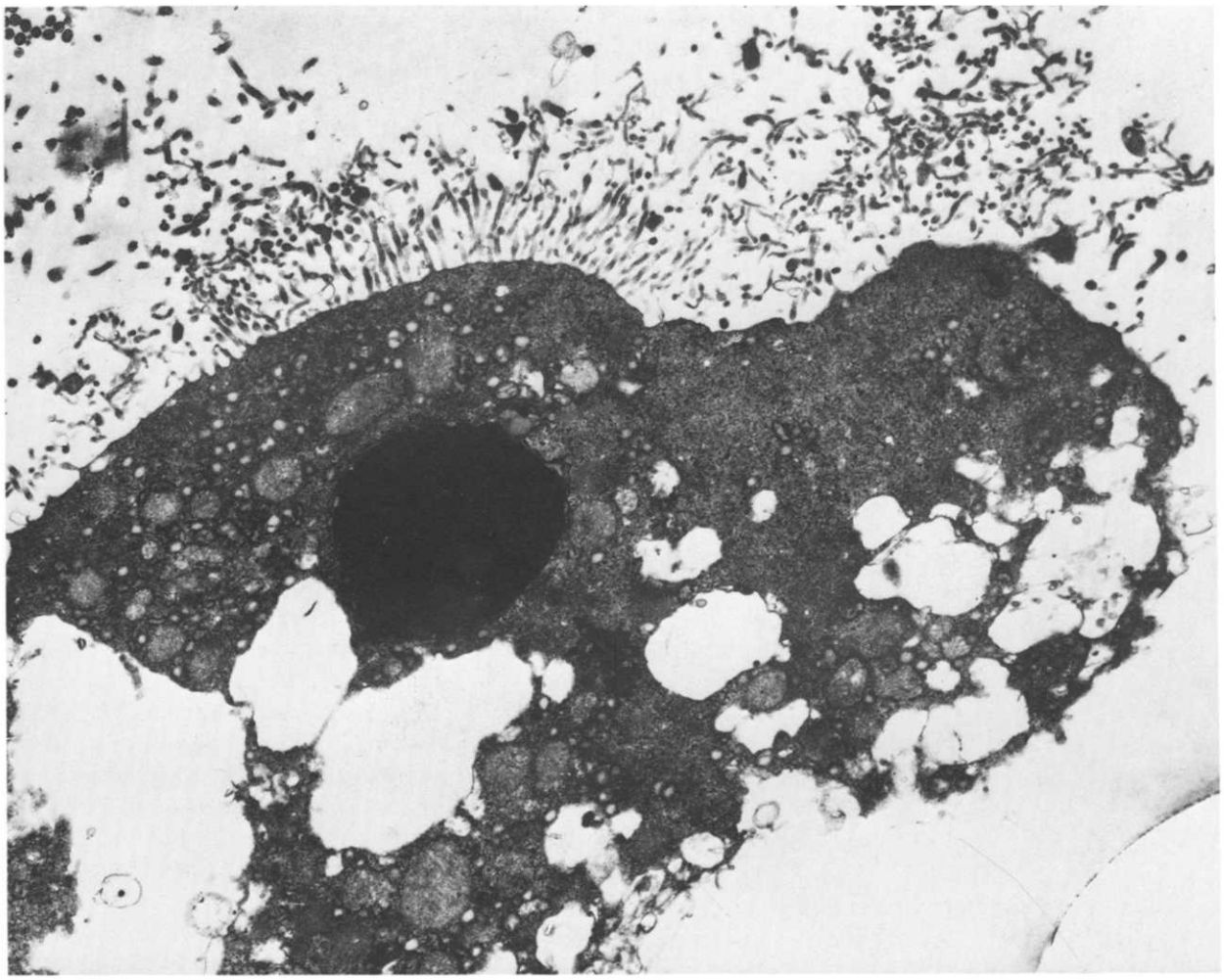

Fig. 1. Electron microscopic examination of isolated jejunal cells: (a) isolated enterocyte having a viable aspect with characteristic, well preserved ribosomes, ergastoplasm, mitochondria and irregularly shaped nucleus with chromatin of granular aspect and intact brush border and basal membrane (arrow); (b) agonizing enterocyte with nucleus chromatin transformed into a dense, amorphous mass (X7200).

extension into the apical cytoplasm. The intracellular organelles such as mitochondria, lamellar ergastoplasm and free ribosomes were numerous. The nucleus was generally of irregular shape and contained chromatin of a granular aspect such as that of viable cells in 88% of instances while in 12% the nucleus was pycnotic with its chromatin content transformed into a dense and amorphous mass. For illustration a viable cell and an agonizing cell are shown in Fig. 1a and b, respectively.

Effect of ouabain on $^{o}K_{Na}$

The validity of the model was supported by the inhibitory effect of ouabain on $^{o}K_{Na}$ of enterocytes. Fig. 2 shows a representative experiment on Na^+ efflux in isolated jejunal cells in the presence and absence of 4.0 mM ouabain. In this experiment the inhibition of $^{o}K_{Na}$ by ouabain was 54% when compared to control cells from the same animal. The mean inhibition of $^{o}K_{Na}$ for all experiments was 44% for 1.0 mM ouabain (3.15 ± 0.30 vs. control 5.63 ± 0.53 h^{-1} (±S.E.), $n = 10$, $P < 0.005$) and 45% for 4.0 mM (2.55 ± 0.56 vs. control 4.65 ± 0.59 h^{-1}, $n = 3$).

Effect of PTH and calcitonin on $^{o}K_{Na}$

Fig. 3 shows the effect of bovine PTH (1.2 I.U./ml) on $^{o}K_{Na}$ in a typical experiment using isolated enterocytes. A 28% inhibition of $^{o}K_{Na}$ was observed in the presence of the hormone when compared to the control experiment containing the vehicle only. 16 such experiments were performed (Table I). A mean inhibitory effect of bovine PTH (1.2 I.U./ml) on $^{o}K_{Na}$ of 26% was observed when compared to control vehicle experiments (3.01 ± 0.19 vs. 4.06 ± 0.36 h^{-1}, $P < 0.02$). Contrary to the bovine PTH inhibitory action on $^{o}K_{Na}$ in enterocytes, no such effect could be obtained using calcitonin from two different animal species (salmon and porcine calcitonin). Table I shows the absence of a significant salmon calcitonin (2.0 M.R.C. U/ml) or porcine calcitonin (2.0 M.R.C. U/ml) effect on $^{o}K_{Na}$ of isolated jejunal cells. Moreover, when increasing the porcine calcitonin concentration in the incubation medium to 20.0 M.R.C. U/ml, $^{o}K_{Na}$ remained similar to that of control experiments (4.5 ± 0.74 vs. 4.89 ± 0.59 h^{-1}, $n = 3$).

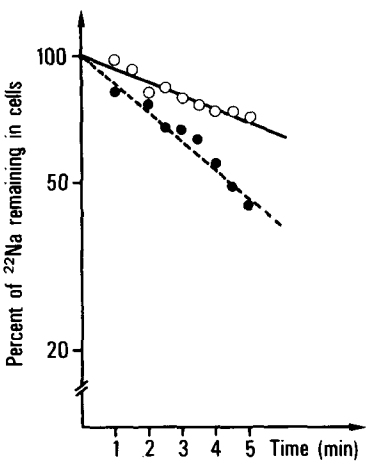

Fig. 2. A representative experiment with isolated enterocytes from one animal. (o———o) Regression line showing $^{o}K_{Na}$ obtained using an incubation medium containing 4.0 mM ouabain ($r = 0.946$; $^{o}K_{Na} = 1.85$ h^{-1}); (•- - - - -•) regression line showing $^{o}K_{Na}$ obtained in control cells in the absence of ouabain ($r = 0.966$; $^{o}K_{Na} = 4.02$ h^{-1}).

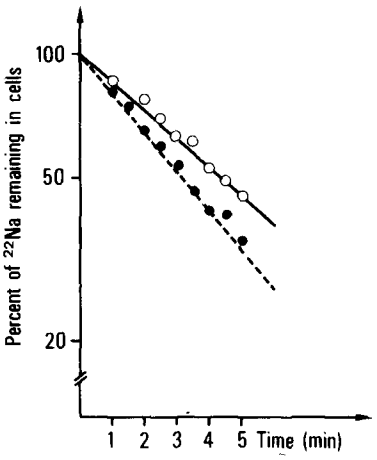

Fig. 3. A representative experiment with isolated enterocytes from one animal. (o———o) Regression line showing $^{o}K_{Na}$ obtained using an incubation medium containing 1.2 I.U./ml bovine PTH ($r = 0.986$; $^{o}K_{Na} = 4.16$ h^{-1}); (•- - - - -•) regression line showing $^{o}K_{Na}$ obtained in control cells in the presence of the hormone vehicle only ($r = 0.991$; $^{o}K_{Na} = 5.76$ h^{-1}).

Effect of PTH on ouabain-independent $^{o}K_{Na}$

To investigate further the site of bovine PTH inhibitory action on $^{o}K_{Na}$, studies with bovine PTH were conducted in the presence of 4.0 mM ouabain. No effect of bovine PTH (1.2 I.U./ml) on ouabain-independent $^{o}K_{Na}$ of enterocytes was observed when

TABLE I

EFFECT OF PTH AND CALCITONIN ON $^{\circ}K_{Na}$

n.s., not significant.

Hormone	Experimental conditions	Experimental values (h^{-1})		P	% inhibition
		Hormone	Vehicle		
Bovine PTH (1.2 I.U./ml)	Total $^{\circ}K_{Na}$	3.01 ± 0.19 (n = 16)	4.06 ± 0.37 (n = 16)	<0.02	26
	Ouabain-resistant $^{\circ}K_{Na}$	2.56 ± 0.27 (n = 7)	2.37 ± 0.21 (n = 7)	n.s.	–
	Ouabain-sensitive $^{\circ}K_{Na}$	0.45	1.69	–	73
Salmon calcitonin (2.0 M.R.C. U/ml)	Total $^{\circ}K_{Na}$	4.73 ± 0.65 (n = 7)	4.50 ± 0.51 (n = 7)	n.s.	–
Porcine calcitonin (2.0 M.R.C. U/ml)	Total $^{\circ}K_{Na}$	4.85 ± 1.242 (n = 3)	4.85 ± 1.11 (n = 3)	–	–

compared to ouabain-exposed enterocytes incubated with the hormone vehicle only. Table I shows the mean $^{\circ}K_{Na}$ values obtained in response to bovine PTH or its vehicle in the absence of ouabain (total $^{\circ}K_{Na}$) and in the presence of ouabain (ouabain-resistant $^{\circ}K_{Na}$). Furthermore, it indicates the calculated inhibitory effect of bovine PTH on the ouabain-sensitive Na^+ transport site obtained by subtracting ouabain-resistant from total $^{\circ}K_{Na}$. This yields an inhibition of 73%.

Effect of PTH and calcitonin on Na^+ uptake

The net uptake of $^{22}Na^+$ by isolated jejunal cells was comparable in the presence or absence of bovine PTH (1.2 I.U./ml) in the incubation medium, at least during the time period of 15 min studied (Fig. 4). Similarly, salmon calcitonin (2.0 M.R.C. U/ml) in the incubation medium induced no change in net enterocyte $^{22}Na^+$ uptake when compared to control (Fig. 5).

Effect of PTH and calcitonin on cyclic nucleotide formation

The effects of bovine PTH (1.0 I.U./ml) and salmon calcitonin (2.0 M.R.C. U/ml) in the presence and absence of theophylline (10 mM) on cyclic AMP and cyclic GMP formation in isolated jejunal cells are shown in Tables II and III. The well known stimulator of intestinal adenyl cyclase, prostaglandin E_1, was also studied in additional experiments. The tables show that neither bovine PTH nor salmon calcitonin induced an increase in the cyclic nucleotide content of isolated enterocytes. However, prostaglandin E_1 led to a significant augmentation of cyclic AMP, but not of cyclic GMP. The increase in cyclic AMP in the

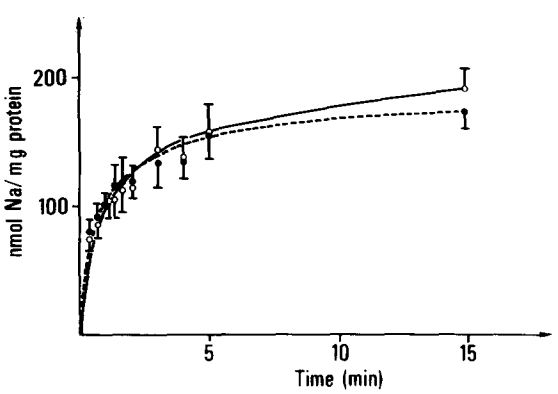

Fig. 4. Na^+ uptake by isolated enterocytes over a time period of 15 min. The values indicated are means ± S.E. of Na^+ uptake when epithelial cells were incubated either with a solution containing 1.2 I.U./ml bovine PTH or its vehicle only. n = 13, P not significant for all time points studied (Student's paired t-test). (●------●) Control, (○———○) bovine PTH.

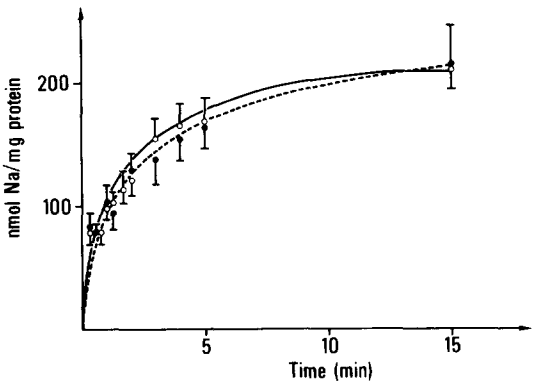

Fig. 5. Same conditions as in Fig. 4 except that bovine PTH was replaced by 2.0 M.R.C. U/ml salmon calcitonin and bovine PTH vehicle by salmon calcitonin vehicle. $n = 11$, P not significant. (●------●) Control, (○———○) salmon calcitonin.

presence of prostaglandin E_1 was already significant at 2 min and still more important at 10 min.

Ca^{2+}-mediated effect of PTH on $^{o}K_{Na}$

To investigate the potential role of Ca^{2+} in the incubation medium in the inhibitory effect of bovine PTH on enterocyte $^{o}K_{Na}$, additional experiments were conducted. In these experiments the effect of bovine PTH or its vehicle was tested on enterocytes incubated in a Ca^{2+}-free solution containing an additional 0.1 mM EGTA concentration. In this situation, bovine PTH exerted no inhibitory effect on $^{o}K_{Na}$ ($3.43 \pm 0.45\ h^{-1}$) when compared to the bovine PTH vehicle ($4.16 \pm 1.31\ h^{-1}$, $n = 7$, P not significant). It should be noted that $^{o}K_{Na}$ in the Ca^{2+}-free medium was comparable to that in the standard Ca^{2+}-containing solution used in the former experiments (4.06 ±

TABLE II

CYCLIC AMP FORMATION IN ISOLATED JEJUNAL CELLS

Results are given as means ± S.E. expressed in pmol/mg DNA.

	Vehicle ($n = 5$)	Prostaglandin E_1 (10 μg/ml) ($n = 5$)	Theophylline (10 mM) ($n = 5$)	Bovine PTH (1.0 I.U./ml)		Salmon calcitonin (2 M.R.C. U/ml)	
				− Theophylline ($n = 5$)	+ Theophylline (10 mM) ($n = 5$)	− Theophylline ($n = 3$)	+ Theophylline (10 mM) (n 3)
Control	265 ± 72	253 ± 66	404 ± 29	327 ± 55	475 ± 78	219 ± 52	347 ± 78
2 min	258 ± 33	682 ± 174 [a]	474 ± 80	347 ± 43	504 ± 67	232 ± 89	281 ± 95
10 min	240 ± 31	857 ± 180 [a]	485 ± 86	490 ± 65	537 ± 84	229 ± 68	370 ± 81

[a] $P < 0.02$.

TABLE III

CYCLIC GMP FORMATION IN ISOLATED JEJUNAL CELLS

Results are given as means ± S.E. expressed in pmol/mg DNA.

	Vehicle ($n = 5$)	Prostaglandin E_1 (10 μg/ml) ($n = 5$)	Theophylline (10 mM) ($n = 5$)	Bovine PTH (1.0 I.U./ml)		Salmon calcitonin (2 M.R.C. U/ml)	
				−Theophylline ($n = 5$)	+ Theophylline (10 mM) ($n = 5$)	− Theophylline ($n = 3$)	+ Theophylline (10 mM) ($n = 3$)
Control	7.9 ± 2.2	9.3 ± 2.3	39.6 ± 13.0	49.5 ± 18.0	60.3 ± 8.8	6.0 ± 2.9	23.0 ± 4.1
2 min	7.3 ± 1.5	11.5 ± 2.7	29.3 ± 6.1	23.7 ± 9.1	62.6 ± 3.8	6.5 ± 1.9	31.7 ± 4.5
10 min	10.5 ± 2.4	12.6 ± 4.0	42.6 ± 6.8	41.1 ± 13.5	87.0 ± 8.5	11.0 ± 4.1	33.3 ± 4.1

0.37 h^{-1}) whereas $^{o}K_{Na}$ in this Ca^{2+}-containing solution was depressed by bovine PTH (3.01 ± 0.19 h^{-1}) as demonstrated above.

Discussion

Besides its well known action on kidney and bone, PTH exerts numerous effects on diverse organs and cell systems such as the nervous system [27,28], the liver [29], myocardium [30–32], the erythropoietic system [33,34], lymphocytes [35] and adipocytes [36]. Calcitonin also exerts its action on several target organs, since multiple effects are well known to exist in the kidney [7,8,37], bone [38] and intestine [10,12–14].

In the kidney, both hormones inhibit the tubular transport of Na^+, bicarbonate and phosphate [3–6]. The mechanisms whereby they exert such an inhibition are uncertain and may vary from one solute to another (decreased lumen-to-bath transport or increased back leakage from tubular epithelium to lumen?). In the case of PTH, recent evidence favors an inhibitory action on transepithelial transport over that of an increase in passive permeability for bicarbonate [6,39–41] and phosphate [42], but not for mannitol and sucrose [43,44].

In the intestine, PTH is generally believed to exert only an indirect action in that it increases calcium absorption by stimulating the renal formation of 1, 25-dihydroxy vitamin D_3. Recently, however, evidence has also been provided by us [9–11] and by others [45] for some direct inhibitory PTH effect on intestinal transport of Na^+ and Ca^{2+}. Calcitonin could inhibit intestinal Na^+ and/or Cl^- [10,12–14] transport either by a direct action on the enterocyte or indirectly. The precise mechanisms of action are not known at present, since most experiments have been conducted using in vivo or in vitro preparations of parts of or the entire intestine.

The use of isolated enterocytes allows more precise investigation of hormone action at the cellular level. The validity of the model in studying transport functions for electrolytes [23,46,47] and electroneutral substances such as sugars [48] has been largely demonstrated in recent years.

The purpose of the present study using isolated enterocytes was to investigate a possible direct action on Na^+ transport, and if so, the mechanism of action of the two hormones, PTH and calcitonin.

Under our experimental conditions, we were unable to demonstrate any direct effect of calcitonin from two different species (salmon and porcine calcitonin) at two different concentrations on $^{o}K_{Na}$ or Na^+ uptake. Thus, it is possible that the previously reported effects on calcitonin on intestinal transport of Na^+ and H_2O [12–14] might be rather indirect [10] or due to excessively high calcitonin concentrations in the incubation medium such as those used by Walling et al. [13]. In contrast to calcitonin, bovine PTH at a concentration of 1.2 I.U./ml induced a 26% decrease in $^{o}K_{Na}$ when compared to control values. This corresponds to approximately half the decrease (45%) in $^{o}K_{Na}$ induced by 4.0 mM ouabain. The inhibitory action of bovine PTH on $^{o}K_{Na}$ of isolated enterocytes appeared to be directed at the ouabain-sensitive Na^+ pump site, since bovine PTH exerted no effect on $^{o}K_{Na}$ in the presence of ouabain. The calculated inhibition of the ouabain-sensitive $^{o}K_{Na}$ by bovine PTH was therefore approx. 73%. In the isolated renal tubule, ouabain has also recently been shown to interfere with the action of PTH in that the former blunted the effect of the latter on phosphate uptake [49]. Moreover, ouabain was also capable of inhibiting the bone-resorbing effect of PTH in mouse calvaria [50]. Thus, the cellular action of PTH appears to involve directly or indirectly the pump responsible for the active extrusion of Na^+ from the cell interior. One might expect that Na^+ entry into the cell would also be altered. However, apparently in contrast to its effect on $^{o}K_{Na}$, bovine PTH exerted no effect on enterocyte Na^+ uptake under the experimental conditions used. However, it must be stressed that the Na^+ uptake examined did not represent an influx of the ion but rather its net accumulation by the cells resulting from the difference between unidirectional influx and efflux. Therefore, a decrease in passive Na^+ permeability of the epithelial cell could well have occurred in the presence of bovine PTH but might have remained undetected due to the experimental design of the Na^+ uptake experiments.

In the present study, the inhibitory action of bovine PTH on the ouabain-sensitive Na^+ pump was independent of a measurable change in enterocyte cyclic AMP or cyclic GMP formation. Conversely, bovine PTH lost its capacity of inhibiting $^{o}K_{Na}$ when the incubation medium was devoid of Ca^{2+}. A com-

parable dependence on the presence of Ca^{2+} in the incubation solution has been recently reported for the effect on PTH on phosphate uptake by renal cortical slices [51]. Therefore, we speculated that bovine PTH inhibition of enterocyte $^{o}K_{Na}$ must involve Ca^{2+}, possibly via an increase in cytosolic Ca^{2+} activity similarly to the hormone's action in other epithelia [52,53]. Furthermore, it has been shown in renal tubular epithelium that an increase in cytosolic Ca^{2+} activity was associated with a decrease in Na^+ transport [54,55]. It is thus possible that cytosolic Ca^{2+} activity, regulated in part through a process of Na^+-Ca^{2+} exchange across the basolateral cell membrane, is important in the physiological regulation of Na^+ transport across epithelial tissues [54].

The effect of bovine PTH on enterocyte $^{o}K_{Na}$ observed in the present study could, therefore, be theoretically explained solely by a modulation of cytos ic Ca^{2+} activity, in the absence of detectable changes of intracellular cyclic AMP or cyclic GMP concentrations, leading to a decrease in the velocity of Na^+ extrusion from the cell (demonstrated) and possibly also of Na^+ entry into the cell (hypothetical, not demonstrated), according to the recently proposed model by Taylor and Windhager [54]. Another possible mode of action of PTH could consist of a primary change in membrane permeability induced by the hormone via structural membrane alterations [56].

Hormonal regulation of the epithelial $(Na^+ + K^+)$-ATPase and hence of transcellular Na^+ transport has been recently proposed for insulin, catecholamines, aldosterone, vasopressin and thyroid hormone [1,2]. In isolated intestinal cells, prostaglandin E_2 has recently been shown to inhibit $^{o}K_{Na}$ [57]. The authors speculated that the observed effect could have been exerted via a direct inhibition of the active transport of Na^+ but they could not exclude a possible primary effect on Na^+ entry at the brush border [57].

Our experiments add a further hormonal candidate, i.e., PTH, for a direct modulation of active Na^+ transport across epithelial cells. The absence of a demonstrable direct effect of calcitonin on Na^+ transport in the present study does not definitely preclude a direct action of this hormone at the intestinal level. Possibly, species differences play a role, or calcitonin exerts an effect on local hormones such as prostaglandins [13] or serotonin [58] which might be stimulated by calcitonin when using Ussing chamber preparations but which might be lost during the procedure of isolating epithelial cells. As to PTH, further studies using isolated cells and cells maintained in culture are required to deepen our understanding of the precise mechanism of action of the hormone on epithelial Na^+ transport and its possible physiological significance.

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