Biochimica et Biophysica Acta 900 (1987) 56–62
Elsevier

BBA 73558

Calcium ionophore-induced changes in HCO_3^- secretion and Cl^- absorption in turtle bladder: relation to action of 3-isobutyl-1-methylxanthine

G. Ehrenspeck [a,b] and C. Voner [b]

[a] *Department of Physiology and Biophysics, Case Western Reserve University, School of Medicine, Cleveland, OH and* [b] *Ohio University, College of Osteopathic Medicine, Athens, OH (U.S.A.)*

(Received 8 December 1986)
(Revised manuscript received 27 February 1987)

Key words: Calcium ionophore; Bicarbonate secretion; Chloride absorption; Isobutylmethylxanthine; Anion transport; cAMP; (Turtle bladder)

The calcium ionophore A23187 stimulates luminal alkalinization and inhibits Cl^- absorption in short-circuited urinary bladders of postprandial or alkalotic turtles. The ionophore appears to mimic the action of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX) by its similar effects on HCO_3^- secretion and Cl^- absorption and by increasing cytosolic cAMP levels of isolated bladder epithelial cells. However, only A23187 (or ionomycin), but not IMBX or cAMP, elevated cytosolic Ca^{2+} of aequorin- or quin2-loaded cells. Since A23187, but not IBMX or cAMP inhibits luminal acidification, we postulate that cytosolic Ca^{2+} (1) regulates the acidification process by a cAMP-independent mechanism and (2) controls HCO_3^- secretion as well as Cl^- absorption, at least in part, via cAMP-mediated pathways.

Introduction

The turtle bladder, like the mammalian cortical collecting tubule [1,2] is capable of alkalinizing its luminal fluid [3–5] and absorbing Cl^- [6]. Luminal alkalinization and Cl^- absorption are independent of Na^+ transport [5,7,8], depend on functional carbonic anhydrase [5,8] and appear to be coupled, in part [9,10], via a $Cl^-:HCO_3^-$ exchange mechanism ([3,4] and Ref. 9 for review). However, the mechanisms of interaction between these two active transport processes and their regulation by intracellular mediators is incompletely understood. That Ca^{2+} and cAMP may influence HCO_3^- and Cl^- transport is suggested by several observations. First, exposure of bladders to the well-known phosphodiesterase inhibitor, IBMX, or IBMX plus exogenous cAMP stimulates luminal HCO_3^- secretion and inhibits Cl^- absorption [5,8,11]. Second, the calcium ionophore, A23187, also accelerates HCO_3^- secretion [12], presumably by elevating cytosolic Ca^{2+} levels. Its action differs, however, from that of maneuvers designed to elevate cytosolic cAMP by also eliciting inhibition of luminal acidification [12,13]. To better understand the actions of A23187 and IBMX on anion transport in the turtle bladder, we more closely examined their effects on HCO_3^- secretion and Cl^- absorption in intact tissues and on cytosolic Ca^{2+} and cAMP levels in isolated bladder cells from turtles with defined metabolic acid-base states.

Abbreviation: IBMX, 3-isobutyl-1-methylxanthine.

Correspondence: G. Ehrenspeck, Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH 44106, U.S.A.

Methods

Pseudemys scripta turtles were kept in flowing water at 30–32°C and fed bovine liver supple-

mented with calcium and multivitamins [5,12]. Turtles, killed 18–48 h after feeding were defined as 'postprandial'; those killed after having been fed by stomach tube 45 mmol/kg per day $NaHCO_3$ for 4 days were defined as 'alkalotic' [5,12].

Methods for measuring transepithelial potential (PD), short-circuit current (I_{sc}), transepithelial resistance (R), and $^{36}Cl^-$ fluxes have been described [7,10]. Depending on the acid-base status of the animal when killed and with Na^+ absorption abolished by ouabain and amiloride, bladders bathed in ($HCO_3^- + CO_2$)-rich medium exhibit either a negative I_{sc} (serosa electronegative to mucosa), which is a measure of the net luminal acidification rate [5,6,8] or a positive I_{sc} (serosa electropositive to mucosa), which is a measure of net electrogenic luminal alkalinization [5,8]. Net Cl^- transport was determined in bladders from postprandial or alkalotic turtles. Time-control experiments confirmed previous findings [7,10] of stable unidirectional $^{36}Cl^-$ fluxes during the 5 h test period. The mucosa-to-serosa and serosa-to-mucosa fluxes were, therefore, determined on adjacent tissue sections from the same bladder.

For cytosolic cAMP and Ca^{2+} determinations bladder epithelial cells were isolated by the method of Schwartz et al. [14], employing collagenase (65 U/ml) and 1% albumin. Cell viability, evaluated with Trypan blue or Acridine orange/ethidium bromide [15] varied from 80 to 95%.

For cAMP determinations, isolated cells were resuspended in the same Na^+ ($Cl^- + HCO_3^-$) medium used for the transport studies and incubated with the test substances at room temperature. The drug exposure interval (7–10 min) coincided with peak changes in the cAMP levels of the isolated cell preparations and was similar to that required for a maximal change in the I_{sc} by IBMX and about 1/3 that for A23187, respectively. Treatment was stopped with the addition of 1 ml ice-cold 6% trichloroacetic acid. Samples were disrupted for 2 min at 0–4° C in a Polytron (Brinkman) and assayed for cAMP by radioimmunoassay (Becton Dickenson). Protein was determined by the method of Bradford [16].

Cytosolic Ca^{2+} was determined by two independent techniques, serving as controls for each other. In one approach, adapted from Borle and Snowdowne [17], packed cells were suspended in an equal volume of Ca^{2+}-free, hypotonic bathing medium containing 10–15 μg aequorin and incubated for 2 min at 4° C. Normal solution osmolarity was then reestablished, trapping aequorin in the cells. After equilibration for 1 h in Ca^{2+}-rich, Na^+ ($Cl^- + HCO_3^-$) medium the aequorin-loaded cells were transferred to a flow-through cuvette, held in place by glass wool, and continuously perfused with or without test agents. Aequorin luminescence was measured by photomultiplier tube [17].

Intracellular Ca^{2+} was also measured with the fluorescent calcium indicator quin2 [18] by a method slightly modified from that of Cannon et al. [19]. Briefly, cells were incubated for 1 h at room temperature in 40 μM quin2/AM in ($HCO_3^- + CO_2$)-free and Ca^{2+}-poor medium (less than 10 μM) to reduce cell clumping, and then washed and resuspended several times in ($HCO_3^- + CO_2$)-free medium containing 2 mM $CaSO_4$. To minimize errors from any quin2 leaking out of cells, the cells ($1–2.5 \cdot 10^6$) were centrifuged for 2 s in an Eppendorf microcentrifuge and resuspended in 2 ml bathing medium immediately before each Ca^{2+} determination. Fluorescence was measured at 22° C with an Aminco-Bowman spectrofluorometer (excitation, 339 nm; emission, 492 nm) equipped with a cuvette stirrer. Calibration of quin2 fluorescence was performed with digitonin (50 μM) and EGTA (25 mM) and cytosolic Ca^{2+} calculated as described by Tsien et al. [18] with a K_d value for quin2 of 105 nM at 22° C [20]. Data were corrected for minor autofluorescence or quench of additives. Because of the intrinsic fluorescence of A23187 [21], the calcium ionophore, ionomycin, was substituted in this series of experiments.

Solutions

Intact bladder epithelia and isolated cells were bathed in Na^+ ($Cl^- + HCO_3^-$) medium containing in mM: NaCl, 21; $NaHCO_3$, 20; Na_2SO_4, 30; KCl, 4; $MgSO_4$, 0.8; $CaSO_4$, 2.0; K_2HPO_4, 0.65; KH_2PO_4, 0.1; glucose 11; osmolality was adjusted to 220 mosM/kg with sucrose; equilibrated with H_2O-saturated 95% O_2/5% CO_2; final pH was 7.2–7.3 at 22–25° C. The cell isolation medium contained in mM: NaCl, 101; KCl, 4; K_2HPO_4, 0.65; KH_2PO_4, 0.1; glucose, 11; sucrose, 20; bub-

bled with 100% O_2 at pH 7.2–7.3 at 22–25°C. In the quin2 experiments substitution of HCO_3^- by SO_4^{2-} and equilibrating the medium with 100% O_2 reduced cell aggregation.

Source of materials

Turtles were obtained from Kons Scientific, Germantown, WI. Ionophore A23187 was a generous gift of Dr. R. Hamill, Eli Lilly & Co., Indianapolis, IN. Ionomycin was purchased from Calbiochem-Behring, San Diego, CA; collagenase (Type IV), bovine serum albumin (Fraction V), and IBMX from Sigma Chemical Co., Saint Louis, MO; and quin2/AM acetoxymethyl ester from Amersham, Arlington Heights, IL.

Results

Effects of A23187 on Cl^- and HCO_3^- transport

Paired bladder sections from postprandial turtles were incubated in Na^+ (Cl^- + HCO_3^-) medium containing 0.2 mM ouabain and 0.1 mM amiloride in the serosal and mucosal fluid, respectively. A23187 added to the mucosal bathing fluid rapidly inhibited the net Cl^- absorption as summarized in Fig. 1 and Table I. The decline of the I_{sc} to zero and its subsequent reversal in polarity together with the fall in the transepithelial resistance illustrate the change from an initial state of net mucosal acidification ($-I_{sc}$) to one of net alkalinization ($+I_{sc}$) [5,8,12]. The calcium ionophore's effect was also examined in bladders of alkalotic turtles (Table I). In these bladders the A23187-sensitive Cl^- transport was (1) similar in magnitude to that of postprandial bladders and (2) comparable to the effect produced by IBMX on Cl^- transport (Fig. 2). IBMX (0.1 mM) also decreased net Cl^- absorption from 13.8 ± 6.4 μA to 2.1 ± 0.9 μA in 90 min ($n = 4$), confirming the results of Durham and Matons [9].

Effect of IBMX and A23187 on cellular cAMP

Since both these agents had similar effects on HCO_3^- secretion and Cl^- absorption, their relative effect on cellular cAMP was compared. As shown in Table II, IBMX or calcium ionophore alone had a small, but statistically insignificant effect on cAMP levels. When present together, however, they significantly increased the level of cAMP.

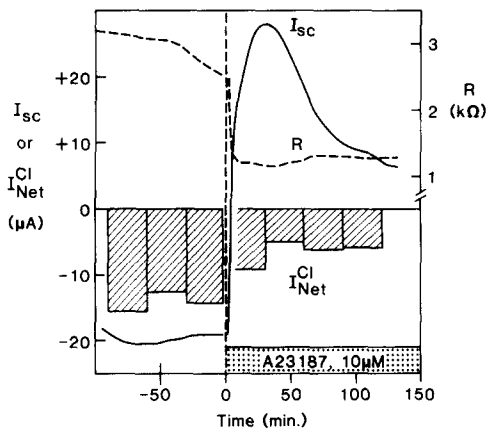

Fig. 1. Effect of A23187 on the anion-dependent I_{sc}, R, and net Cl^- flux. Paired bladder sections (1.5 cm^2) from a postprandial turtle were bathed by symmetrical Cl^--rich, HCO_3^--rich medium containing 0.2 mM ouabain in serosal fluid and 0.1 mM amiloride in mucosal fluid. Ionophore (10 μM) was added to the mucosal fluid. A negative value of I_{sc} denotes a net flow of negative charge from mucosal to serosal fluid or positive charge in the reverse direction. Potential difference can be estimated from $I_{sc} \times R$. Net Cl^- absorption, plotted as columns, was calculated from the unidirectional Cl^- fluxes measured in 30 min intervals [8].

Effects of IBMX and A23187 on cytosolic Ca^{2+}

A typical tracing of the light emission from aequorin loaded cells is shown in Fig. 3. Perfusion of cells for 25–35 min with IBMX failed to increase the aequorin photoluminescence. A23187, on the other hand, produced a rapid rise in the aequorin signal followed by a decline in luminescence upon perfusion of cells with medium devoid of ionophore. IBMX plus 1 mM 8-*p*-chlorophenyl cAMP also had no effect on the aequorin signal (data not shown). The ionophore also elicited an increase in aequorin luminescence when cells were perfused by Ca^{2+}-free medium (Fig. 4). This indicates (1) the existence of an intracellular source of exchangeable Ca^{2+} and (2) that the lack of effect by IBMX on Ca^{2+} levels was not due to a complete depletion of intracellular, exchangeable pools of Ca^{2+} during cell isolation and aequorin incorporation.

The amount of aequorin in the bladder cell preparation was too low for determining baseline Ca^{2+} levels. It was possible, therefore, that the lack of effect of IBMX on cytosolic Ca^{2+} reflected an already elevated cytosolic Ca^{2+} activity result-

TABLE I

EFFECT OF A23187 ON TRANSEPITHELIAL I_{sc}, RESISTANCE, AND Cl^- FLUXES

Mean values ± S.E. for n = number of mated pairs of hemi-bladder sections (1.5 cm^2). Values of unidirectional and net Cl^- flux before ionophore addition were determined from the two 30 min sampling periods immediately prior to addition of drug; values of Cl^- flux after ionophore addition were determined from the data of the two sampling periods 90–150 min after ionophore addition. Net Cl^- fluxes were calculated from the individual flux differences of mated hemi-bladder sections.

State of turtle	Before/after	MS flux (μA)	SM flux (μA)	Net flux (μA)	I_{sc} (μA)	R (kΩ)
Postprandial	before	17.1 ± 6.1	1.8 ± 0.3	15.2 ± 6.0	−20.7 ± 2.4	2.1 ± 0.2
(n = 7)	after	8.8 ± 2.2	3.1 ± 0.7	5.7 ± 2.1	+1.6 ± 4.4	1.4 ± 0.2
	difference	8.3 ± 3.9	1.3 ± 0.5	9.5 ± 4.0	21.3 ± 4.0 *	0.7 ± 0.2
	difference (%)	40.7 ± 5.4 *	72.0 ± 17.9 **	58.6 ± 6.3 *	–	30.8 ± 4.5 *
Alkalotic	before	17.3 ± 4.9	1.9 ± 0.9	15.4 ± 4.5	−12.0 ± 3.3	3.1 ± 0.2
(n = 5)	after	7.1 ± 2.2	2.1 ± 0.6	5.0 ± 3.9	+4.2 ± 4.1	1.9 ± 0.2
	difference	10.2 ± 3.1	0.2 ± 0.5	10.4 ± 2.7	16.2 ± 4.1 **	1.2 ± 0.2
	difference (%)	63.2 ± 9.9 *	26.6 ± 19.6	74.6 ± 2.2 *	–	37.6 ± 6.5 *

* $P < 0.001$;
** $P < 0.01$ according to Student's t-test of paired data.

ing from an imperfectly resealed plasma membrane. This argument is, however, not supported by the results obtained with quin2. Loading of cells with this calcium indicator does not involve osmotic stress.

The mean resting cytosolic Ca^{2+} level of quin2-loaded cells was 112.1 ± 7.3 nM (N = 12), a value similar to that reported for cells of turtle and toad bladder [19,20] and of other systems [18].

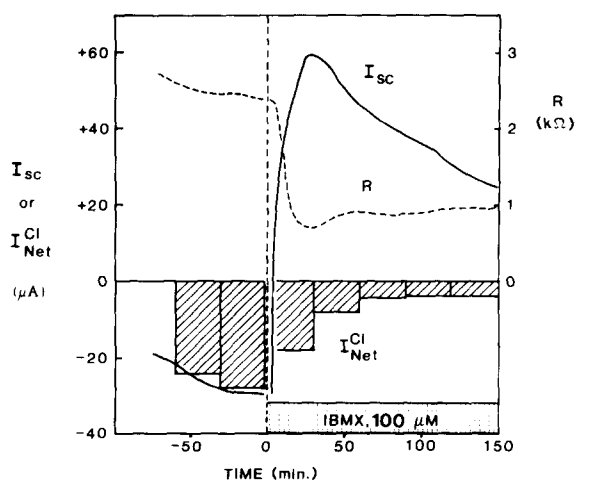

Fig. 2. Effect of IBMX on the anion-dependent I_{sc}, R, and net Cl^- flux of an alkalotic turtle. Sign convention and other experimental details as described in Fig. 1.

Moreover, calcium ionophore again increased the light emission of quin2-loaded cells, whereas IBMX did not (Fig. 5). As verified on suspensions of unloaded cells (Fig. 5) and cell-free bathing media (data not shown), the small elevation in fluorescence intensity after addition of IBMX was caused by IBMX autofluorescence. Forskolin, a potent activator of adenylate cyclase [22] and stimulator of HCO_3^- secretion in the turtle bladder

TABLE II

EFFECT OF IBMX AND A23187 ON CELLULAR cAMP LEVELS

Mean values ± S.E. for n = 7 duplicate cAMP determinations on five separate cell isolations. Relative cAMP levels represent the ratio of cAMP levels in the presence of the indicated agent(s) to that in cells simultaneously incubated without drugs (controls). Control cAMP level was 23.0 ± 6.4 pmol/mg protein. Cells (about $5 \cdot 10^6$ in 1 ml medium) from bladders of four postprandial turtles were incubated 7–10 min at 22–25 °C with 100 μM IBMX and/or 10 μM A23187. At these concentrations the drugs elicited maximal changes in the electrophysiological parameters (See also Refs. 8 and 12).

Condition	Relative cAMP levels
Control	1
+ IBMX	2.0 ± 0.7
+ A23187	1.6 ± 0.4
+ IBMX + A23187	3.1 ± 0.8 *

* $P < 0.05$ compared to control.

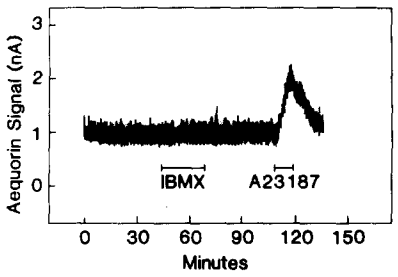

Fig. 3. Effect of IBMX and calcium ionophore on cytosolic Ca^{2+} of aequorin-loaded cells (representative of five experiments). Cells were perfused at room temperature with Na^+ ($Cl^- + HCO_3^-$) medium at 1 ml/min. IBMX (100 μM) or A23187 (1 μM) were added to the perfusate during the time marked by the horizontal lines below the trace. Background current was 0.2 μA.

(unpublished data), but which does not fluoresce, at a concentration of 1 μM also failed to alter the Ca^{2+} levels (data not shown). The relative Ca^{2+} level after IBMX, i.e., the quin2 fluorescence emission after the addition of IBMX to that before IBMX, corrected for IBMX fluorescence, was 1.00 $\pm$ 0.01 ($n = 12$).

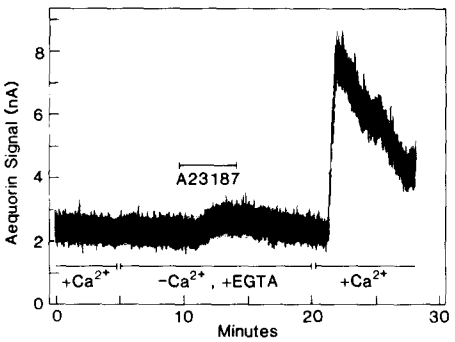

Fig. 4. Effect of A23187 on cytosolic Ca^{2+} of aequorin-loaded cells bathed by Ca^{2+}-free medium (representative of two experiments). Conditions were similar to those described under Fig. 3. Cells were initially perfused with Na^+ ($Cl^- + HCO_3^-$) medium containing Ca^{2+} (2 mM), then in the same medium devoid of Ca^{2+} and containing EGTA (2 mM), and finally with Ca^{2+}-rich medium again. A23187 (10 μM) was added 5 min after cells were exposed to Ca^{2+}-free medium. The increased aequorin signal after replacement of the Ca^{2+}-free perfusion medium with Ca^{2+}-rich solution indicates retention of ionophore by the cells. The lag times of about 1 min in the aequorin signal represent, in part, the time required for replacement of the perfusate in the sample cuvette. The decay in the ionophore-elicted signal is probably due the loss of ionophore from the cells, consumption of aequorin, and reuptake of Ca^{2+} by calcium storage sites.

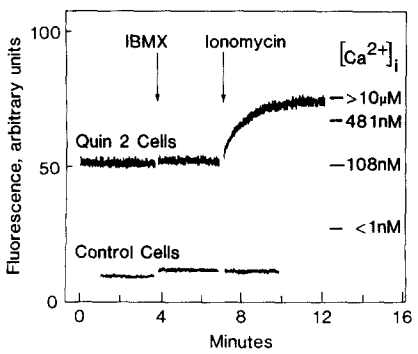

Fig. 5. Effect of IBMX and calcium ionophore on cytosolic Ca^{2+} of quin2-loaded cells. Cuvette containing $1.2 \cdot 10^6$ cells in 2 ml HCO_3^--free NaCl bathing medium. Similar results were obtained with cells bathed by HCO_3^--rich NaCl medium. However, in the HCO_3^--free NaCl bathing medium the cells seemed to aggregate less or more slowly than in HCO_3^--rich medium, resulting in significantly lower noise in the quin2 signal. IBMX and ionomycin were added to give final concentrations of 100 and 1 μM, respectively.

Discussion

In the intact epithelium of the turtle bladder the calcium ionophore A23187 stimulated mucosal alkalinization and inhibited Cl^- absorption in the absence of transepithelial gradients of these anions, thus mimicking the effects of IBMX and cAMP on these transport processes [5,8,9]. The ionophore's stimulation of the alkalinization current and decrease in transepithelial resistance in the presence of ambient Cl^- are similar to its effects on these parameters in the absence of Cl^- [12]. These results are predicted by the hypothesis of Cl^--independent, electrogenic and active HCO_3^- secretion in this tissue [8–10,12].

How Ca^{2+} and cAMP modify HCO_3^- and Cl^- transport is unclear. The fall in transepithelial resistance evoked by A23187 and IBMX in the present study was similar in magnitude to that elicited by these agents under Cl^--free bathing conditions and is consistent with other evidence [5,12] for an increase in a HCO_3^--selective conductance in the apical membrane [11,23]. An increase in apical Cl^- permeability could give rise to the increased serosa-to-mucosa Cl^- flux after A23187 (Table I) or IBMX/cAMP [9], but cannot account for the major inhibition of the mucosa-to-serosa Cl^- flux. A cAMP-sensitive HCO_3^--selective con-

ductance [11], a relatively nonselective conductance for Cl^- and HCO_3^- [23], and a Cl^- pump [9] have been postulated for the apical membrane. The postulated plasma membrane location of these anion pathways, however, is based on indirect methods [9–11,23]. Conclusive evidence of their localization requires the development of specific techniques for measuring the electrical parameters and Cl^- levels of cells that make up less than 15% of the turtle bladders' epithelium [14].

Although in the intact epithelium IBMX or A23187 each changed electrical transport parameters in less than 1 min, in isolated cells they did not elevate cAMP to significant levels, when added separately. This result, however, is not unexpected: (1) Resting adenylate cyclase activity is probably relatively low so that inhibition of phosphodiesterase by IBMX would result in localized increases of cAMP sufficient to modify transport, but too small to detect by a measurement of total cellular cAMP. (2) In the presence of functional phosphodiesterase, probably activated by A23187 via Ca-calmodulin (24), a stimulation of adenylate cyclase by A23187-elevated Ca^{2+} would be expected to yield a transient increase in cAMP, difficult to detect when averaged over the entire cell population [25,26]. Conversely, upon inhibition of phosphodiesterase by IBMX, a Ca^{2+}-mediated stimulation of adenylate cyclase would be expected to produce a measurable increase in cellular cAMP content. This prediction was confirmed by the data of Table II.

Of major interest are the findings that (1) A23187 in the presence of IBMX caused a small, but significant increase in cellular cAMP, and (2) IBMX and cAMP did not alter the cytosolic Ca^{2+} levels of aequorin- or quin2-loaded cells. The apparent additive nature of the actions by ionophore and IBMX on cellular cAMP is consistent with the increased stimulation of the ionophore-induced alkali secretion by IBMX [12]. How Ca^{2+} increases cAMP in the turtle bladder is not known. Based on its actions in other systems, one route could be activation of adenylate cyclase via a calmodulin dependent pathway [24]. A norepinephrine-sensitive adenylate cyclase and cAMP-dependent protein kinase have been found in the apical membrane fraction of turtle bladder epithelial cells [27], but their involvement in the regulation of anion transport has not been established. Regulation of Na^+ transport, however, appears unlikely, since IBMX was found to have no effect on the Na^+-dependent I_{sc} [8].

Whether the change in cAMP associated with a change in Ca^{2+} is a unique or essential step in the sequence of events leading to stimulation of alkali secretion and inhibition of Cl^- absorption or whether Ca^{2+} can also alter these transport processes directly or by other routes (e.g., via Ca^{2+}-dependent protein kinase C) remains unclear. Pretreatment of bladders with cordycepin or N^6-phenylisopropyl adenosine, inhibitors of adenylate cyclase in fat cells [28], failed to demonstrate a requirement for cAMP, since these compounds did not inhibit the alkali secretion current.

A major problem at present is the sparsity of firm information on hormonal messengers that might influence HCO_3^- secretion and Cl^- absorption in the turtle bladder by cAMP or Ca^{2+}-mediated pathways and which would help in the identification of the type of synarchic regulation [24] present. It was recently reported, however, that vasoactive intestinal peptide stimulated HCO_3^- secretion. Since vasoactive intestinal peptide mimicked the effect of exogenous cAMP, including its failure to inhibit acidification [11], it would not be expected to elevate Ca^{2+} levels. This prediction, consistent with the hormone's action in other systems [29] was confirmed in quin2-loaded cells in which the relative Ca^{2+} activity (Ca^{2+} after/Ca^{2+} before vasoactive intestinal peptide) was 0.98 ± 0.02 ($n = 3$).

Although cytosolic Ca^{2+} was determined in cell suspensions consisting of several epithelial cell types [14,31], available evidence [19] suggests that the Ca^{2+} levels observed with quin2, in large part, reflect those of the cells thought to be responsible for acid-base transport, the so-called mitochondrion-rich cells [11,14,19,31]. These cells, which also contain high levels of carbonic anhydrase [14], a potent esterase, were found by Cannon et al. [19] to exhibit a preferential and acetazolamide-sensitive loading of quin2. The membrane-permeant ester of this Ca^{2+} indicator is cleaved by cytoplasmic esterase activity, trapping the dye inside the cells [18].

In summary, the results indicate a correlation between changes in cellular cAMP and Ca^{2+} and

changes in HCO_3^- secretion and Cl^- absorption. In light of earlier findings that (1) calcium ionophore A23187 [12,13], but not IBMX and/or exogenous cAMP [5,8,9,23,30], inhibits mucosal acidification, (2) whereas both A23187 [12] and IBMX (and cAMP) [5,8,23] stimulate mucosal alkalinization, we propose as a working hypothesis that cytosolic Ca^{2+} regulates the acidification process by cAMP-independent paths and regulates HCO_3^- secretion and Cl^- absorption, in part, by modulating cAMP levels. The present findings form the basis for subsequent biochemical analyses of the sequence of molecular events leading to the regulation of HCO_3^- and Cl^- transport in this urinary epithelium.

Acknowledgements

The authors thank Dr. K.W. Snowdowne for the gift of aequorin and help in the aequorin studies and Dr. A.B. Borle for the use of his laboratory, Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261. This study was supported by a grant-in-aid from the American Heart Association with funds contributed, in part, by the Central Ohio Chapter.

References

1 McKinney, T.D. and Burg, M.B. (1978) J. Clin. Invest. 61, 1421–1427
2 Stone, D.K., Seldin, D.W., Kokko, J.P. and Jacobson, H.R. (1983) J. Clin. Invest. 71, 1505–1508
3 Cohen, L. (1980) Am. J. Physiol. 239, F167–174
4 Leslie, B.R., Schwartz, J.H. and Steinmetz, P.R. (1973) Am. J. Physiol. 225, 610–617
5 Satake, B., Durham, J.H., Ehrenspeck, G. and Brodsky, W.A. (1983) Am. J. Physiol. 244, C259–269
6 Brodsky, W.A. and Schilb, T.P. (1966) Am. J. Physiol. 210, 987–996
7 Brodsky, W.A., Durham, J. and Ehrenspeck, G. (1979) J. Physiol. 287, 559–573
8 Ehrenspeck, G. (1982) Biochem. Biophys. Acta 684, 219–227
9 Durham, J.H. and Matons, C. (1984) Biochim. Biophys. Acta 769, 297–310
10 Ehrenspeck, G. and Voner, C. (1985) Biochim. Biophys. Acta 817, 318–326
11 Brodsky, W.A., Matons, C. and Durham, J.H. (1985) Fed. Proc. 44, 1365
12 Ehrenspeck, G. (1983) Biochim. Biophys. Acta 732, 146–153
13 Arruda, J.A.L. (1979) Pflugers Arch. 381, 107–111
14 Schwartz, J.H., Bethencourt, D. and Rosen, S. (1982) Am. J. Physiol. 242, F627–F633
15 Lee, S.-K., Singh, J. and Taylor, R.B. (1975) Eur. J. Immunol. 5, 259–262
16 Bradford, M. (1976) Anal. Biochem. 72, 248–254
17 Borle, A.B. and Snowdowne, K.W. (1984) Am. J. Physiol. 247, C396–C408
18 Tsien, R.Y., Pozzan, T. and Rink, T.J. (1982) J. Cell. Biol. 94, 325–334
19 Cannon, C., Van Adelsberg, J., Kelly, S. and Al-Awqati, Q. (1985) Nature 314, 443–446
20 Crutch, B. and Taylor, A. (1983) J. Physiol. 295, 109P.
21 Case, G.D., Vanderkooi, J.M. and Scarpa, A. (1974) Arch. Biochem. Biophys. 162, 174–185
22 Seamon, L.B. and Daly, J.W. (1981) J. Cyclic Nucleotide Res. 7, 201–224
23 Stetson, D.L., Beauwens, R., Palmisano, J., Mitchell, P.P. and Steinmetz, P.R. (1985) Am. J. Physiol. 249, F546–F552
24 Rasmussen, H. (1981) Calcium and cAMP as synarchic messengers. pp. 86–92, John Wiley & Sons, New York
25 Field, M., Sheerin, H.E., Henderson, A. and Smith, P.L. (1975) Am. J. Physiol. 229, 86–92
26 Schimmel, R.J. (1984) Am. J. Physiol. 246, C63–C68
27 Brodsky, W.A., Cabantchik, Z.I., Davidson, M., Ehrenspeck, G., Kinne-Saffran, E.M. and Kinne, R. (1979) Biochim. Biophys. Acta 556, 490–508
28 Fain, J.N., Pointer, R.H. and Ward, W.F. (1972) J. Biol. Chem. 247, 6866–6872
29 Said, S.I. (1980) in Gastrointestinal Hormones (Glass, G.J.G., ed.), pp. 246–265, Raven Press, New York
30 Sabatini, S. (1985) Kidney Int. 27, 25–30
31 Stetson, D.L. and Steinmetz, P.R. (1985) Am. J. Physiol. 249, F553–F565