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EFFECT OF CALCIUM IONOPHORE A23187 ON ELECTROGENIC ACID-BASE TRANSPORT IN TURTLE BLADDER

INHIBITION OF ACIDIFICATION AND STIMULATION OF ALKALINIZATION

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Turtle bladders bathed on both surfaces with identical HCO_3^- / CO_2-rich, Cl^--free Na^+ media and treated with ouabain and amiloride exhibit a transepithelial potential serosa electronegative to mucosa and a short-circuit current (I_{sc}) which is a measure of the net luminal acidification rate. Addition of calcium ionophore A23187 (10 μM) to the mucosal side of the epithelium rapidly reverses the direction of the potential difference and I_{sc} and decreases tissue resistance. The resulting positive I_{sc} resembles that previously observed in response to isobutylmethylxanthine (IBMX) and cAMP analogs. Reversal of the I_{sc} is enhanced in bladders from severely alkalotic turtles. In contrast, in severely acidotic turtles, ionophore A23187 decreases, but does not reverse, the I_{sc}. The data suggest that, like IBMX and cAMP analogs, the Ca ionophore stimulates an electrogenic alkalinization mechanism, but, unlike the former agents, inhibits the concurrent acidification process as well.

Introduction

The urinary bladder of the fresh water turtle is capable of acidifying [1,2] and alkalinizing [3,4] the luminal fluid in vitro. Moreover, its acid-base transport [3,4], like that of the toad bladder [5], the rabbit cortical collecting tubule [6] and the isolated kidney [7], is conditioned by the acid-base state of the donor animal. However, the mechanisms for acidification and alkalinization and their physiologic control processes have been only partly delineated.

The recent use of the phosphodiesterase inhibitor, 3-isobutyl-1-methyl-xanthine (IBMX), and cAMP derivatives provided evidence that net acid-base transport in the turtle bladder includes a cAMP-mediated alkali secretion [4,8]. These findings prompted the present study of the effect of the ionophore A23187 on acid-base transport in the turtle bladder. The rationale was that the use of ionophore A23187, which is specific for divalent cations [9,10] and is a widely used pharmacologic tool for studying the influence of elevated cytosolic Ca^{2+} levels on physiologic processes, might help to: (1) characterize further the physiologic control of acid-base transport; and (2) provide another experimental approach for examining the properties of the acid-base transport processes in the turtle bladder.

Abbreviations: IBMX, 3-isobutyl-1-methylxanthine; SITS, 4-acetamido-4′-isothiocyano-2,2′-disulfonic stilbene.

Methods

Pseudemys scripta turtles were kept for 2–4 weeks in flowing water maintained at 30–32°C, which is near the turtles' preferred body temperature [11], fed vitamin and calcium-supplemented beef liver twice weekly, and exposed to artifical sunlight on a 12 h cycle. Donor turtles weighing 750-1000 g were killed under three conditions: (1) 1–4 days after the previous feeding of meat, when the turtles were in a post-prandial state characterized by a post-prandial alkaline tide [12]; (2) after giving turtles 45 mmol/kg per day $NaHCO_3$ to induce severe metabolic alkalosis; (3) after giving turtles 15 mmol/kg per day NH_4Cl to induce severe metabolic acidosis. $NaHCO_3$ and NH_4Cl were given by gavage for 4 days [4].

Electrophysiologic measurements. All techniques and procedures for surgical removal, mounting and short-circuiting of the urinary bladders, together with those for evaluating transepithelial potential, short-circuit current (I_{sc}), and tissue resistance have been described [8,17]. Except where noted, bladders were bathed on both surfaces with identical HCO_3^--rich or HCO_3^--poor Na^+-Ringer solutions devoid of exogenous Cl^-. Routinely, ouabain (0.2 mM) was added to the serosal fluid and amiloride (5 μM) to the mucosal fluid. These agents inhibit Na^+ transport without inhibiting acid-base transport in short-circuited bladders [13–15]. Increases in the amiloride concentration to 100 μM in some experiments did not modify the effect of the Ca ionophore.

Solutions. The Ringer solutions used had the following composition (mM): *Solution A* (complete HCO_3^--rich medium): Na_2SO_4, 40.5; $NaHCO_3$, 20; K_2SO_4, 2.0; $MgSO_4$, 0.8; K_2HPO_4, 0.65; KH_2PO_4, 0.1; $CaSO_4$, 2.0; glucose, 11; osmolality was adjusted to 220 mosM/kg with sucrose; equilibrated with H_2O-saturated 98% 0_2/2% Co_2; final pH 7.6 ± 0.1 at 22–25°C. *Solution B* (modified HCO_3^--rich medium): Similar to the above medium, except for the omission of the salts of K^+, Mg^{2+} and P_i. After confirming the finding that the lack of K^+, Mg^{2+}, and P_i had no deleterious effect on the electrical parameters associated with the acid-base transport [8], solution B was routinely used. *Solution C* (HCO_3^--poor medium): composition was similar to that of solution A, except that $NaHCO_3$ was replaced by Na_2SO_4 without changing the concentration of Na^+; osmolality was readjusted with sucrose; equilibrated with humidified 100% 0_2; final pH 7.6 ± 0.1 at 22–25°C. This solution, when bathing the bladder tissue, is not HCO_3^--free, despite vigorous bubbling with 0_2, because small quantities of HCO_3^- are unavoidably introduced by fine adjustments of the pH with NaOH and the continuous influx of metabolic CO_2 [16]. The free Ca^{2+} in solutions A,B and C, measured with a radiometer Ca^{2+}-selective electrode, was about 0.8 mM.

Calcium ionophore was routinely added to the mucosal fluid. Stock solutions of ionophore A23187 (or monensin) were prepared in absolute ethanol. The final concentration of ethanol in the bathing fluid generally was 0.2% and did not exceed 2%. Preliminary experiments indicated that addition of ethanol alone at these concentrations had no effect on the measured electrical parameters.

Source of materials. Ionophore A23187 was a generous gift of Dr. R. Hamill, Eli Lilly&Co., Indianapolis, IN. Monensin was a gift of Dr. M. Charlton, Ohio University, Athens, OH. Turtles were obtained from Lemberger Assoc., Germantown, WI. IBMX and ethoxyzolamide were purchased from Sigma Chemical Co., Saint Louis, MO. Oxygen and analyzed 2% CO_2 in O_2 mixture were obtained from De Lille Oxygen Co., Columbus, OH.

Analysis of Data. Results are presented as means ± S.E., and statistical significance was determined by Student's *t*-test of paired or unpaired variates, with values of $P < 0.05$ considered significant.

Results

Effect of ionophore A23187 on potential, I_{sc}, and tissue resistance and its independence of Na^+ transport

Bladders from post-prandial turtles bathed by HCO_3^--rich, Cl^--free media containing ouabain and amiloride generated a spontaneous potential difference serosa negative to mucosa (Fig. 1 and 2). The associated I_{sc} provided a measure of the luminal acidification rate and HCO_3^- absorption [17–19]. Addition of the ionophore A23187 (10 μM) to the mucosal fluid resulted in a rapid

decline of the potential and I_{sc} to zero followed by a reversal in their polarity within 30 min. The changes in the potential and I_{sc} were accompanied by a decrease in resistance. The results are summarized in Table I (top row).

Addition of (10 μM) ionophore A23187 to the serosal fluid of seven bladders made the I_{sc} of -13.5 ± 3.3 μA less negative by 2.1 ± 0.5 μA ($P < 0.001$) after 30 min. The subsequent addition of ionophore to the mucosal fluid or the elevation of its concentration in the serosal fluid 5- to 10-fold reversed the I_{sc}. Thus, the ionophore is more effective when added on the mucosal side of the bladder.

The reversibility of the the ionophore A23187-elicited effect was examined in three experiments. Serial dilution of the mucosal fluid by more than 10^4-fold with fresh medium made the reversed I_{sc} less positive, but failed to restore the I_{sc} to control values.

Since ionophore A23187 allows Ca^{2+}:H^+ exchange, another carboxylic ionophore, monensin, which permits the exchange of monovalent alkali metal ions and H^+ (9) was tested. It had essentially no effect on the I_{sc} (Fig. 1). The I_{sc} before and 30 min after exposure of four bladders to 10 μM monensin was -14.4 ± 3.9 μA and -13.9 ± 3.9 μA, respectively ($P > 0.1$). Nor did higher concentrations of monensin (100 μM) reproduce the effect of ionophore A23187. These results suggest that the effects of ionophore A23187 are evoked primarily by changes in intracellular Ca^{2+} and not H^+ distribution.

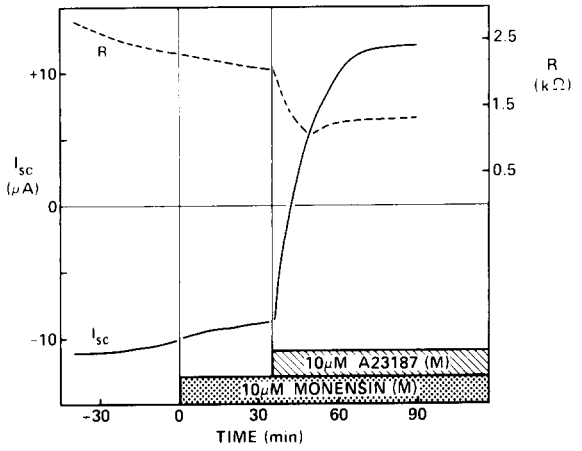

Fig. 1. Effect of monensin and ionophore A23187 on the I_{sc} and R of a bladder from a post-prandial turtle. Bladder was bathed by symmetrical HCO_3^--rich, Cl^--free bathing medium containing 0.2 mM ouabain in serosal fluid (S) and 5 μM amiloride in mucosal fluid (M). Values of I_{sc} (solid line) and resistance (R) (dashed line) shown are for 1.5 cm^2 area of tissue. A negative value of control I_{sc} indicates that serosa is negative relative to mucosa and denotes a net flow of negative charges from mucosal to serosal fluid or positive charges in the reverse direction. Potential difference has been omitted for clarity but can be estimated from $I_{sc} \cdot R$.

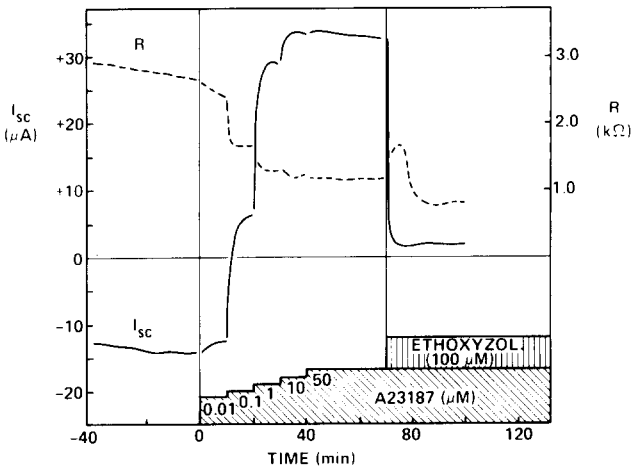

Fig. 2. Concentration-response relationship for the effect of ionophore A23187 on the I_{sc} and resistance (R) of a bladder from a severely alkalotic turtle. I_{sc} is depicted by the solid line and resistance by the dashed line. A23187 was added to mucosal fluid; ethoxyzolamide to mucosal and serosal fluid. Sign convention and other experimental details as described in Fig. 1.

The dose-dependent manner of action by ionophore A23187 is illustrated in Fig. 2. As shown, exposure of bladders to 10 μM ionophore usually resulted in a maximal or near-maximal physiologic response. This was checked in some experiments by raising the ionophore concentration 5-fold.

Effect of ethoxyzolamide on the reversed I_{sc}

Also shown in Fig. 2. is that the inhibitor of carbonic anhydrase, ethoxyzolamide (0.1 mM) in both mucosal and serosal bathing fluids rapidly decreased the reversed I_{sc}. In general, the reversed I_{sc} declined $90.7 \pm 2.8\%$ ($n = 16$) in post-prandial turtles and $88.7 \pm 1.8\%$ ($n = 8$) in alkalotic turtles in 10–15 min. The degree of inhibition is identical to that produced by this inhibitor as well as acetazolamide in IBMX-treated bladders [8].

Effect of ionophore A23187 on bladders bathed by Ca^{2+}-free, mucosal fluid

Bladders were bathed by solution B on the serosal surface and an identical solution on the mucosal surface, except for the omission of Ca^{2+} and the addition of EGTA (2mM) for about 45 min before the addition of ionophore. The data indicate that luminal Ca is not essential for the ionophore-evoked reversal of the I_{sc} (Table I, row 2). (The larger S.E.'s reflect the existence of a positive control I_{sc} in two bladders and an ionophore-evoked, positive I_{sc} of +29 and +46 μA in two others.)

Effect of ionophore A23187 before and after sequential isohydric additions of HCO_3^-

The experiments described above provide no information on the relative effects of ionophore A23187 on the two discrete acid-base ion flows believed to coexist during net mucosal acidification or alkalinization in post-prandial bladders and generating the net charge flow, or I_{sc} [17,18]. Therefore, an experimental protocol used previously [8] was adopted. In what follows, the two postulated ion flows, which may involve the active translocation of H^+, OH^- or HCO_3^-, for convenience will be referred to as HCO_3^- absorption and HCO_3^- secretion.

Paired bladder sections were initially bathed on both surfaces by HCO_3^--poor solution C (plus ouabain and amiloride); one section was exposed to the ionophore, the other served as the control. After 35–45 min exposures of bladders to ionophore, 20 mM HCO_3^- was added isohydrically first on the mucosal side and after another 35–45 min also on the serosal side of both experimental and control tissues. The results are shown in Fig. 3, which illustrates that ionophore A23187: (1) decreased the residual net HCO_3^- absorption defined by the I_{sc} prevailing when the bathing fluids

TABLE I

EFFECT OF IONOPHORE A23187 ON THE TRANSEPITHELIAL POTENTIAL, I_{sc} AND RESISTANCE OF BLADDERS FROM POST-PRANDIAL OR ALKALOTIC TURTLES

Mean values ± S.E. of parameters are for 1.5 cm^2 area of tissue. Sign convention for potential and I_{sc}: polarity of serosa relative to mucosa. Solution described in Methods. Values of potential, I_{sc} and resistance (R) after ionophore addition were obtained at the time of maximal response, 24 ± 3 min for tissues bathed by HCO_3^--rich media and 40–45 min for tissues bathed by HCO_3^--poor media.

Condition of turtle	Bathing medium	Before/after ionophore	Potential (mV)	I_{sc} (μA)	R (kΩ)
Post-prandial	HCO_3^--rich ($n = 19$)	before	−17.0 ± 2.9	−8.3 ± 1.7	2.4 ± 0.2
		after	+6.5 ± 2.0	+4.3 ± 1.6	1.5 ± 0.1
		difference	23.6 ± 3.4	12.7 ± 1.6	0.9 ± 0.2
	Medium B, Mucosal soln.: $-Ca^{2+}$, + EGTA	before	−6.4 ± 5.0	−2.9 ± 2.2	1.6 ± 0.3
		after	+14.5 ± 3.0 [a]	+13.3 ± 6.2 [b]	1.1 ± 0.2 [a]
	($n = 7$)	difference	20.9 ± 6.5	20.0 ± 7.2	0.5 ± 0.2
	HCO_3^--poor ($n = 5$)	before	−12.0 ± 1.8	−3.5 ± 0.4	3.4 ± 0.2
		after	−2.4 ± 0.8	−1.2 ± 0.4	2.1 ± 0.2
		difference	9.7 ± 1.7	2.4 ± 0.6	1.3 ± 0.2
Alkalotic	HCO_3^--rich ($n = 10$)	before	−9.1 ± 4.6	−3.8 ± 2.2	2.4 ± 0.2
		after	+28.0 ± 3.9	+22.3 ± 3.4	1.3 ± 0.1
		difference	37.2 ± 6.0	26.1 ± 3.8	1.1 ± 0.2

[a] According to the Student's t-test of paired data different from control ($P < 0.02$).

[b] Different from control ($P < 0.05$). All other effects of the ionophore were significant at the $P < 0.001$ level.

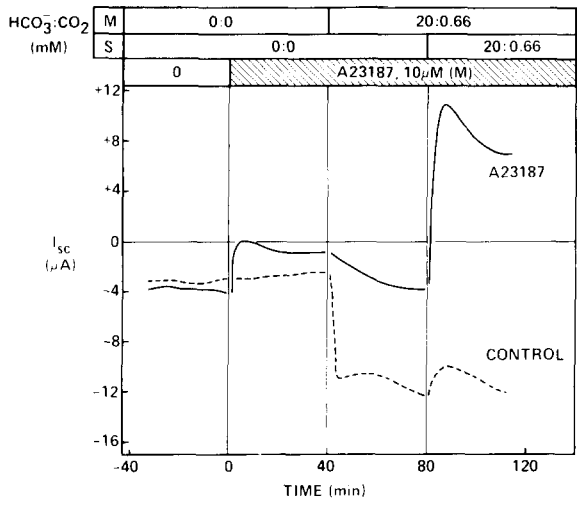

Fig. 3. Effect on the I_{sc} by sequential isohydric additions of HCO_3^- and CO_2 to mucosal (M) and serosal (S) fluids of an ionophore A23187-treated bladder. Data shown are from paired bladder sections from a post-prandial turtle; one section was exposed to A23187 (solid line), the other received diluent (dashed line). Sign convention and other experimental details as in Fig. 1.

TABLE II

COMPARISON OF BLOOD AND URINE pH VALUES OF POST-PRANDIAL, NH_4Cl-LOADED AND $NaHCO_3$-LOADED TURTLES

Mean values ± S.E. for (n) animals in each group. Turtles were considered acidotic when at the optional time of death the blood and urine pH values were less than those of post-prandial turtles; similarly, turtles were judged alkalotic when the blood and urine pH were higher than those of post-prandial turtles [4].

Condition of turtle	Blood pH	Urine pH
Acidotic ($n = 6$)	7.27 ± 0.04 [a]	4.34 ± 0.44 [b]
Post-prandial control ($n = 22$)	7.69 ± 0.02	5.68 ± 0.42
Alkalotic ($n = 6$)	7.81 ± 0.03	7.99 ± 0.09

[a] Different from post-prandial and alkalotic turtles ($P < 0.001$).
[b] Different from post-prandial turtles ($P < 0.05$) and alkalotic turtles ($P < 0.001$).

were HCO_3^--poor; (2) depressed the accelerated net HCO_3^- absorption in the presence of 20 mM HCO_3^- and 2% CO_2 in the mucosal fluid; but (3) did not reverse the I_{sc} when exogenous HCO_3^- and CO_2 were lacking in the serosal fluid. Additional data for the initial period, when bladders were bathed in HCO_3^--poor fluids, are given in Table I, row 3. As shown in the final period of Fig. 3 and replicated in other bladders, the isohydric addition of $HCO_3^- + CO_2$ to the serosal fluid elicited a rapid reversal of the I_{sc} in the absence of transepithelial HCO_3^-, p_{CO_2}, and pH gradients. This phenomenon resembles that observed in IBMX-treated bladders [8].

Effect of ionophore A23187 on bladders from acidotic and alkalotic turtles

To characterize more precisely the effects of A23187 on HCO_3^- absorption and secretion, A23187 was added to bladders from turtles made severely alkalotic or acidotic and interposed between identical ($HCO_3^- + CO_2$)-rich solutions. The rationale was that bladders from severely alkalotic turtles would be preconditioned for amplified net HCO_3^- secretion and those from severely acidotic turtles would be preconditioned for amplified net HCO_3^- absorption, so that the effects of the ionophore could be evaluated under conditions in which one of the two transport processes predominated, the other making only a small or negligible contribution to the I_{sc}. The blood and urine pH values of such preconditioned animals are summarized in Table II.

In bladders from alkalotic turtles (Table I, row 4), the initial negative I_{sc} was on average half the control I_{sc} generated by bladders from post-prandial turtles. Two tissues exhibited a positive control I_{sc} for 220 min. The ionophore caused a dramatic change in the I_{sc}, supporting the notion that one effect of ionophore A23187 is the stimulation of alkali secretion. A mere ionophore-induced unmasking of HCO_3^- secretion due to inhibition of HCO_3^- absorption (see below) would be inconsistent with the fact that in the alkalotic turtles in which HCO_3^- absorption should be greatly reduced or nearly abolished, the ionophore-induced change in the I_{sc} was greatest.

Bladders from acidotic turtles (Table III) exhibited a mean negative control I_{sc} which is 3-times that of bladders from post-prandial turtles. The

TABLE III

COMPARISON OF EFFECTS OF IONOPHORE A23187 AND IBMX ON POTENTIAL, I_{sc}, AND RESISTANCE OF BLADDERS FROM ACIDOTIC TURTLES

Mean values ± S.E. ($n = 6$) of potential, I_{sc}, and resistance (R) before and 45 min after addition of either ionophore A23187 or IBMX. Sign convention, experimental conditions, and statistical analysis are given in Table I.

Agent	Period	Potential (mV)	I_{sc} (μA)	R (kΩ)
Ionophore A23187 (10 μM)	Before	−52.6 ± 13.3	−26.4 ± 6.3	2.1 ± 0.2
	After	−20.5 ± 6.7 [a]	−13.4 ± 3.9 [a]	1.6 ± 0.2 [a]
	Difference	32.1 ± 6.8	13.1 ± 2.5	0.5 ± 0.2
IBMX (100 μM)	Before	−43.7 ± 13.9	−26.0 ± 8.4	1.8 ± 0.2
	After	−30.2 ± 9.0 [b]	−26.6 ± 9.1 [c]	1.3 ± 0.2 [a]
	Difference	13.6 ± 5.3	0.5 ± 1.6	0.5 ± 0.2

[a] Different from control ($P < 0.001$).
[b] Different from control ($P < 0.01$).
[c] Not different from control ($P > 0.4$).

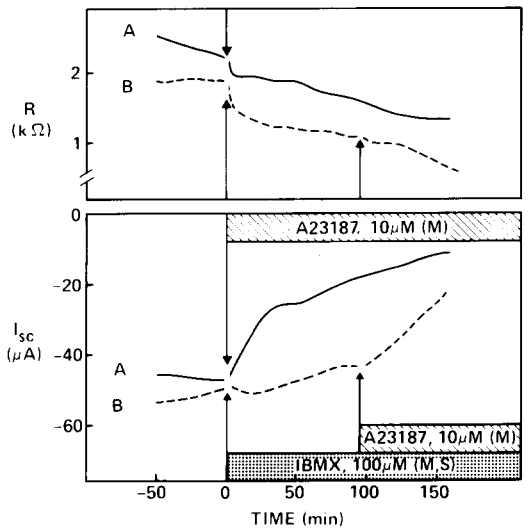

Fig. 4. Comparison of the effects of ionophore A23187 and IBMX on the I_{sc} and resistance (R) of a bladder from a severely acidotic turtle. Data shown are from paired tissue sections A and B of a bladder, with the changes in the I_{sc} elicited by A23187 (curve A) and by IBMX alone and then IBMX plus ionophore A23187 (curve B) shown in the lower panel. IBMX was added to both mucosal and serosal fluid. The corresponding changes in resistance of tissue sections A and B are depicted in the upper panel. Sign convention and other experimental details as in Fig. 1.

addition of ionophore to one member of paired membranes made the I_{sc} less negative by 53.8 ± 3.9% in 45 min. The other paired section received a supramaximal dose of IBMX (0.1 mM). Since IBMX stimulates HCO_3^- secretion without inhibiting the concurrent HCO_3^- absorption [4,8], a failure by IBMX to make the I_{sc} less negative would tend to exclude HCO_3^- secretion as a major component of the I_{sc} in these bladders. This was found: IBMX in no instance made the I_{sc} less negative (Fig. 4, curve B; Table III, row 2). To verify the inability of IBMX to make the I_{sc} less negative, the 8-(4-chlorophenylthio) derivative of cAMP (1 mM in serosal fluid) which rapidly increases the positive I_{sc} of IBMX-treated post-prandial or alkalotic bladders [4,8], was added to four IBMX-treated acidotic bladders. Like IBMX, the cAMP analog had essentially no effect, with the mean I_{sc} becoming insignificantly more negative (1.8 ± 4.6%, $P > 0.9$), which confirms previous findings [4]. The final addition of ionophore to the IBMX- or (IBMX + cAMP)-treated bladders, however, made the I_{sc} less negative by 50.6 ± 10.0% in 45 min ($n = 6$). These results further support the conclusion that a second effect of A23187 is the inhibition of the acidification mechanism.

Finally, the ionophore-induced change in tissue resistance depended on acid- or alkali-loading of the turtles. The mean ionophore-evoked decrease in resistance after 30 min was 21.6 ± 6.2% ($n = 6$) in bladders of acidotic turtles and 45.8 ± 4.0% ($n = 10$), in those of alkalotic turtles, which is significantly larger ($P < 0.01$).

Effect of IBMX on the ionophore A23187-elicited reversed I_{sc}

Since the ionophore A23187-evoked I_{sc} resembled that produced by IBMX [8], it was determined whether IBMX could further increase the reversed I_{sc} of ionophore-treated bladders. In bladders from post-prandial turtles, IBMX (0.1 mM) increased the reversed I_{sc} of +5.0 ± 1.5 μA by 4.1 ± 2.2 μA ($P < 0.02$, $n = 5$). In bladders from alkalotic turtles, IBMX increased the reversed I_{sc} of +16.2 ± 4.1 μA by 6.7 ± 2.6 μA ($P < 0.001$, $n = 7$).

Discussion

Calcium ionophore A23187 in the mucosal bathing solution of bladders of post-prandial or alkalotic turtles produces a rapid reversal in the (HCO_3^- + CO_2)-dependent, (amiloride + ouabain)-insensitive I_{sc}. The resulting reversed, positive I_{sc} closely resembles that evoked by maneuvers to increase cellular cAMP levels by the phosphodiesterase inhibitor, IBMX, and cAMP derivatives [4,8]. Since previous pH-stat studies demonstrated that the generation of or increase in the positive I_{sc} by IBMX and cAMP is associated with a parallel increase in the rate at which titratable alkali enter the mucosal fluid [4], it is concluded that the reversed, positive I_{sc} after addition of ionophore A23187 reflects active, mucosal alkalinization (HCO_3^- secretion or H^+ absorption).

The notion of active, conductive alkali secretion stimulated by ionophore A23187 is consistent with the dependence of the reversed I_{sc} on the physiologic state of the turtles, i.e., the ionophore-evoked positive I_{sc} was absent in acidotic turtles, intermediate in magnitude in post-prandial turtles, and reached maximum values in alkalotic turtles. Moreover, since (1) the transepithelial resistance in the presence of amiloride is predominantly a function of the apical cell membrane resistance [20], (2) SO_4^{2-} is known not to be transported [17], (3) K^+, Mg^{2+}, P_i, and Cl^- were deleted from both mucosal and serosal fluid and Ca^{2+} from the mucosal fluid, and (4) the reduction in resistance after ionophore A23187 was smallest in acidotic bladders and largest in alkalotic bladders, it is inferred that the decrease in resistance reflects primarily a conductance increase in the apical transport paths of the predominantly transported ions, namely, HCO_3^-, OH^- or H^+.

The collective findings are best explained by the turtle bladder model in which the relative acidification/alkalinization state is controlled by the relative magnitudes of discrete, electrogenic processes for HCO_3^- absorption and HCO_3^- secretion [4,8,17,18]. Accordingly, the magnitude and direction of the (HCO_3^- + CO_2)-dependent I_{sc} are determined by the net sum of absorptive and secretory HCO_3^- flows, irrespective of the ion species (H^+, OH^- or HCO_3^-) actually translocated by the pump mechanisms. This analysis, however, does not exclude involvement of electrically silent symport or antiport of HCO_3^- with some other ion in series or parallel with the conductive, potential-generating transport elements [4,21].

That both absorptive and secretory HCO_3^- transport processes can exist in the absence of ionophore, or IBMX and cAMP, is suggested by the observations that (1) the polarity of the (HCO_3^- + CO_2)-dependent I_{sc} of post-prandial bladders is initially positive and spontaneously declines to finite positive or negative values in 1–2 h [4], and (2) the inhibition of acidification by SITS can unmask a small, but finite, positive I_{sc} [17,18].

Although the inhibition of both the positive I_{sc} and luminal alkalinization [4] and the negative I_{sc} and luminal acidification [2,17,19] by ethoxyzolamide or acetazolamide points to a common carbonic anhydrase-mediated control of intracellular HCO_3^-/H^+ levels, the possibility that the positive I_{sc} is caused by an ionophore-elicited reversal of one-and-the same H^+ secretion/HCO_3^- absorption mechanism appears unlikely. This is because of (1) the apparent requirement of serosal HCO_3^- (Fig. 3), as discussed previously [8], (2) the fact that neither ionophore A23187 nor IBMX [8] reverses the negative I_{sc} of bladders bathed by isohydric, HCO_3^--poor solutions, and (3) the different actions of ionophore and IBMX on the negative I_{sc} of acidotic bladders bathed by HCO_3^--rich solutions.

A regulatory role of Ca^{2+} in urinary acid-base transport was first suggested by Arruda who showed that ionophore A23187 added to bladders bathed by HCO_3^--poor solutions decreased the acidification rate. This result is confirmed by the ionophore's inhibition of the negative I_{sc} in post-prandial bladders bathed by HCO_3^--poor solutions and the dependence of the I_{sc}'s reversal on abundant serosal HCO_3^-. The suggestion of impaired mitochondrial function [22], on the other hand, is not compatible with the sustained, reversed I_{sc} and its high sensitivity to oxidative uncouplers and inhibitors of respiration [8].

The positive I_{sc} evoked by A23187 in Ca^{2+}-free, EGTA-containing mucosal fluid suggests release of Ca^{2+} from intracellular storage sites. This inference is supported by the finding that A23187, but not IBMX, in isolated turtle bladder cells

loaded with the Ca^{2+}-sensitive protein, aequorin [23], raised cytosolic Ca^{2+} levels in the absence of exogenous Ca^{2+} (Ehrenspeck, G., Snowdowne, K.W. and Borle, A., unpublished data). In the study by Arruda [22], ionophore A23187 in the serosal fluid was found to reduce the acidification current by about 50% in 2 h, but failed to inhibit it when both mucosal and serosal fluids were essentially free of Ca^{2+} and Mg^{2+}. Such bathing conditions, however, if prolonged, might have led to a depletion of intracellular exchangeable pools of Ca^{2+} [24] and to morphologic and physiologic changes [25] reflected by a control acidification rate 50% that of bladders bathed by 1.8 mM Ca^{2+} [22]. Different levels of exchangeable stored Ca^{2+} may also explain the ionophore's failure to elicit a positive I_{sc} in bladders of acidotic turtles and its generation of a large positive I_{sc} in those of alkalotic turtles. Thus Studer and Borle [26] found that the intracellular Ca^{2+} concentration of isolated rat kidney cells was depressed in acidosis and elevated in alkalosis.

In conclusion, the present study provides new evidence for the coexistence of discrete electrogenic pathways for mucosal acidification and alkalinization. Such processes under physiologic control involving cytosolic Ca^{2+} and cAMP would have an important adaptive value in omniverous, ectothermic vertebrates, such as turtles, in which the acid-base metabolism is affected by (1) a varied acid-ash or alkaline-ash diet, (2) prolonged periods of diving, and (3) environmental temperature. Such discrete transport and control mechanisms may also operate in the renal control of mammalian acid-base balance.

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