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EFFECT OF 3-ISOBUTYL-1-METHYLXANTHINE ON HCO_3^- TRANSPORT IN TURTLE BLADDER

EVIDENCE FOR ELECTROGENIC HCO_3^- SECRETION *

GERHARD EHRENSPECK

Department of Zoology and Microbiology and College of Osteopathic Medicine, Ohio University, Athens, OH 45701 (U.S.A.)

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Ouabain-treated turtle bladders bathed on both surfaces by identical HCO_3^- /CO_2-containing, Cl^--free Na^+ media exhibit a short-circuit current (I_{sc}) and transepithelial potential (p.d.) serosa electronegative to mucosa. Addition of 3-isobutyl-1-methylxanthine (IBMX), an inhibitor of cyclic nucleotide phosphodiesterase, rapidly reverses the direction of the I_{sc} and p.d.. The IBMX-induced reversal of I_{sc} and p.d. is (1) dependent on the presence of HCO_3^- (and CO_2) in the serosal bathing fluid, (2) independent of Na^+ and other ions in the bathing medium, (3) decreased by inhibitors of carbonic anhydrase or oxidative metabolism, (4) increased by the serosal addition of cyclic AMP or the disulfonic stilbene, SITS. The results constitute evidence that the reversed I_{sc} elicited by IBMX represents electrogenic secretion of HCO_3^-.

Introduction

The urinary bladder of the fresh water turtle is capable of acidifying [1,2] or alkalinizing [3,4] the luminal fluid, but little is known about the physiological factors that influence or regulate the relative rates of the acid-base flows. A possible role of cyclic AMP in the control of the luminal acidification in this epithelium is suggested by the finding of a norepinephrine-sensitive adenyl cyclase and a cyclic AMP-sensitive protein kinase in isolated apical cell membranes of turtle bladder epithelial cells [5]. In addition, the mucosal addition of agents known to elevate cellular cyclic AMP levels such as histamine, norepinephrine and cholera toxin (for review, see Refs. 6 and 7) increases the acidification of the luminal fluid by short-circuited turtle bladders [8–11]. In contrast, the serosal addition of theophylline and cyclic AMP decreases luminal acidification [12].

These enzymatic and physiological findings prompted the present study of the effects of 3-isobutyl-1-methylxanthine (IBMX), a more potent inhibitor of phosphodiesterase than theophylline [13], on the I_{sc} or 'acidification current' generated by ouabain-treated turtle bladders. The underlying rationale was that the physiological control of the luminal acidification or alkalinization in this epithelium might, in part, be mediated by changes in the cellular levels of cyclic nucleotides; thus IBMX might serve as a tool to (1) manipulate the bladder's acidification/alkalinization state in vitro and (2) further characterize the transport processes involved.

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

Methods

For the present experiments, *Pseudemys scripta* turtles were maintained in a large tank containing continuously flowing water at a depth of 20 cm and temperature of 29–32°C [14], which is near the turtles' preferred body temperature [15]. The turtles were fed vitamin-supplemented bovine liver [15] and were used for experiments within a 1–4 day period after the previous feeding. All procedures and techniques for surgical removal, mounting and short-circuiting of the urinary bladders, together with those for evaluating transepithelial potential (p.d.), short-circuit current (I_{sc}), and tissue resistance (R) have been described in detail previously [16–18].

The isolated bladders were bathed by three different Ringer solutions, the composition of which was as follows (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% O_2/2% CO_2; final pH 7.6 ± 0.1 at 22–25°C. In some experiments all Na^+ in this medium was replaced by choline without changing the concentration of the other ions.

Solution B (modified HCO_3^--rich medium): Na_2SO_4, 40.5; $NaHCO_3$, 20, glucose, 11, osmolality was adjusted to 220 mosM/kg with sucrose; gassed with H_2O-saturated 98% O_2/2% CO_2; pH 7.6 ± 0.1 at 22–25°C. Tissue viability was maintained by supplementing the medium bathing the serosal tissue surface with 2 mM $CaSO_4$.

Solution C (HCO_3^--poor medium): composition was similar to that of the complete medium, except that $NaHCO_3$ was replaced by Na_2SO_4 without changing the concentration of Na^+; osmolality was readjusted with sucrose; equilibrated with H_2O-saturated 100% O_2; final pH 7.6 ± 0.1 at 22–25°C. As detailed previously by Brodsky and Schilb [19,20], Solution C, when bathing the bladder tissue, is not HCO_3^--free, despite vigorous gassing with O_2 (this is 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).

Results are presented as means ± S.E., and statistical significance was determined by Student's t-test of paired or unpaired variates.

Turtles were obtained from Lemberger Assoc., Germantown, WI and Carolina Biological Supply Co., Burlington, NC. The disodium salt of SITS was a product of Pierce Chemical Co., Rockford, IL. IBMX, acetazolamide, and ethoxyzolamide were purchased from Sigma Chemical Co., Saint Louis, MO. The cyclic AMP analog, 8-(aminooctyl)-amino cyclic AMP, was obtained from ICN Nutritional Biochemicals, Cleveland, OH. Oxygen and analysed 2% CO_2 in O_2 mixture were obtained from DeLille Oxygen Company, Columbus, OH.

Results

Reversal of the p.d. and I_{sc} by IBMX and its independence of Na^+

Bladders were bathed on both surfaces by identical HCO_3^--rich, Cl^--free medium (Solution A) supplemented with 0.2 mM ouabain in the serosal fluid. Under these bathing conditions the tissues generated a p.d. serosa negative to mucosa. The associated I_{sc}, carried either by the active absorption of HCO_3^- or active secretion of protons, provides a measure of the luminal acidification rate and net HCO_3^- absorption [17,21–23].

Fig. 1 shows the result of 0.05 mM IBMX present in both the mucosal (M) and serosal (S) bathing fluids. After the addition of IBMX, the I_{sc} and p.d. rapidly decreased to zero and then reversed in orientation so that the serosa became positive to the mucosa while the transepithelial resistance (R) decreased. This 'reversed I_{sc}' was also elicited maximally or near-maximally by 0.05 mM IBMX added to either M or S alone; but in all experiments reported here IBMX was present in both M and S. The results of thirteen such experiments are summarized in Table I (top row). Not apparent from the mean values of the data shown, however, is that in four bladders of this first set of experiments, IBMX reduced the p.d. and I_{sc} to values near zero, but did not reverse the parameters (criterion for reversal: a true spontaneous p.d. greater than +1 mV). The reason for this variability is unknown, but may be related, in part, to variations in the physiological state of the turtle (e.g. the magnitude of the postprandial alkaline

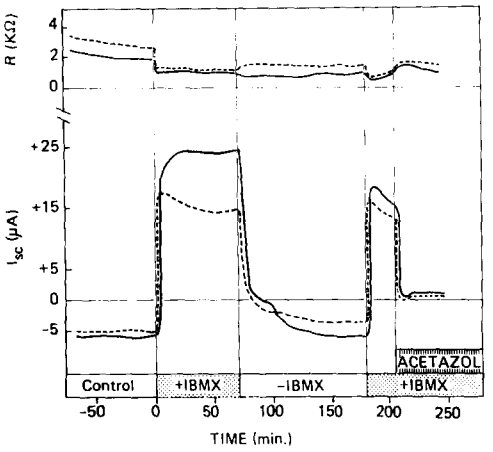

Fig. 1. Effect of 0.05 mM IBMX on I_{sc} and R of ouabain-treated bladders in HCO_3^--rich, Cl^--free bathing media. Values of I_{sc} (lower panel) and R (upper panel) shown are for 1.5 cm^2 area of tissue. Negative values of control I_{sc} indicated that serosa is negative relative to mucosa. p.d. has been omitted for clarity but can be estimated from $I_{sc} \times R$. Data from paired sections of a bladder are shown, one bathed in Na^+ medium (solid line), the other in Na^+-free choline medium (dashed line). IBMX, present in both M and S was rapidly removed (5 min) in the period denoted (−IBMX) by 4–5 complete replacements of S with fresh medium and serial dilution of M (7–9 consecutive replacements of 1/2 the 10 ml chamber volume with fresh medium). The serial dilution procedure minimized the mechanical perturbations which in a complete replacement and rinsing of the mucosal compartment decreases transepithelial resistence [18]. Ouabain (0.2 mM) was present in S; acetazolamide (1 mM) was added to S.

tide) at the time of bladder excision for experiment. For the nine bladders that met the criterion for reversal of the electrical parameters, the mean values of the p.d. and the reversed p.d. were -17.5 ± 3.8 mV and $+16.2 \pm 2.7$ mV, respectively; the mean values of the I_{sc} and the reversed I_{sc} were -11.5 ± 2.4 μA and $+16.0 \pm 2.3$ μA, respectively.

Also shown in Fig. 1 is that the IBMX-induced, reversed I_{sc} was sustained, but that the replacement of the bathing fluids with fresh, IBMX-free media restored the I_{sc} to the control orientation and magnitude. In addition, IBMX had essentially the same effect on I_{sc} when ouabain-treated bladders were bathed on both surfaces by Na^+-free (choline-substituted) solutions. In other experiments exposure of ouabain-treated bladders to 5 μM amiloride either before or after the adition of IBMX did not prevent or diminish the reversed I_{sc}. Thus, this reversed I_{sc} is independent of extracellular Na^+ and is not carried by a residual, ouabain-resistant Na^+ absorption; these data confirm an earlier study showing that ouabain completely inhibits the net Na^+ flux in turtle bladder [24].

Lack of effect by IBMX on Na^+ transport

IBMX was added to bladders bathed by Na^+-rich, HCO_3^--poor media (Solution C) without

TABLE I

EFFECT OF ION SUBSTITUTIONS ON I_{sc} AND R OF OUABAIN-TREATED BLADDERS EXPOSED TO IBMX

Mean values ±S.E. of I_{sc} and R for 1.5 cm^2 area of tissue. Sign convention for I_{sc} and p.d.: polarity of serosa relative to mucosa. IBMX was added simultaneously to mucosal (M) and serosal (S) bathing fluids. Except for ouabain (0.2 mM) present in S, the M and S fluids were identical. Values of p.d., I_{sc}, and R after IBMX addition were obtained at the time of maximal response, 12±3 min for tissues bathed by HCO_3^--rich media and 30–40 min for tissues bathed by HCO_3^--poor media. All effects of IBMX were significant at the $P<0.001$ level according to the Student's t-test of paired data.

Ions deleted or substituted	Period	p.d. (mV)	I_{sc} (μA)	R (kΩ)
None	Before IBMX	−17.3±3.0	−11.3±2.2	1.6±0.2
(Complete medium)	After IBMX	+10.8±3.1	+10.4±2.6	1.2±0.2
($N=13$)	Difference	28.0±4.4	21.7±3.5	0.4±0.1
M: P_i, K^+, Mg^{2+}, Ca^{2+}	Before IBMX	−15.2±4.1	−11.8±3.8	1.3±0.1
S: P_i, K^+, Mg^{2+}	After IBMX	+13.9±4.6	+19.6±8.7	0.9±0.1
($N=6$)	Difference	29.2±6.3	31.4±9.2	0.4±0.2
M: HCO_3^-	Before IBMX	−9.9±1.5	−4.4±0.7	2.3±0.2
S: HCO_3^-	After IBMX	−7.6±1.3	−3.9±0.7	2.1±0.3
($N=10$)	Difference	2.2±0.3	0.5±0.2	0.3±0.1

ouabain. Under these bathing conditions the I_{sc} is a measure of net Na^+ absorption, since the concomitantly occurring bicarbonate (or proton) transport carries only a small fraction of the total I_{sc} [25–27]. In six bladders 0.05 mM IBMX in M and S did not change the rate of Na^+ transport. The control I_{sc} and R were 125.0 ± 23.9 μA and 0.5 ± 0.1 kΩ, respectively; after a 60 min exposure of bladders to IBMX, the I_{sc} and R were 125.2 ± 22.7 μA ($P > 0.9$) and 0.4 ± 0.1 kΩ ($P < 0.01$), respectively.

Independence of the reversed I_{sc} of ions other than Na^+

For the study of which ions other than Na^+ were involved in the generation of the IBMX-induced, reversed I_{sc}, ion deletion or substitution experiments were conducted (Table I, central and bottom rows).

When ouabain-treated bladders were bathed by the HCO_3^--rich medium (Solution B) from which P_i, K^+, and Mg^{2+} were deleted from M and S and Ca^{2+} from M, IBMX also reversed the direction of the I_{sc}. In one bladder the I_{sc} changed from -11 μA to $+62$ μA. The subsequent addition of EGTA to M at a final concentration of 2 mM in several experiments (not shown) to sequester residual Ca^{2+} did not lower the reversed I_{sc}. Table I also shows that the mean value of the I_{sc} of bladders bathed by the modified HCO_3^--rich medium (central row) was not significantly different ($P > 0.9$) from that of bladders bathed by the complete HCO_3^--rich medium (top row). In contrast, as shown in the bottom row, when the concentration of exogenous HCO_3^- and CO_2 was low (bladders bathed by Solution C), the I_{sc} was decreased 60% ($P < 0.01$) and R increased ($P < 0.02$), confirming previous evidence on the dependence of the acidification current on HCO_3^- and CO_2 [17,22,23,28,29]; more importantly, IBMX did not cause a reversal in I_{sc} under these ($HCO_3^- + CO_2$)-poor bathing conditions. IBMX merely decreased the I_{sc} by $12.3 \pm 2.7\%$ ($P < 0.01$) while the I_{sc} (3.7 ± 0.6 μA) of six untreated, paired bladder sections remained unchanged at 3.6 ± 0.6 μA ($P > 0.05$). Since SO_4^{2-} is not transported by the turtle bladder [17], these data on ion deletion or substitution suggest that HCO_3^- is the predominant ionic carrier of the reversed I_{sc}, but do not exclude a contribution by protons.

Dependence of the reversed I_{sc} on serosal HCO_3^-

If the IBMX-induced reversal in I_{sc} reflected electrogenic HCO_3^- secretion, one would expect that the generation of the reversed I_{sc} would depend on the availability of substrate from the uptake side of the transport process, i.e. the reversed I_{sc} should depend on HCO_3^- in S. This prediction was confirmed in the experiments described below (Fig. 2 and Table II).

Ouabain-treated bladders were first incubated on both surfaces by HCO_3^--poor Solution C. After exposure of the bladders to IBMX, HCO_3^- was first added to M and subsequently also to S while the pH was held constant by simultaneously increasing the CO_2 of the gas phase in M and then S from 0 to 2%.

Shown in Fig. 2 are paired bladder sections. At time zero, one section was exposed to IBMX, the other served as a control. The isohydric addition of HCO_3^- and CO_2 to M of untreated and IBMX-treated tissues produced within 1 min a transient increase in the I_{sc} (in the direction of the imposed transepithelial HCO_3^- concentration gradient). After HCO_3^- and CO_2 were added to S (resulting in zero transepithelial HCO_3^- concentration gradient), the orientation of the I_{sc} of the untreated tissue remained the same. The I_{sc} of the IBMX-exposed tissue, however, was rapidly reversed in direction. The effects of the isohydric additions of

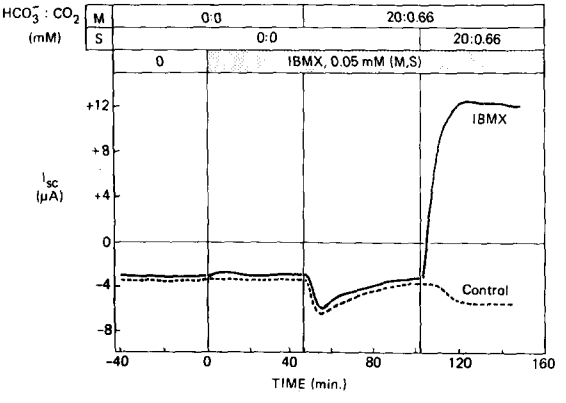

Fig. 2. Effect on I_{sc} by sequential isohydric additions of HCO_3^- to mucosal and serosal fluids of IBMX-treated bladders. Data from paired sections of a bladder are shown; one section was exposed to IBMX (solid line), the other received diluent (dashed line). Sign convention and other experimental details are given in Table II.

TABLE II

EFFECT ON p.d., I_{sc}, and R BY SEQUENTIAL ISOHYDRIC ADDITIONS OF HCO_3^- TO MUCOSAL AND SEROSAL FLUIDS OF IBMX-TREATED BLADDERS

Mean values ±S.E. ($N=5$) of p.d., I_{sc}, and R before and after addition of IBMX. Sign convention is given in Table I. Ouabain-treated tissues were initially bathed in HCO_3^--poor medium (Solution C) for 90–120 min and then exposed to IBMX for 35–45 min (top row); HCO_3^- was added first to M by a one-step replacement of half the chamber volume by isosmotic, isohydric medium containing 40 mM HCO_3^- (otherwise similar to Solution A), and aspiration in M was switched from 100% O_2 to 98% O_2/2% CO_2. After a new steady state was attained in 35–45 min, a similar maneuver was performed on S (bottom row). Mean values of p.d., I_{sc}, and R before and after the HCO_3^- additions were significantly different from each other ($P<0.001$) by Student's t-test of paired data. Values marked with an asterisk were significantly different at $P<0.02$.

Period	HCO_3^- present (mM)	p.d. (mV)	I_{sc} (μA)	R (kΩ)
1	M=0			
	S=0	−6.2±1.9 *	−3.4±0.9	1.9±0.4
2	M=20			
	S=0	−9.1±2.4 *	−7.3±2.0	1.4±0.3 *
3	M=20			
	S=20	+7.2±2.1	+6.9±2.0	1.1±0.2 *

HCO_3^- to IBMX-treated bladders are summarized in Table II. In two other IBMX-treated bladders, when HCO_3^- and CO_2 were first added to S and then to M, the response of the I_{sc} was qualitatively similar to that described above.

Effects of inhibitors on the reversed I_{sc}

If the IBMX-induced I_{sc} inversion reflected a change in the bladders' state from net acidification to net alkalinization, one would expect a sensitivity of the reversed I_{sc} to inhibitors of carbonic anhydrase. This notion was tested by exposing the serosal surface of IBMX-treated bladders to acetazolamide or ethoxyzolamide, which have been shown to inhibit the mucosal acidification of turtle bladders [22,23,30].

As shown in Fig. 1, 1 mM acetazolamide decreased the reversed I_{sc} by 90–95% in 10 min. In other experiments 0.1 mM ethoxyzolamide, which is more lipid soluble than acetazolamide [31], lowered the reversed I_{sc} by 89±4% ($N=6$) in 10–15 min.

In another set of bladders the effect of 0.2 mM SITS added to S on the reversed I_{sc} was studied. This disulfonic stilbene decreases mucosal acidification of turtle bladders [17,32,33] and under certain conditions reveals a small reversed I_{sc} (2–3 μA) that can be stimulated by theophylline [17,34]. The effect of SITS on the reversed I_{sc} is shown in Fig. 3. In eight such experiments SITS increased the reversed I_{sc} of +10.4±2.6 μA by 9.4±1.1 μA in 30 min. The effect of IBMX and SITS additions to ouabain-treated bladders in the reverse order is shown in Fig. 4. In five such experiments SITS decreased the I_{sc} from −7.4±1.7 μA to near zero values; the subsequent addition of IBMX elicited a reversed I_{sc} of +10.1±5.0 μA. The final addition of acetazolamide (1 mM) decreased the reversed I_{sc} by 85±3%.

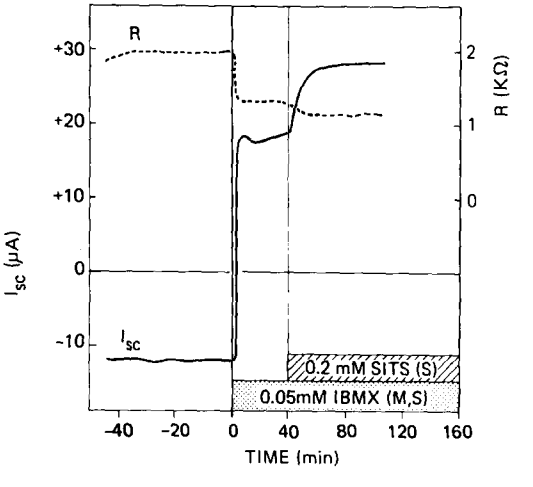

Fig. 3. Effect of SITS on I_{sc} and R of IBMX-treated bladders. Bladders were bathed in HCO_3^--rich, Cl^--free bathing medium (Solution A) plus 0.2 mM ouabain. I_{sc} is depicted by the solid line and R by the dashed line.

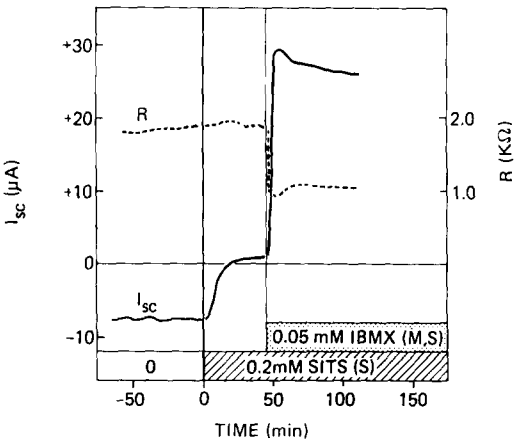

Fig. 4. Effect of IBMX on I_{sc} and R of SITS-treated bladders. Experimental conditions and notations as described in Fig. 3.

In several experiments a few of the bladders which were not treated with carbonic anhydrase inhibitors were exposed to respiratory inhibitors or uncoupling agents. The following agents present in S, 1 mM KCN ($N = 2$), 0.1 mM rotenone ($N = 1$), or 0.1 mM 2,4-dinitrophenol ($N = 2$), all decreased the reversed I_{sc} to near zero values.

Effects of cyclic AMP analogs on the reversed I_{sc}

If the observed actions of IBMX resulted from submaximal increases in the cellular cyclic AMP concentrations, the exposure of IBMX-treated bladders to high concentrations of exogenous cyclic AMP would be expected to further stimulate the reversed I_{sc}. The serosal addition of 1 mM 8-(aminooctyl)-amino cyclic AMP to IBMX-treated bladders bathed by HCO_3^--rich media further accelerated the reversed I_{sc} of $+3.5 \pm 1.8$ μA by 14.2 ± 2.1 μA ($N = 3$) and in (IBMX + SITS)-treated bladders further increased the reversed I_{sc} of $+12.1 \pm 3.6$ μA by 5.2 ± 1.5 μA ($N = 4$). Other cyclic AMP analogs, the bromo- and *p*-chlorophenylthio derivatives, had the same effect.

Discussion

An important finding of this study is that IBMX produces a rapid reversal in the Na^+-independent I_{sc}. The most consistent interpretation of this result is based on a general model of the turtle bladder epithelium in which the I_{sc} of ouabain-treated bladders bathed by HCO_3^--rich, Cl^--free media equals the algebraic sum of two active, conductive HCO_3^- flows, HCO_3^- absorption and HCO_3^- secretion, the relative magnitude of which determines the net direction of the I_{sc} [17,34,35]. For the present analysis it is not critical whether (1) the mechanisms responsible for HCO_3^- absorption and secretion are located in the same cell or spatially separate in different cell types [4,36,37] and (2) the actual net charge translocation across the plasma membrane occurs via a primary HCO_3^- or proton pump. Whereas HCO_3^- has been concluded to be the ion translocated in HCO_3^- secretion [3,4], both HCO_3^- [1,14,38,39] and H^+ [23,40,41] have been implicated in HCO_3^- absorption.

Based on the above model, it is proposed that IBMX, at the concentration used, gives rise to the reversed I_{sc} by accelerating the HCO_3^- secretion process without abolishing the HCO_3^- absorption mechanism. Accordingly, the change in the I_{sc} induced by IBMX, i.e., the reduction to lower or near zero values or the reversal in direction, would depend on the magnitude of the accelerated HCO_3^- secretion relative to the magnitude of the concurrent HCO_3^- absorption. If the reversed I_{sc} consisted of the algebraic sum of an enhanced HCO_3^- secretion and a residual HCO_3^- absorption, it should be increased upon the inhibition of any remaining HCO_3^- absorption by SITS [17,34,35]. This prediction was confirmed by the data (Fig. 3). Moreover, under conditions which would not be consistent with high rates of HCO_3^- secretion, i.e. when 20 mM $NaHCO_3$ is omitted from S or both S and M, IBMX should not abolish or reverse the residual HCO_3^- absorption (I_{sc}). This prediction was confirmed by the data (Fig. 2 and Tables I and II). The small IBMX-induced decrease in the I_{sc} observed under HCO_3^--poor conditions is assumed to be due to an acceleration of HCO_3^- secretion limited in magnitude by the low availability of HCO_3^- substrate, although a small inhibition of HCO_3^- absorption cannot be excluded. Finally, HCO_3^- in S but not M, would be expected to provide the primary source of substrate for the HCO_3^- secretion process. This notion is consistent with the data (Fig. 2 and Table II).

An alternative hypothesis that the I_{sc} of ouabain-treated bladders is generated by a secre-

tion of protons and that HCO_3^- secretion occurs via an active, electroneutral HCO_3^--Cl^- exchange mechanism [3,23,30,40,41] cannot account for the existence of the reversed I_{sc}. An electroneutral HCO_3^- secretion coupled to Cl^- absorption would not generate a current and would be inoperative under Cl^--free bathing conditions. Moreover, an IBMX-induced decrease in proton secretion analogous to that proposed for theophylline (and cyclic AMP) [12] would be consistent with the decrease in the I_{sc} toward zero, but not with its sustained reversal.

If it were postulated that the reversed I_{sc} represented a reversal in proton transport from secretion to absorption, one would predict that an increase of the pCO_2 in either M or S would symmetrically influence this transport process, since the availability of metabolic and exogenous CO_2 is considered a major control factor of the rate of proton transport in the above model. The fact that only the isohydric addition of 2% CO_2 (in 20 mM HCO_3^-) to S gave rise to the reversed I_{sc}, despite the prior equilibration of the tissue for 35–45 min with 2% CO_2 (in 20 mM HCO_3^-) in M, does not support this postulate; but the possibility of an IBMX-induced reversal of a proton-translocating mechanism cannot be rigorously excluded. On the other hand, regardless of whether net HCO_3^- secretion of H^+ absorption is responsible for the IBMX-induced, reversed I_{sc}, the validity of the assumption that luminal alkalinization by the turtle bladder occurs primarily via an electroneutral HCO_3^- secretion [4,23,30,42] may be questioned.

Since the turtle bladder is capable of lactate secretion into the mucosal fluid, the possible contribution of a flow of this organic anion to the reversed I_{sc} should be discussed. Although lactate production by the turtle bladder was not measured, a major contribution by lactate to the reversed I_{sc} seems unlikely because under the aerobic conditions used lactate production is low and only 10–20% of the lactate produced enters the mucosal fluid, the majority appearing in the serosal fluid [43]. In addition, whereas lactate production is increased during anaerobiosis [43] and remains unchanged by acetazolamide [44], the reversed I_{sc} was decreased by inhibitors of oxidative metabolism and carbonic anhydrase.

The underlying rationale for this study was that changes in cellular concentration of cyclic nucleotides may, in part, be involved in the regulation of the bladders' acidification/alkalinization state. If the postulated stimulation of a HCO_3^- secretory mechanism by IBMX were mediated by increased cellular cyclic AMP levels, the exposure of bladders to high concentrations of exogenous cyclic AMP should accelerate the reversed I_{sc}. This was observed after the addition of the cyclic AMP derivatives to IBMX-treated and (IBMX + SITS)-treated bladders. The additional enhancement of the postulated HCO_3^- secretion by cyclic AMP is qualitatively consistent with the 55% reduction of the mucosal acidification (pH-stat) rate by 10 mM theophylline and 10 mM cyclic AMP in bladders bathed by HCO_3^--poor media [12].

In previous experiments exposure of the mucosal but not the serosal surface of bladders to certain adenyl cyclase activators, e.g. histamine, norepinephrine, or cholera toxin reversibly accelerated the luminal acidification rate (as defined by the I_{sc} under the present conditions) [8–11]. These results contrast with the effects of IBMX and serosally applied cyclic AMP reported here and the effects of serosally applied theophylline and cyclic AMP reported previously [12]. On the basis of this recent evidence and the fact that other amine or guanidine-containing compounds such as arginine, amiloride, AMP or GMP stimulated the acidification in a similar manner [10,11,18] it was concluded that these substances do not increase cellular cyclic AMP, but increase the I_{sc} by some other means [10,11].

The present results constitute the first demonstration in a urinary tissue of a ouabain and amiloride-insensitive I_{sc} and p.d. (serosa positive to mucosa) that are (1) reversibly elicited by IBMX, (2) primarily dependent on HCO_3^- in the serosal fluid, and (3) sensitive to inhibitors of carbonic anhydrase. The results are consistent with the following claims: (i) the reversed p.d. and I_{sc} elicited by IBMX represent electrogenic secretion of HCO_3^-, and (ii) IBMX-induced elevations in cellular cyclic AMP play a role in mediating a shift in the turtle bladder's function from net HCO_3^- absorption to net HCO_3^- secretion.

Recently, Gunter and White have provided evidence for a theophylline-induced electrogenic

HCO_3^- secretion in *Amphiuma* small intestine. Unlike the reversed I_{sc} in the turtle bladder, however, the HCO_3^--dependent and acetazolamide-sensitive reversed I_{sc} in this epithelium depends on the presence of Na^+ in the bathing medium [45,46].

A net secretion of HCO_3^- has also been demonstrated in the urinary bladder of alkalotic toads [47] and the cortical collecting tubule of alkali-loaded rabbits [48]. Except for its independence of extracellular Na^+, the reversed I_{sc} or presumptive HCO_3^- secretion induced by IBMX in the turtle bladder resembles the HCO_3^- secretion of the cortical collecting tubule, e.g. ouabain-insensitivity, acetazolamide-sensitivity, and Cl^--independence. In the light of these findings, it is suggested that the acidification/alkalinization status of turtle bladders and possibly that of other urinary epithelia is determined in large part by the regulation of HCO_3^- secretion. A similar view has recently been advanced by others on the basis of measurements of the changes in the rate of mucosal alkalinization by bladders of alkali- and acid-loaded turtles [4].

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