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Effect of furosemide on ion transport in the turtle bladder: evidence for direct inhibition of active acid-base transport

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The diuretic furosemide inhibits acid-base transport in the short-circuited turtle bladder. It inhibits luminal acidification when present in either mucosal or serosal bathing fluids, but decreases alkalinization only from the serosal side of the tissue. The inhibition of both acid-base transport processes is independent of ambient Cl^-; and the disulfonic stilbene, SITS, an inhibitor of Cl^--HCO_3^- exchange, fails to prevent the furosemide-elicited inhibition of alkalinization. These results preclude an absolute requirement of a furosemide-sensitive Cl^--HCO_3^- exchange by these transport processes. The drug also interferes with the CO_2-induced stimulation of acidification and alkalinization. The inhibition of the residual acidification in acetazolamide-treated, acidotic bladders, however, suggests an action at sites other than cytosolic carbonic anhydrase. Although active Na^+ and Cl^- reabsorption and tissue oxygen uptake are also decreased by furosemide, the rate of oxygen consumption uncoupled by 2,4-dinitrophenol is not diminished, indicating a primary inhibition of the various ion transport processes, not of metabolism. It is proposed that inhibition of transepithelial acid-base transport by furosemide in the turtle bladder includes inhibition of the acid-base pumps.

Introduction

The potent diuretic, furosemide, inhibits salt absorption in the thick ascending limb of the loop of Henlé [1,2], Cl^- transport in the early amphibian distal tubule [3] as well as anion and cation transport in many other systems [4–6]. Less well characterized is the effect of furosemide on renal tubular acid-base transport. Several lines of evidence suggest a furosemide-evoked inhibition of tubular acidification after acute administration [7] as well as stimulation after chronic administration [8].

A well-known model of the mammalian distal nephron is the turtle urinary bladder. It actively reabsorbs Na^+ and Cl^- [9] and, like the cortical collecting tubule [10,11] is capable of acidifying [12,13] or alkalinizing [14,15] the luminal fluid in vitro. Reports on whether furosemide inhibits the bladder's acidification rate and whether from the mucosal or serosal side have been conflicting, however [16–18]. Moreover, it has been proposed that the decrease in the acidification rate elicited by furosemide present in the serosal bathing medium is due to the inhibition of a Cl^--HCO_3^- exchange mechanism in the basolateral membrane [18].

One purpose of this study was to test the valid-

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

ity of this hypothesis by examining whether the inhibition of acidification by furosemide (1) is caused by its interference with metabolic or carbonic anhydrase activity, (2) is selectively elicited by the drug in the serosal fluid, and (3) requires the presence of Cl^- in the medium. Also of interest was the effect by furosemide on luminal alkalinization. This transport process, like that of acidification, (1) is independent of Na^+ [19–21], (2) depends on functional carbonic anhydrase [20,21], and (3) is frequently [14,15,18], but not uniformly [20,22], thought to be obligatorily coupled to Cl^--HCO_3^- exchange; it differs, however, by its insensitivity to inhibition by SITS [19–21] and vanadate [23].

Methods

Pseudemys scripta turtles were prepared for experiment as described previously [21,24]. Postprandial turtles were defined as those fed beef liver 1–2 days prior to death. Acidotic turtles were defined as those which had been given 15 mmol/kg per day NH_4Cl by gavage for 4 days prior to death.

Electrical and Cl^- flux measurements. Methods for mounting sections of urinary bladder and measuring transepithelial potential (PD), short-circuiting current (I_{sc}), transepithelial resistance (R), and $^{36}Cl^-$ fluxes have been described previously [19]. Briefly, for measurements of acid-base and Cl^- transport bladders were bathed in (HCO_3^- + CO_2)-rich medium with 0.2 mM ouabain and 0.1 mM amiloride in the serosal and mucosal fluid, respectively. Under these bathing conditions net Na^+ absorption is abolished and bladders from postprandial or acidotic turtles exhibit a negative I_{sc} (serosa electronegative to mucosa), which provides a measure of the luminal acidification rate [19–21]. For measurements of luminal alkalinization bladders from postprandial turtles were exposed on both surfaces to 0.1 mM IBMX [20] and 1 μM forskolin, which maximally stimulated the alkalinization process (Ehrenspeck, G., unpublished data). To inhibit any concurrent residual acidification bladders were preincubated with 0.1 mM SITS in the serosal fluid [19–21]. The resulting positive I_{sc} (serosa electropositive to mucosa) is a measure of the electrogenic luminal alkalinization rate [20,21]. Preliminary experiments demonstrated that SITS did not abolish the effect of furosemide on the alkalinization current. Net Cl^- absorption was determined in bladders from postprandial turtles; bladders from acidotic turtles exhibit little or no net Cl^- transport [22]. Since time-control experiments in this as in previous studies [19,25] confirmed that the unidirectional $^{36}Cl^-$ fluxes remained stable for at least 4–5 h, the mucosa-to-serosa and serosa-to-mucosa fluxes were determined on adjacent tissue sections from the same bladder (I_{sc} and R each differed less than 25%). To measure Na^+ transport postprandial bladders were bathed by Na^+-rich, HCO_3^--free media without amiloride or ouabain. Under these bathing conditions the positive I_{sc} is a measure of net Na^+ absorption since, in the absence of ambient CO_2 and HCO_3^-, acid-base transport carries only a small fraction of the total I_{sc} [13,20].

Solutions. Stock medium, composition 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 mosmol/kg with sucrose; equilibrated with H_2O-saturated 98% O_2/2% CO_2; final pH was 7.6 at 22–25°C.

Cl^--free medium: same as above, except that NaCl and KCl were replaced by the appropriate sulfate salts and osmolality readjusted with sucrose. Cl^- concentration analysed with a Cl^--selective electrode (Model 94-17B, Orion Research Inc., MA), was less than 10 μM.

HCO_3^--free medium: same as stock or Cl^--free Ringer, except that HCO_3^- was replaced by SO_4^{2-}; equilibrated with humidified 100% O_2; final pH was 7.4–7.6.

Stock solutions (100 mM) of furosemide were prepared in absolute methanol. Control studies with methanol indicated no effect by this solvent on the measured electrical or flux parameters. When added to HCO_3^--free bathing fluids, furosemide was first titrated to neutral pH in an aliquot of bathing fluid.

Oxygen consumption. Intact bladder tissue was incubated in stock medium with or without ouabain and amiloride for 60–90 min to simulate the incubation conditions of the electrophysiologic experiments. The tissue was then diced into roughly 1×1 mm pieces according to LeFevre et al. [26] who demonstrated that the mucosal fraction of the

bladder wall is responsible for 80% of the total O_2 consumption. The minced tissue (0.1 g wet weight) was suspended in 5 ml medium thermostatted at 25°C and equilibrated with CO_2 in air at pH 7.4–7.6 in an oxygen monitor (Model 53, Yellow Springs Instrument Co., Yellow Springs, OH). All measurements were made between a p_{O_2} of 100% and 70% of air. Results are expressed as μl O_2/h g wet tissue, based on the solubility of O_2 in Ringer at 25°C of 5.78 μl/ml.

Source of materials. Turtles were obtained from Kons Scientific, Germantown, WI. The disodium salt of SITS was a product of Pierce Chemical Co., Rockford, IL. Furosemide, IBMX, acetazolamide, and ethoxyzolamide were purchased from Sigma Chemical Co., Saint Louis, MO. and forskolin from Calbiochem-Behring, San Diego, CA.

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 furosemide on the acidification current and net Cl^- reabsorption

Paired bladder sections from postprandial turtles were incubated in $Na^+(Cl^- + HCO_3^-)$ medium containing amiloride and ouabain. Furosemide was added to both mucosal and serosal bathing fluids. As shown in Fig. 1, furosemide at 1 mM, the concentration used previously [18], decreased the acidification current (negative I_{sc}) and net Cl^- reabsorption and increased the transepithelial resistance (R), which subsequently declined with time. The results are summarized in Table I.

As shown in Fig. 2 (lower panel), furosemide

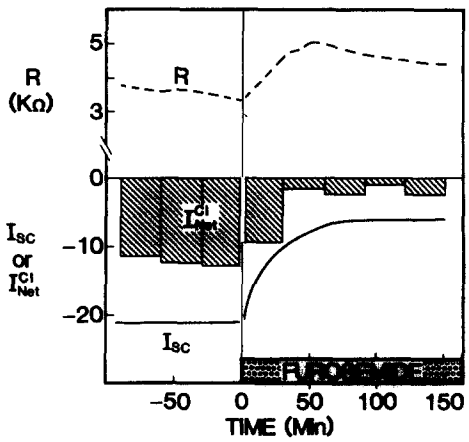

Fig. 1. Effect of 1 mM furosemide on the anion-dependent I_{sc}, R (kΩ), and net Cl^- flux. Bladder was bathed by symmetrical Cl^--rich, HCO_3^--rich medium containing 0.2 mM ouabain in serosal fluid (S) and 0.1 mM amiloride in mucosal fluid (M). Values of parameters are for 1.5 cm^2 area of tissue. A negative value of I_{sc} indicates that serosa is electronegative 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 $I_{sc} \cdot R$. Net Cl^- reabsorption, plotted as columns, was calculated from the unidirectional Cl^- fluxes across matched, paired sections of the bladder [19].

inhibited the I_{sc} from the mucosal or serosal side of the epithelium. On average it decreased the I_{sc} about equally from the mucosal (50.6 ± 7.4%, N = 9) or serosal side (55.7 ± 8.8%, $N = 5$). With respect to Cl transport, furosemide on the serosal side inhibited the mucosal-to-serosal Cl^- flux by 58.6 ± 7.8% ($N = 5$), which is not significantly different ($P > 0.3$) from its effect when present on both sides (Table I). In contrast, the drug on the mucosal side alone had an inconsistent effect on

TABLE I

EFFECT OF FUROSEMIDE ON THE ACIDIFICATION CURRENT AND NET Cl^- REABSORPTION

Before/after furosemide (1 mM)	I_{sc} (μA)	R (kΩ)	MS Cl^- flux (μA)	SM Cl^- flux (μA)	Net MS Cl^- flux (μA)
Before	−24.2±3.6	2.3±0.2	19.4±6.8	3.2± 1.1	16.2± 6.0
After	−5.6±0.9	2.3±0.3	6.4±1.7	2.9± 0.8	3.4± 1.7
Δ (%)	−76.0±2.2 *	+2.9±4.8	−66.5±3.9 *	−2.6±11.7	−80.1±10.1 *

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

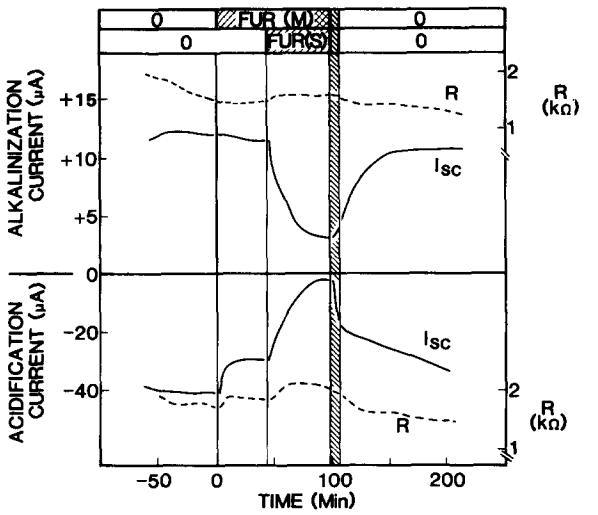

Fig. 2. Relative mucosal and serosal effectiveness and reversibility of furosemide-elicited inhibition of acidification and alkalinization currents. Data from paired sections of a bladder are shown. Shaded column denotes period during which the furosemide-containing fluid was replaced by drug-free medium. Sign convention and other experimental details as in Fig. 1.

Cl^- transport, resulting in an average decrease of 23.5 ± 13.1 ($P(\Delta = 0) > 0.1$, $N = 4$). Replacement of the bathing media by fresh medium, as illustrated in Fig. 2, resulted in either partial or complete restoration of the I_{sc} to control values.

Effect of furosemide on the acidification current in the absence of exogenous Cl^-

Furosemide evoked a dose-dependent inhibition of the acidification current from about 50 μM to 1 mM (the highest dose used) which was reversed by washing of tissue with fresh medium. As in the case of bladders bathed by Cl^--rich media, furosemide inhibited the acidification current from either surface of the bladders. Mucosal furosemide decreased the negative I_{sc} $56.3 \pm 3.9\%$ ($N = 6$) after 30–40 min; the subsequent addition of the drug to the serosal fluid caused a further decline, resulting after 30–40 min in a total decrease of $78.1 \pm 4.3\%$. The reverse sequence of drug addition resulted in similar decreases in I_{sc}.

In these experiments Cl^- diffusing from the tissues could have raised the solution Cl^- to levels near or above the apparent K_m of 0.13–0.4 mM Cl^- [18,22] of the postulated basolateral Cl^--HCO_3^- exchange mechanism so that its operation could have influenced the results. This possibility was examined on tissues that were more extensively washed so that the Cl^- concentration of the bathing fluids was below 10 μM. As shown in Table II, furosemide inhibited the I_{sc} at levels of ambient Cl^- less than 1/10 the K_m of Cl^--HCO_3^- exchange.

Effect of furosemide on the acidification current of acetazolamide-treated acidotic bladders

Since furosemide is a weak inhibitor of carbonic anhydrase [27,28], its effect on the acidification current might have been due to inhibition of carbonic anhydrase. This possibility was examined by the following approach [21].

Bladder sections from acidotic turtles were bathed by $NaHCO_3$-rich media and exposed to acetazolamide (0.1 mM) in the serosal fluid. Although at this concentration acetazolamide completely inhibits the intracellular carbonic anhydrase [29], it decreases the acidification rate much less than in non-acidotic turtles [21], thereby permitting a more accurate determination of any further drug-induced decreases in the I_{sc}. Since furosemide has an I_{50} for inhibition of carbonic anhydrase in vitro much greater than that of acetazolamide [27,28], a furosemide-evoked decrease in the I_{sc} after acetazolamide (Fig. 3) would then indicate a primary action other than inhibition of carbonic anhydrase.

In five such experiments acetazolamide decreased the I_{sc} by $49.6 \pm 4.1\%$. The subsequent addition of furosemide decreased the I_{sc} further, resulting in a total decline in the I_{sc} by $75.7 \pm 1.6\%$, identical to that produced by furosemide alone,

TABLE II

EFFECT OF FUROSEMIDE ON THE ACIDIFICATION CURRENT AND R OF BLADDERS BATHED BY Cl^--FREE MEDIA

Before/after furosemide (1 mM)	I_{sc} (μA)	R (kΩ)
Before	-7.8 ± 1.9	3.4 ± 0.3
After	-0.9 ± 0.5	3.6 ± 0.5
Δ (%)	-92.8 ± 4.0 *	$+3.4 \pm 6.0$

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

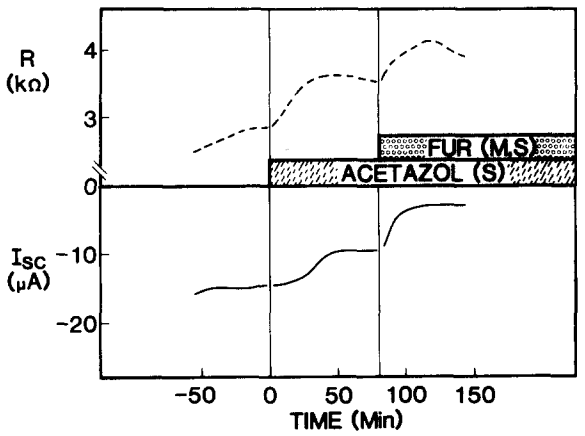

Fig. 3. Effect of furosemide in acetazolamide-treated bladders. Bladders from acidotic turtles (prepared as described in Methods) were bathed in Cl^--free, HCO_3^--rich medium plus ouabain and amiloride. Sign convention and other experimental details as in Fig. 1.

$77.3 \pm 3.8\%$ ($N = 5$, $P > 0.7$). Similarly, in two acidotic bladders pretreated with ethoxyzolamide (0.1 mM), furosemide lowered the I_{sc} further. The inhibitory effects by the carbonic anhydrase inhibitors and furosemide were similar for bladders bathed by Cl^--free or Cl^--rich media.

Effect of furosemide on the alkalinization current

As shown in Table III, furosemide inhibited the alkalinization current (positive I_{sc}) in the presence of 25 mM or < 10 μM exogenous Cl^-. As shown in Fig. 2 (top panel), furosemide exhibited a distinct difference in its inhibitory effectiveness, depending on the side of its addition. It had no effect from the mucosal side, decreasing the I_{sc} by only $5.0 \pm 2.8\%$ ($N = 8$) in 30–40 min. The major decrease in the I_{sc} occurred after the addition of furosemide to the serosal fluid. Replacement of the bathing fluids with fresh medium reversed the inhibition. The drug's differential effectiveness from the two tissue surfaces and reversibility of inhibition existed in the presence or absence of medium Cl^-.

TABLE III

EFFECT OF FUROSEMIDE ON THE ALKALINIZATION CURRENT AND R OF BLADDERS BATHED BY Cl^--RICH OR Cl^--FREE BATHING MEDIA

Chloride Conc. (mequiv./l)	Before/after furosemide	I_{sc} (μA)	R (kΩ)
25	Before	$+14.4 \pm 1.5$	1.2 ± 0.1
($N = 3$)	After	$+6.1 \pm 2.5$	1.4 ± 0.1
	Δ%	-59.6 ± 12.5 *	$+14.9 \pm 5.0$
< 0.01	Before	$+11.0 \pm 2.6$	3.7 ± 0.5
($N = 5$)	After	$+5.7 \pm 1.2$	4.1 ± 0.5
	Δ%	-44.0 ± 6.6 *	$+11.6 \pm 5.3$

* $P < 0.01$ according to Student's t-test, but not significantly different from each other ($P > 0.1$).

Inhibition of CO_2 stimulated acid-base transport by furosemide

The similar degree of inhibition of acidification and alkalinization at 25 mM or less than 10 μM ambient Cl^-, and in the case of alkali secretion, in the presence of serosal SITS, suggested a site of furosemide action other than the Cl^--HCO_3^- exchanger. In an attempt to further distinguish between an inhibition of the pump sites for H^+ (or its equivalent, OH^- or HCO_3^-) and the passive HCO_3^- paths in series with the ion pumps (see model, Fig. 6), we used a protocol slightly modified from that of Fisher et al. [18].

Mated pairs of bladder sections (one control, the other furosemide-treated) from postprandial turtles were bathed in phosphate buffered, (Cl^- + HCO_3^-)-free media and equilibrated with 100% O_2. We then stimulated mucosal acidification or alkalinization by adding 2% CO_2 in 98% O_2 first to the mucosal fluid (on the side with, presumably, the smaller diffusion barrier between bulk and cell fluids) and then to the serosal fluid. The rationale was that mucosally added CO_2 would rapidly diffuse into the cells to form H^+ and HCO_3^-, one or the other of which would then be translocated by the acidification or alkalinization pumps. Depending on the relative transport rates of the pumps and membrane permeabilities to H^+ and HCO_3^-, the CO_2 addition should give rise to transient and/or new higher steady-state values [30] of acidification or alkalinization in controls and little or no change in bladders with inhibited ion pumps.

Fig. 4 (bottom panel) shows one of three such experiments on mucosal acidification. Addition of CO_2 to mucosal fluid stimulated the acidification current, which is compatible with the observations

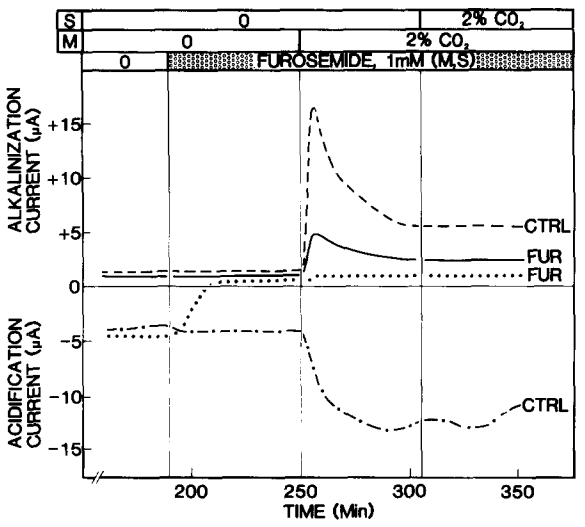

Fig. 4. Inhibition of CO_2-stimulated acid-base transport by furosemide. Data from two bladders bathed in Cl^--free and initially nominally HCO^- 8d3-free medium (plus ouabain and amiloride) and equilibrated with 100% O_2 are shown. To accelerate the acid-base transport processes CO_2 was first added to the mucosal (M) and then to the serosal (S) fluid, which lowered fluid pH from 7.6 to 5.9. Lower panel: Acidification current of control (·–·–·) and furosemide-treated (·····) paired tissues. Upper panel: Alkalinization current of control (- - - - - -) and furosemide-treated (———) paired tissues. Prior to the addition of CO_2, tissues were exposed for 90 min to 0.1 mM SITS in serosal fluid, 0.1 mM IBMX plus 1 μM forskolin in mucosal and serosal fluids (not shown), and for 60 min to furosemide (as indicated). baseline alkalinization current was low due to low levels of substrate for transport.

of others [31]. The subsequent addition of CO_2 to the serosal fluid of control bladders, which annulled transepithelial CO_2, HCO_3^-, and pH gradients, neither increased nor decreased the Isc. Since CO_2 in the serosal fluid alone also stimulates acidification [13,15,18], the above data suggest that the formation of HCO_3^- and H^+ in the hydration of CO_2 supplied from the mucosal fluid was maximal. More importantly, there was no CO_2-induced stimulation of I_{sc} in furosemide-treated tissues.

The effect of furosemide on the CO_2-accelerated alkalinization current (reproduced in three experiments) is shown in Fig. 4 (top panel). In control tissue, mucosal CO_2 stimulates the alkalinization current consisting of a transient phase and plateau phase higher than control. Serosal CO_2, subsequently, did not change the I_{sc}. In the furosemide-treated tissue, moreover, both the transient and steady-state stimulation of the I_{sc} was largely abolished.

Effect of furosemide on Na^+ transport

The effect of furosemide on the positive I_{sc} of bladders bathed in (HCO_3^- + CO_2)-free Na^+ media is shown in Fig. 5. After 60 min the I_{sc} of $+41.5 \pm 7.5$ μA ($N = 6$) of bladders in Cl^--rich medium was decreased by $48.2 \pm 12.2\%$ and R of 1.1 ± 0.2 kΩ increased by $36.5 \pm 10.7\%$.

In Cl^--free medium the effect was similar, the decline in I_{sc} being $59.6 \pm 16.5\%$ ($N = 3$), but the increment in R being higher, $127.4 \pm 46.3\%$, which is consistent with the removal of the shunt conductance of a highly permeable ion, i.e. Cl^-. In four experiments it was observed that the inhibition was due to furosemide on the serosal side (ΔI_{sc}, $-40.4 \pm 9.4\%$), not on the mucosal side (ΔI_{sc}, $+9.3 \pm 8.6\%$). Washing of the serosal tissue surface failed to restore Na^+ transport during a period of 2 h.

Effect of furosemide on O_2 consumption

Since furosemide can interfere with metabolism in vitro [5,32], a decrease in ATP synthesis could have led to the decline of the aforementioned ion transport processes. To distinguish between a direct effect on metabolism and ion transport, the 'dinitrophenol method' of Weiner [5] was used, the rationale being that an inhibition of metabolism will inhibit tissue O_2 consumption in the presence

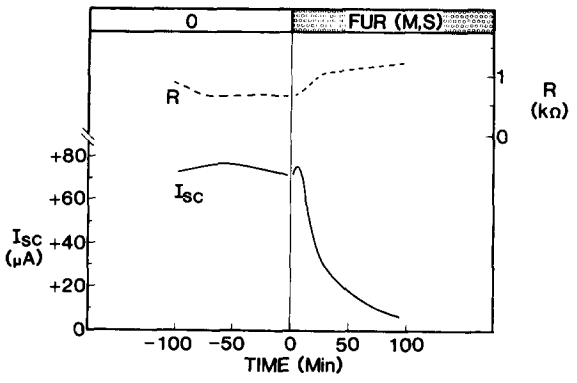

Fig. 5. Effect of furosemide on the Na^+-dependent I_{sc} and R. Bladders were bathed in Cl^--free, HCO_3^--free Na^+ medium. Furosemide (1 mM) was present in both mucosal and serosal fluids. Sign convention as in Fig. 1.

TABLE IV

EFFECT OF FUROSEMIDE ON 2,4-DINITROPHENOL-UNCOUPLED (DNP) O_2 CONSUMPTION

Period	O_2 consumption		
	Control (μl/h per g)	Experimental	
		μl/h per g	% of control
Control	133.1 ± 7.8	134.7 ± 7.5	100
Furosemide (1 mM)	120.5 ± 6.7	97.1 ± 5.0	77.3 ± 5.7 *
DNP (0.1 mM)	327.5 ± 17.8	331.2 ± 26.2	100.9 ± 5.4

* Significantly different from control, $P < 0.01$ ($N = 10$).

of 2,4-dinitrophenol, whereas a direct inhibition of transport will not affect the 2,4-dinitrophenol-uncoupled O_2 uptake.

As shown in Table IV O_2 uptake data from bladder sections bathed by $Na^+(Cl^- + HCO_3^-)$ medium plus ouabain and amiloride are consistent with a direct effect by furosemide on active anion transport. In two other experiments, in which bladder pieces were not treated with ouabain and amiloride, the results were identical to those obtained when Na^+ transport was inhibited.

Discussion

The results of this study show that furosemide inhibits active Na^+, Cl^-, and acid-base transport by the turtle urinary bladder. The inhibition of these transport process appears not to be due to a generalized inhibition of bladder metabolism, but to direct interactions of the drug with elements of the cation and anion transport processes.

Na^+ absorption. The irreversible inhibition of Na^+ transport by serosal furosemide suggests a direct action on the $(Na^+ + K^+)$-ATPase in the basolateral cell membrane, and is consistent with the drug's modest inhibition of microsomal $(Na^+ + K^+)$-ATPase from this tissue [33]. The failure of mucosal furosemide to inhibit Na^+ transport is consistent with other evidence [34,35] suggesting that the turtle bladder, unlike some other epithelia [2,3] lacks a similar sodium-chloride cotransport mechanism in the apical membrane.

Cl^- absorption. The inhibition of Cl^- transport by serosal furosemide is consistent with the operation of a Cl^-/HCO_3^- exchanger in the basolateral membrane as described by Durham and Matons [22] in their comprehensive model of Cl^- and acid-base transport for the turtle bladder (see also Fig. 6). The basolateral Cl^-/HCO_3^- exchanger may be similar to the furosemide-sensitive anion exchange mechanism in erythrocytes [4], since SITS, another inhibitor of anion exchange in red cells [36], also inhibits Cl^- reabsorption in the turtle bladder [19]. An effect by furosemide on a Cl^- pump in the apical membrane [22] cannot be ruled out, however, without information on intracellular Cl^- activity.

Acidification. The inhibition of the acidification current by serosal furosemide in bladders bathed by Cl^--rich media is consistent with the view that a furosemide-sensitive [18] Cl^-/HCO_3^- exchanger [22] in the basolateral membrane is involved in acid secretion. However, such an electroneutral exchange process, cannot be the sole basolateral pathway for reabsorbed HCO_3^- as recently postulated by Fisher et al. [18], nor can this be the only site at which furosemide inhibits acidification since under essentially Cl^--free bathing conditions (1) a significant acidification current was generated by the bladders, consistent with the results of previous studies [18–22], and (2) serosal furosemide inhibited the acidification current. These results support the model described in Fig. 6.

Other findings point to a furosemide action distinct from inhibition of the basolateral anion pathways, i.e. (1) the Cl^--independent inhibition of acidification from the mucosal side, and (2) the total inhibition of CO_2-stimulated acidification. In contrast to the results, a rate-limiting modification of the basolateral anion pathways by furosemide would be expected to give a transient CO_2-induced stimulation of the I_{sc} similar to that observed when bladders are exposed to serosal SITS [37]. Therefore, in the absence of evidence for carbonic

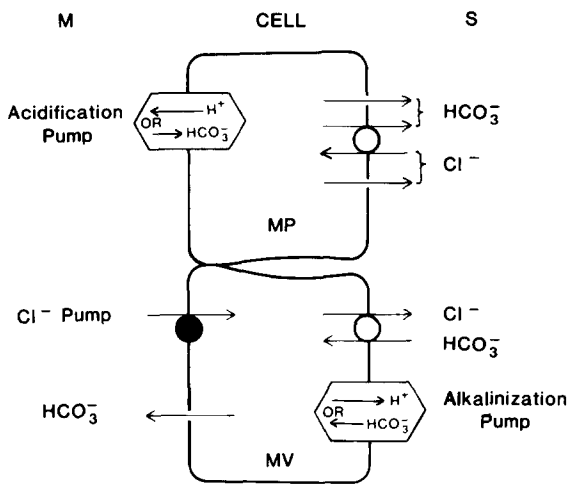

Fig. 6. Model of transepithelial acidification, alkalinization, and Cl^- absorption in the turtle bladder. This model depicts the conclusions of electrophysiological studies by Durham and Matons [22] and Brodsky et al. [40]. Consistent with morphologic evidence [39,45], it incorporates the acidification and alkalinization processes in separate carbonic anhydrase-rich cell types, characterized by apical microplicae (MP) or microvilli (MV), respectively. The MP cell contains an electrogenic acidification pump (H^+ secretion or OH^- or HCO_3^- absorption, the actual moiety transported by the pump not yet being established, but frequently through to be H^+) in the apical membrane and a Cl^-/HCO_3^- exchanger and conductive pathways for HCO_3^- and Cl^- in the basolateral membrane. The MV cell contains an electrogenic Cl^- pump and conductive HCO_3^- pathway in the apical membrane and an electrogenic alkalinization pump (H^+, OH^-, or HCO_3^- translocation) in the basolateral membrane. It is proposed that furosemide inhibits both Cl^--HCO_3^- exchange and acid-base pumps.

anhydrase inhibition, the lack of CO_2-stimulated acidification current in furosemide-treated bladders is ascribed to an inhibition of the acidification pumps. A direct effect on luminal H^+ secretion has been postulated by Radtke et al. [38] for furosemide's inhibition of volume absorption in the proximal tubule.

Alkalinization. Unlike an earlier model of the turtle bladder [14,15] proposing electroneutral luminal alkalinization in exchange for Cl^- absorption, a recent model, incorporating conductive HCO_3^- (or H^+) pathways in both apical and basolateral membranes, proposes electrogenic alkalinization without an absolute requirement for Cl^--HCO_3^- exchange [22]. The present data support the latter model insofar as the alkalinization current and its inhibition by furosemide occurred in the absence of ambient Cl^- and in the presence of another Cl^--HCO_3^- exchange inhibitor, SITS (Table III). Since furosemide inhibited the CO_2-stimulated alkalinization current, it is suggested that furosemide inhibits the alkalinization pump. This pump was earlier postulated to be located in the apical membrane [22], but is now thought to be located in the basolateral membrane, with the passive pathways for HCO_3^- located in the apical membrane [40,41] (see Fig. 6). If furosemide inhibited the apical pathways (presumably from the cytoplasmic side, since it is ineffective from the luminal side) one would expect no change in the magnitude of the transient CO_2-increment in the I_{sc} (Fig. 5), but primarily a change in the rates at which the CO_2-induced Isc increases or decays (see Ref. 30). This was not observed. A simultaneous inhibition of the alkalinization (or acidification) pumps and series conductive pathways cannot be ruled out, however. The resolution of this problem requires the development of specific techniques for measuring the apical and basolateral membrane conductances of the acid-base transporting cells, which make up a mere 11–15% of bladder epithelial cells [39].

The reason for furosemide's ability to inhibit acidification from both sides of the tissue, but alkalinization only from the serosal side is not known with certainty, but probably is related to the presence of two subpopulations of carbonic anhydrase-rich epithelial cells. We postulate that the cells with microplicae, the apical surface area of which decreases upon serosal SITS treatment [39], are responsible for the SITS-sensitive luminal acidification [19,37] and those, the apical surface of which appears not to be affected by SITS [39] are responsible for the SITS-insensitive luminal alkalinization [19–21]. With the assumption that furosemide acts on the cytoplasmic side of the acid-base pumps, its different effectiveness in inhibiting acidification and alkalinization from the mucosal and serosal sides of the bladder can be assigned to cellular differences in apical (low) and basolateral (high) membrane permeabilities to the anionic form of the drug (pK_a 3.9 [4]). Moreover, because of the lower pH microclimates at the cell surfaces near the acid-base pumps, furosemide at elevated levels in its undissociated form will diffuse across the apical membrane of cells responsi-

ble for luminal acidification and the basolateral membranes of cells responsible for luminal alkalinization.

The interpretation that furosemide acts on the acidification and alkalinization pumps is compatible with several lines of evidence: (1) furosemide has been shown to inhibit ATPases [33,42] and (2) vanadate-sensitive [43] and resistant [43,44] ATPases with apparent proton translocating activity isolated from turtle bladder epithelial cells. However, the mechanism(s) by which furosemide might affect the acid-base transport pumps remain(s) unknown.

In conclusion, the results presented here may be relevant for analysing acid-base transport and the possible action of furosemide thereon in the mammalian collecting duct, the intercalated cells of which resemble the carbonic anhydrase-rich cells of the turtle bladder [45] and are thought to be responsible for urinary acid-base transport.

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