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Mercury Blockade of Thiazide-Sensitive NaCl Cotransport in Flounder Urinary Bladder

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The effects of $HgCl_2$ on ion transport were investigated using isolated sheets of flounder urinary bladder, a model epithelium that is capable of electrically silent NaCl absorption and electrogenic K secretion. Exposure of the mucosal surface of the bladder to submicromolar doses of $HgCl_2$ reduced K secretion, but the effect was not due to blockade of apical K channels. Rather, the effects of $HgCl_2$ were virtually identical to those seen with experimental maneuvers that blocked the thiazide-sensitive NaCl cotransporter in the apical membrane, e.g., hydrochlorothiazide, Cl-free solutions, and Na-free solutions. Mucosal $HgCl_2$ also blocked ^{22}Na absorption, suggesting that the effect of the metal was mediated by blockade of NaCl entry. The effects of $HgCl_2$ had a rapid onset and were readily reversed by washing, suggesting a noncovalent binding reaction. The abundance of polyanionic Hg complexes in salt solutions prompts the speculation that one of these may bind to the Cl-binding site on the cotransporter, thereby blocking it. The results provide the first evidence that the thiazide-sensitive NaCl cotransporter is a specific site of action for inorganic mercury.

Membrane proteins, such as those involved in the transport of ions across cell membranes, are expected to be targets for inorganic mercury, a highly reactive species that can form mercaptide bonds with exposed sulfhydryl groups, thereby altering membrane transport or permeability (Rothstein, 1981). On the other hand, in aqueous, chloride-containing solutions, mercury exists predominantly in the form of neutral or charged polyanionic complexes that could, in principle, interact with membrane proteins by means of noncovalent, reversible binding reactions (Webb, 1966), and perhaps enter cells via anion transporters (Knauf and Rothstein, 1971b), or by solubility diffusion through the lipid bilayer (Gutknecht, 1981).

We present here evidence for a specific action of inorganic mercury: blockade of thiazide-sensitive NaCl cotransport. We used the urinary bladder of the Winter flounder for these experiments because it is the *only* flat-sheet, model epithelial tissue that is known to express the thiazide-sensitive cotransporter. The isolated bladder, mounted as a flat sheet in an Ussing chamber, exhibits two principal transport activities, NaCl absorption and K secretion, as shown schematically in Fig. 1. NaCl entry into the epithelial cells appears to be due entirely to a 1:1 NaCl cotransporter that is blocked by thiazide diuretics (Stokes *et al.*, 1984; Dawson and Frizzell, 1989). This electrically neutral entry step renders the salt absorptive process electrically silent. The apical membrane also contains a population of barium-sensitive K channels so that cellular K, accumulated by the basolateral Na/K ATPase, exits across the apical membrane giving rise to K secretion. Active K transport creates a mucosa-positive transepithelial potential and, under voltage clamp conditions, the short-circuit current is a quantitative measure of K secretion (Dawson and Frizzell, 1989).

For the purpose of the present experiments, it is important to note that the rates of NaCl entry and K exit across the apical membrane behave as if they are coupled; reducing NaCl entry, by means of a blocker such as hydrochlorothiazide (Stokes *et al.*, 1984), or by removing mucosal Na or Cl, also reduces K secretion. Although the mechanism for this apparent coupling is unknown, one factor could be decreased turnover of the basolateral Na/K ATPase. We show here that mercury blockade of NaCl entry also reduces K secretion, even though K channels are not blocked. The blockade of the cotransporter was highly specific for mercury and the ready reversibility of the effect suggests that interaction of the mercury with the cotransporter does not involve formation of a mercaptide bond, but rather the reversible binding of a polyanionic complex, perhaps $HgCl_3^-$.

METHODS

Urinary bladders were removed from flounder (*Pseudopleuronectes americanus*) that had been maintained for several days to 2 weeks in flowing seawater. Flat sheets of bladder were glued to rubber rings and mounted either in a perfusion chamber similar to that described by De

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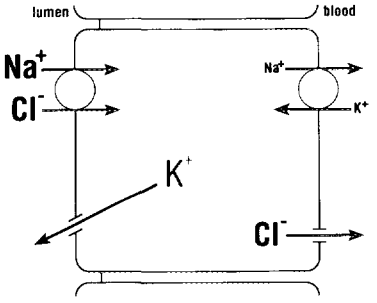

FIG. 1. Schematic model for NaCl absorption and K secretion in flounder urinary bladder showing ion transport under short-circuit conditions ($V_t = 0$), so that K secretion produces an electrical current. This current is presumed to be carried across the basolateral membrane by Cl exit. Other as yet unidentified conductive processes at the basolateral membrane are required to meet the requirements of mass and charge balance in the steady state.

Wolf and Van Driessche (1986) or in a standard Ussing chamber (Dawson and Frizzell, 1989). Perfusion chambers were used for determining the dose response to $HgCl_2$ and for electrophysiological studies because continuous perfusion permitted rapid changes in the composition of the solutions bathing the bladder and minimized the loss of mercury from the solutions due to nonspecific binding of mercury to the Lucite. In a preliminary study we noted that, in Ussing chambers having a constant 10-ml vol, after a rapid onset (see below) the inhibitory effect of $HgCl_2$ gradually diminished and disappeared completely in 20–30 min (Venglarik and Dawson, 1986). Readdition of the mercury resulted in a repetition of the same process, suggesting that the mercury was being removed from the solution by binding to the Lucite chamber. In spite of this difficulty, it was necessary to use standard, Lucite Ussing chambers for radioactive tracer studies, so somewhat higher concentrations of mercury were used to avoid problems due to binding.

In either chamber, the bladder was initially bathed on both sides by solutions containing (mmol/liter): Na, 147.5; Cl, 152.5; K, 2.5; Ca, 1.5; Mg, 1.0; Hepes, 15.0 (added as 50% Na Hepes and 50% Hepes acid); and glucose, 5.0, pH 7.5. Mucosal and serosal sides were either continuously perfused or vigorously stirred with air. All experiments were conducted at room temperature, which averaged about 20°C.

The transepithelial electrical potentials (V_t) of mounted bladders were voltage-clamped to zero mV (short-circuited), and the short-circuit currents (I_{sc}) were monitored continuously on a chart recorder. Positive current indicated cation movement from serosa to mucosa. Transepithelial slope conductance (g_t) was determined periodically by recording the current deflections in response to clamping V_t to −10 mV briefly (~1 sec). To determine the spectral characteristics of spontaneous fluctuations in the I_{sc} due to stochastic opening and closing of apical K^+ channels, the fluctuations were filtered and amplified as described by Fisher and Van Driessche (1991) and monitored continuously on an oscilloscope. Power density spectra (PDS) were obtained and interpreted as described by Wilkinson *et al.* (1993). Briefly, PDS exhibiting a spontaneous Lorentzian component were fitted with the sum of single Lorentzian and $1/f$ components: $S(f) = [S_o/(1 + (f/f_c)^2)] + S(1)/f^a$, where S_o is the power of the Lorentzian low frequency plateau, f_c is the Lorentzian corner frequency, $S(1)$ is the power of the $1/f$ background noise at 1 Hz, and a is the exponent that defines the slope of the $1/f$ noise. The Lorentzian component was interpreted according to a two-state reaction scheme,

$$\text{Closed} \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} \text{Open},$$

where α and β are the rate constants for the closing and opening reactions, respectively. In this scheme, the Lorentzian corner frequency is given by the sum of the rate constants, $2\pi f_c = \alpha + \beta$. The power of the Lorentzian plateau depends on the density of open channels (N_o), the single-channel current $(i)^2$, and the rate constants, $S_o = 4N_o\, i^2\, \alpha/(2\pi f_c)^2$. Single-channel current is related to the channel conductance (γ) and the driving force for ion flow according to Ohm's law, $i = \gamma\,(E_a - V_a)$, where E_a is the Nernst equilibrium potential for the permeant ion and V_a is the electrical potential across the apical membrane, using the mucosal bath as reference.

^{22}Na fluxes were measured across sheets of bladder mounted in Ussing chambers using a sample-and-replace paradigm as previously described (Dawson and Frizzell, 1989; Post and Dawson, 1992). Briefly, ^{22}Na (5 μCi) was added to the hot side, and after a 20- to 30-min period to ensure that steady-state intracellular tracer concentration had been achieved and that transcellular fluxes were in a steady state, the cold side was sampled (0.5 or 1.0 ml) at 10-min intervals. Data were expressed as the rate coefficient (λ_{Na}) for tracer flow, calculated as tracer flow divided by tracer concentration on the hot side in units of cm/hr (Post and Dawson, 1992).

In either perfusion chambers or Ussing chambers, bladders were exposed to 10^{-5} M Verapamil on the serosal side to eliminate contraction of smooth muscle. Drugs and reagents were obtained from Sigma. Verapamil, $HgCl_2$, $BaCl_2$, and dithiothreitol were prepared as concentrates in distilled water and added to the perfusion solution or chamber volume (4 ml) as a small volume of concentrate. Hydrochlorothiazide was prepared similarly but in DMSO.

RESULTS

Hg^{2+} Reversibly Inhibits K^+ Secretion

Figure 2 shows the results of a representative experiment illustrating the effect of mucosal $HgCl_2$ on K^+ secretion by isolated flounder urinary bladder. In the presence of symmetric bathing solutions (flounder Ringer's), the I_{sc} generated by flounder bladders is due entirely to net K^+ secretion but can vary widely from one animal to another (Dawson and Frizzell, 1989). In the experiment shown I_{sc} was initially 11–12 μA/cm^2. The addition of 0.5 μM $HgCl_2$ to the

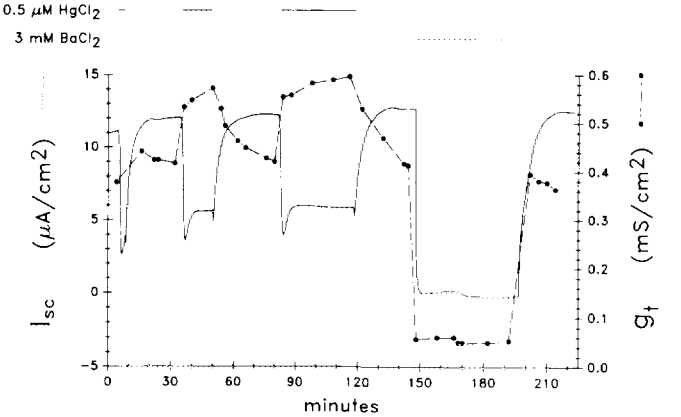

FIG. 2. Effects of 0.5 μM mucosal $HgCl_2$ on short-circuit current (I_{sc}) and transepithelial conductance (g_t) in isolated flounder urinary bladder. A flat sheet of bladder was perfused on both sides by NaCl solutions and short-circuited (V_t clamped to zero mV). I_{sc} was recorded continuously, and g_t was determined periodically by recording the current deflection in response to a 10-mV pulse. Positive values of I_{sc} indicate current flow from serosa to mucosa due to active K secretion. Mucosal $HgCl_2$ reduced I_{sc} and increased g_t.

mucosal perfusion solution rapidly and reversibly reduced I_{sc}. This dose was chosen on the basis of preliminary experiments indicating that 0.5 μM $HgCl_2$ in a perfusion chamber produced about 50% inhibition of I_{sc}. Shown are the results of three sequential exposures to mucosal $HgCl_2$, ranging in duration from less than 3 min to more than 30. In each case $HgCl_2$ rapidly reduced I_{sc}, and the new steady-state current was about 50% of the initial value. Perfusing with $HgCl_2$-free Ringer's rapidly restored the current to its preinhibition value. As shown previously (Dawson and Frizzell, 1989), mucosal barium rapidly reduced I_{sc} to near zero due to blockade of apical K channels. In the presence of 3 mM $BaCl_2$, the mercuric chloride had a small but reproducible effect, consistent with the notion that the $HgCl_2$-induced changes in I_{sc} can be attributed to an attenuation of K secretion, rather than the induction of another current in the absorptive direction.

If the effect of $HgCl_2$ on K^+ secretion were due to blockade of apical K^+ channels, the reduction in I_{sc} should be accompanied by a decrease in tissue conductance (g_t) reflecting the reduced K^+ conductance of the apical membrane. As illustrated in Fig. 2, however, exposure of the bladder to $HgCl_2$ *increased* tissue conductance and this effect was not seen in the presence of mucosal barium. Table 1 contains the results obtained from 10 tissues exposed to 0.5 μM mucosal $HgCl_2$. This dose produced nearly a 50% reduction in K secretion but actually *increased* total tissue conductance by 22%. Changes in *total* bladder conductance are often difficult to interpret, however, due to a highly variable contribution from the paracellular shunt pathway that can be influenced by, among other things, the state of the serosal smooth muscle (Dawson and Frizzell, 1989). For this reason we estimated apical K conductance by determining the change in the I_{sc} (ΔI_{sc}) induced by an increase in mucosal K concentration. Figure 3 shows a representative experiment for a tissue with an initial secretory K current of about 13 $\mu A/cm^2$. Raising the mucosal K concentration by 10 mM (from 2.5 to 12.5 mM) reduced the K current as expected due to the reduction in E_K across the apical membrane, where E_K is the equilibrium potential for K^+ given by $(RT/F)\ \ln([K]_i/[K]_o)$ and R, T, F, $[K]_i$, and

TABLE 1
Mucosal $HgCl_2$ Decreased I_{sc} but Increased g_t

	I_{sc} ($\mu A/cm^2$)	g_t (mS/cm^2)
Control	10.3 ± 2.2	0.49 ± 0.11
0.5 μM $HgCl_2$ (M)	5.2 ± 0.9*	0.58 ± 0.12*
% change	−42 ± 7%*	+22 ± 7%*

Note. Means ± SEM (n = 10). * Indicates statistically significant change ($p < 0.01$, two tailed, paired t tests). Values for % change are the means of the changes observed in individual experiments.

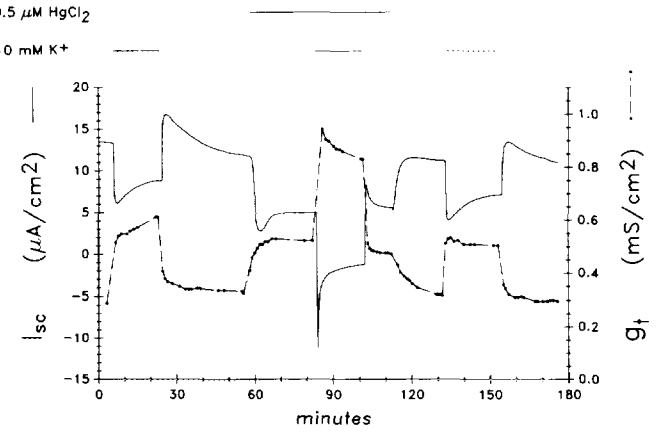

FIG. 3. Effect of 0.5 μM mucosal $HgCl_2$ on the changes in I_{sc} (ΔI_{sc}) and g_t (Δg_t) induced by a 10 mM increase in mucosal K concentration.

$[K]_o$ have their usual significance. The total resistance of the transcellular path represents the sum of that due to the apical and basolateral membranes, respectively. The fractional resistance of the apical membrane approaches unity (Dawson and Frizzell, 1989), however, so that the decrease in E_K is not expected to alter markedly the apical membrane potential (Wilkinson and Dawson, 1990), and the ΔI_{sc} as a result of the change in E_K provides an assessment of apical K conductance. Returning the mucosal K concentration to 2.5 mM resulted in a small overshoot and a return of I_{sc} to the original value. Barium (2 mM) in the mucosal bath reduced I_{sc} to zero and abolished the change in I_{sc} due to an increase in mucosal K, as expected if these current transients reflect the properties of barium-sensitive apical K channels (data not shown). In the presence of 0.5 μM mucosal $HgCl_2$, I_{sc} was reduced, but the change in I_{sc} in response to raising mucosal K was, if anything, somewhat greater under this condition. The time course of the mucosal K-induced change in I_{sc} was also altered, exhibiting a rapid transient and relaxation in the first several minutes. The significance of this transient is not clear at present but, like the responses seen under control conditions, it was abolished by mucosal barium. Washing out the mucosal $HgCl_2$, after approximately 1 hr, returned the current and the response to mucosal K to the pre-$HgCl_2$ values. Also shown in Fig. 3 is the tissue conductance. As reported previously (Dawson and Frizzell, 1989), g_t was increased by raising mucosal K and this effect was, if anything, increased in the presence of mucosal $HgCl_2$. Table 2 shows the average results of five such experiments. In the presence of mercury, the ΔI_{sc} induced by a 10 mM change in $[K^+]_m$ was *increased* by 136%, and the increase in g_t was augmented by 97%.

In some experiments we determined the spectral characteristics of spontaneous fluctuations in I_{sc} due to the stochastic opening and closing of the apical K^+ channels. These measurements can reveal blocker–channel interactions as the induction of a new component in the noise

TABLE 2

Mucosal $HgCl_2$ (0.5 μM) Enhanced the Changes in I_{sc} (ΔI_{sc}) and g_t (Δg_t) Produced by a 10 mM Increase in Mucosal [K^+]

	ΔI_{sc} ($\mu A/cm^2$)	Δg_t (mS/cm^2)
Control	−5.3 ± 1	+0.18 ± 0.02
0.5 μM $HgCl_2$ (M)	−12.0 ± 2*	+0.35 ± 0.04*
% change	136 ± 12%*	97 ± 5%*

Note. Means ± SEM (n = 5). * Indicates statistically significant change ($p < 0.01$, two-tailed, paired t tests). ΔI_{sc} was measured at the peak of the response. % change values are the means of individual changes.

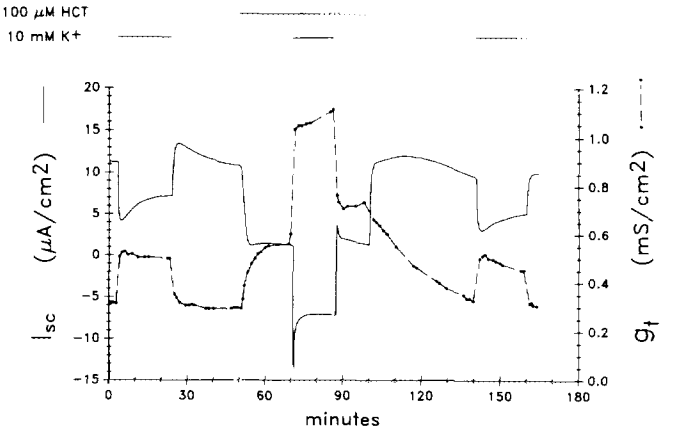

FIG. 4. Effect of 100 μM mucosal hydrochlorothiazide (HCT) on I_{sc} and g_t and the response to increased mucosal K.

spectrum with a distinct corner frequency (Wilkinson and Dawson, 1990). In the case of Ba^{2+}, for example, reversible blockade is readily detectable as a reduction in the power of the spontaneous fluctuations and induction of a new, low frequency component in the spectrum (Van Driessche *et al.*, 1985). During inhibition of I_{sc} by $HgCl_2$, however, no additional component was evident in the power density spectrum (Table 3). The f_c of the spontaneous fluctuations was unchanged, but the fluctuation amplitudes and, hence, the power of the Lorentzian plateau (S_0) were reduced, suggesting a reduction in either the number of open K^+ channels or the driving force for K^+ exit across the apical membrane. Taken together, the increased macroscopic K^+ conductance and the decreased power of the spontaneous fluctuations suggested a reduction in the driving force for apical K^+ exit due to hyperpolarization of the apical membrane potential or a reduction in the intracellular K^+ concentration. It seems clear that apical K channels were not blocked in the presence of $HgCl_2$ as suggested in preliminary studies (Venglarik and Dawson, 1986).

$HgCl_2$ Effect Is Mimicked by Maneuvers That Attenuate NaCl Cotransport

NaCl absorption by flounder urinary bladder proceeds by way of an electrically silent, apical NaCl cotransporter that is blocked by thiazide diuretics, and inhibition of NaCl absorption is expected to diminish K^+ secretion by depriving the basolateral Na^+/K^+-ATPase of intracellular Na^+, thereby reducing active K^+ uptake into the cells. To explore the possibility that the effects of mucosal $HgCl_2$ could be due to a single action, inhibition of NaCl entry across the apical membrane, we used three different experimental maneuvers previously shown to inhibit NaCl cotransport and reduce K secretion (Stokes *et al.*, 1984). Apical NaCl cotransport was attenuated by (1) blocking the cotransporter with hydrochlorothiazide (HCT), (2) removal of mucosal Na^+, or (3) removal of mucosal Cl^-. The effects of each of these maneuvers on K^+ secretion and apical K^+ conductance were compared with the effects of $HgCl_2$ in the same tissue. Figure 4 shows the results obtained when the same tissue used to test the effect of $HgCl_2$ (Fig. 3) was subsequently exposed to mucosal HCT (100 μM). The effect of the diuretic was remarkably similar to that of $HgCl_2$. This dose of HCT reduced I_{sc} nearly to zero, but the increase in g_t and a comparison of the responses to increased mucosal K showed that apical K conductance was elevated.

Figure 5A shows the results of a representative experiment in which I_{sc} was reduced by perfusing the mucosal surface with chloride-free Ringer's solution (gluconate substitution). This maneuver reduced I_{sc} nearly to zero, although a small component of barium-sensitive I_{sc} remained. In the presence of Cl-free solution, however, exposure of the apical surface to 0.5 μM $HgCl_2$ was without effect. Inspection of g_t and the effect of increased mucosal K suggested that although I_{sc} was reduced, K channels were not blocked. Furthermore, changes in I_{sc} in response to the increase in mucosal K concentration were attenuated by mucosal barium. During a subsequent exposure to elevated mucosal K, once again $HgCl_2$ had no effect. Figure 5B shows that in the same tissue, after restoring mucosal chloride, $HgCl_2$ exerted its typical effect, i.e., a reduction in I_{sc}

TABLE 3

Mucosal $HgCl_2$ Reduced the Power of the Spontaneous Lorentzian Plateau (S_0) but did not Change the Corner Frequency (f_c)

	f_c (Hz)	S_0 (10^{-20} A^2 sec/cm^2)
Control	23.8 ± 1.2	0.306 ± 0.092
0.5 μM $HgCl_2$ (M)	23.6 ± 2.0	0.116 ± 0.021*
% change	−2 ± 3%	−47 ± 11%*

Note. Mean ± SEM (n = 7). *Indicates statistically significant change ($p < 0.05$, two-tailed, paired t tests). % change values are the means of individual changes.

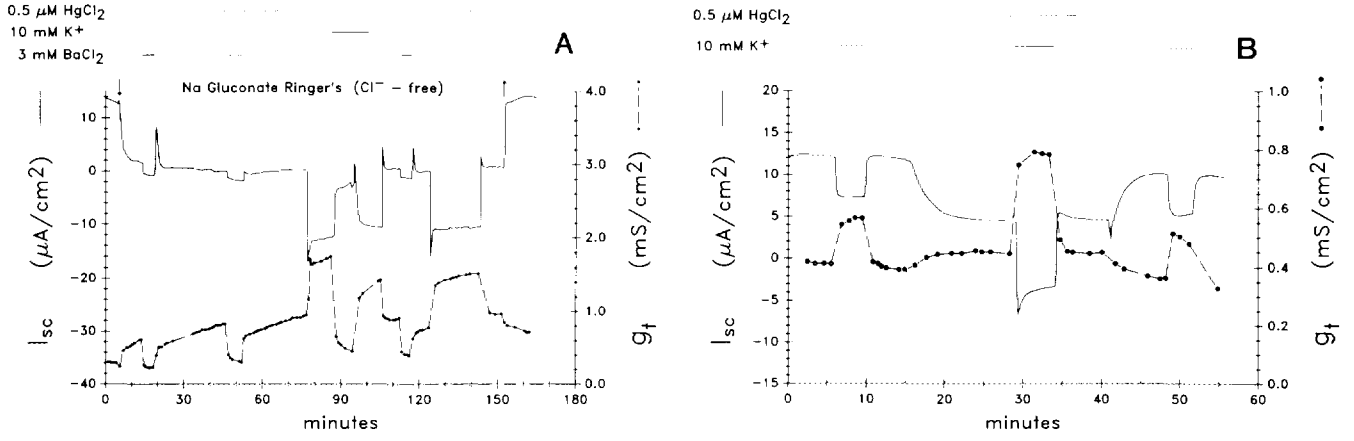

FIG. 5. (A) Effect of perfusing the mucosal bath with Cl-free (gluconate) Ringer's on I_{sc} and g_t and responses to mucosal $HgCl_2$, K^+, and $BaCl_2$. (B) Effect of $HgCl_2$ in the same tissue in the presence of Cl-containing Ringer's.

accompanied by an increase in apical g_K. This result was consistent with the notion that the site of $HgCl_2$ action was the cotransporter, but the attenuation of the $HgCl_2$ effect could also be related to an alteration in the abundance of Cl complexes of Hg^{2+} (see Discussion). Bathing tissues in Cl-free solutions often led to slow increases in conductance such as that which is evident in Fig. 5A. The reason for this behavior is unknown but may be related to the effects of Cl deprivation on intracellular solute composition or cell volume.

Figure 6 shows the effects of reducing the mucosal Na concentration by perfusing with Na-free, NMDG–Ringer's. This tissue responded to $HgCl_2$ and Cl-substitution in the typical fashion (not shown). Perfusion with Na-free mucosal solution reduced I_{sc} as expected and, if anything, increased apical K conductance, duplicating the effect of mercury. In addition, despite an abundance of chloride ion (145 mM), the effect of $HgCl_2$ was abolished.

These results create the strong impression that reducing NaCl entry by blockade of the cotransporter or removal of Na or Cl attenuates K secretion without reducing apical K conductance. The striking similarity of these effects to that produced by $HgCl_2$ and the fact that these maneuvers abolish the effect of $HgCl_2$ supports the notion that the heavy metal salt acts by blocking the thiazide-sensitive NaCl cotransporter.

^{22}Na Fluxes Confirm Inhibition of NaCl Absorption

As a more direct assay for inhibition of NaCl absorption by $HgCl_2$, we measured transmural fluxes of Na in the presence and absence of the heavy metal. Previous studies (Stokes *et al.*, 1984; Dawson and Frizzell, 1989) indicated that the rate of ^{22}Na movement from mucosa to serosa provides a measure of NaCl absorption. To reduce the effects of mercury binding to Lucite Ussing chambers, a dose of 1.5 μM $HgCl_2$ was utilized. As shown in Fig. 7, the inhibi-

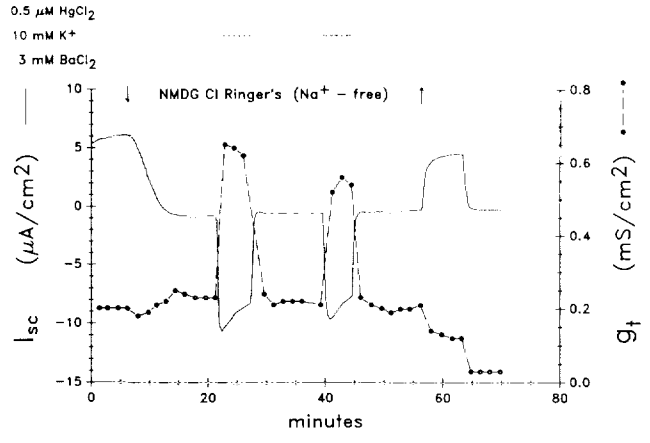

FIG. 6. Effect of perfusing the mucosal bath with Na-free (*N*-methyl-D-glucamine) Ringer's on responses of I_{sc} and g_t to $HgCl_2$, K, and $BaCl_2$ in the mucosal perfusate.

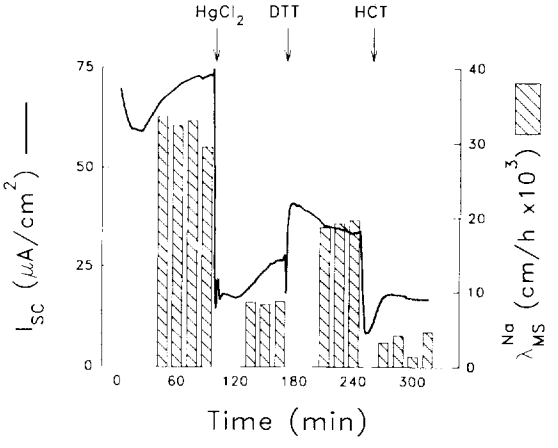

FIG. 7. Effect of $HgCl_2$ on I_{sc} and Na absorption. All additions were made sequentially to mucosal bath: $HgCl_2$ (1.5 μM) followed by DTT (1 mM) and HCT (100 μM).

TABLE 4
Inhibition of Na Absorption by $HgCl_2$

Condition	λ_{ms}^{Na} (cm/hr × 10^3)[a]
Control	26.6 ± 4.3
$HgCl_2$	8.3 ± 2.2
HCT	5.0 ± 2.2
% inhibition	84.7[b] ± 1.9

[a] All values are x ± SE, n = 5.
[b] % inhibition was calculated as $[\lambda_{ms}^{Na}$ control − λ_{ms}^{Na} $HgCl_2]/[\lambda_{ms}^{Na}$ control − λ_{ms}^{Na} HCT].

tion of I_{sc} by mucosal $HgCl_2$ was accompanied by a rapid reduction in the transmural flux of ^{22}Na. After about a 1-hr exposure, the effect on I_{sc} was partially reversed by the addition of 1 mM dithiothreitol. It is noteworthy that the ^{22}Na flux also exhibited proportionate, partial reversal. Addition of 100 μM HCT to the mucosal bath reduced both I_{sc} and the ^{22}Na flux. The serosa to mucosa ^{22}Na flux (not shown) was unaffected by these maneuvers. Summarized in Table 4 are the results of five experiments in which λ_{ms}^{Na} was determined under control conditions, in the presence of mucosal $HgCl_2$, and again after the addition of mucosal HCT. $HgCl_2$ produced about 85% inhibition of the thiazide-sensitive ^{22}Na flux.

Specificity and Reversibility of $HgCl_2$ Effect

Inhibition of NaCl cotransport, evaluated using I_{sc}, was highly specific for $HgCl_2$. Addition of a variety of other compounds (Table 5) to the mucosal bath resulted in either no effect or only a small change in I_{sc}, even at greatly elevated concentrations. Particularly noteworthy is the observation that the organic mercurial, PCMBS, was without effect, except at higher concentrations.

The reversibility of the effect of $HgCl_2$ was, not surprisingly, dependent on the length of exposure. For times up to about 90 min following addition of from 0.5 to 1.5 μM $HgCl_2$ to the mucosal bath, the effect was completely reversed by simply washing the mucosal bath with Hg^{2+}-free Ringer's. After 90 min, however, reversal was often incomplete.

DISCUSSION

NaCl Cotransporter as a Target for Inorganic Mercury

The toxic effects of mercury compounds on the human body are widespread, including effects on the kidneys, gastrointestinal tract, and brain (Venugopal and Luckey, 1978). Analysis of the molecular mechanisms underlying these effects is complicated by the fact that the three molecular forms of mercury, inorganic, organic, and vapor, can have different targets. We chose to study the action of inorganic mercury with the expectation that the molecular interactions of mercury salts might be the simplest. The results indicate that the thiazide-sensitive NaCl cotransporter is a specific target for inorganic mercury.

The actions of inorganic mercury originally attracted our attention when we found that $HgCl_2$ was a potent, reversible inhibitor of active K secretion. This observation raised the possibility that Hg^{2+}, like other divalent cations (e.g., barium) might be a blocker of K channels (Venglarik and Dawson, 1986). A closer examination of the effects of $HgCl_2$, however, revealed that the inhibition of K secretion was not due to K channel blockade, but rather to an indirect effect, probably on the apical NaCl cotransporter.

The possibility that the thiazide-sensitive NaCl cotransporter could be a specific target for inorganic mercury is of interest for several reasons. First, the effect is highly specific for $HgCl_2$. Neither other divalent ion salts nor an organic mercury compound elicited the effect, even at substantially higher doses. Second, the effect is readily and completely reversible after moderate exposures, suggesting that the effects do not involve the formation of a mercaptide bond as might be presumed (see also below). Finally, this transporter appears to be important in distal salt and water transport in the mammalian kidney so that acute renal effects of $HgCl_2$ could stem, at least in part, from the inhibition of thiazide-sensitive NaCl cotransport.

Site and Mechanism of Action of Inorganic Mercury

Any attempt to formulate a working hypothesis for the mechanism of action of inorganic mercury on the thiazide-sensitive cotransporter must begin with an assessment of the forms of mercury that are likely to be abundant in salt solutions such as those used to bathe the isolated sheets of urinary bladder. The propensity of mercury to form halide complexes results in the existence of a variety of forms that vary in abundance as a function of Cl concentration and pH (Webb, 1966; Gutknecht, 1981). In a salt solution like

TABLE 5
Effects of Metal Ions and an Organic Mercurial on I_{sc}

Ion or compound	% inhibition
$BaCl_2$ (5 mM)	100
$HgCl_2$ (5 μM)	100
NiCl (5 μM)	0
$CuSO_4$ (3 μM)	0
$CoCl_2$ (2 μM)	0
CdCl2 (2 μM)	0
PCMBS	
(50 μM)	0
(150 μM)	15
(300 μM)	55

that used here ([NaCl] = 145 mM), the concentration of *divalent* mercury (Hg^{2+}) is expected to be vanishingly small, of the order of 10^{-18} M for a 1 μM total salt concentration. The majority of the mercury exists in three forms: the neutral complex, $HgCl_2$; the univalent anionic complex, $HgCl_3^-$, and the divalent anionic complex, $HgCl_4^{2-}$, in roughly equal amounts. The cotransporter protein, recently cloned (Gamba *et al.*, 1993), presumably has binding sites for Na^+ and Cl^-. It is tempting to speculate that the anion binding site can accommodate the univalent-anionic mercury complex $HgCl_3^-$. If the mercury complex binds with high affinity, but does not promote translocation, the catalytic cycle of the transporter would be blocked. The rapid onset and ready reversibility of the mercury effect are consistent with a noncovalent ion binding reaction with a site on the outside of the apical membrane. The chloride binding site of the thiazide-sensitive transporter seems a likely candidate for this site. In this context, it is of interest that the organic mercurial PCMBS, although not a potent blocker of NaCl cotransport, is apparently transported as an anion by the Cl/HCO_3 exchanger of human red blood cells (Knauf and Rothstein, 1971a,b).

This hypothesis is reminiscent of the model proposed by Tran *et al.* (1990) for the mechanism of action of metolazone, another thiazide-like diuretic, in the mammalian kidney. They found that the binding of [^{3}H]metolazone to rat kidney membranes was competitively inhibited by chloride ions and enhanced by sodium, and they reasoned that the diuretic bound to the Cl-binding site on the cotransporter. The availability of a variety of putative ligands for the cotransporter may facilitate the evaluation of the possible binding of an anionic form of mercury.

Mechanism for Increased Apical K Conductance

The experiments reported here shed no light on the mechanism by which a decrease in apical NaCl entry leads to a striking increase in apical K conductance. One possibility would be a change in steady-state cell volume induced by reducing salt entry. It is now well known that some epithelial K channels are modulated by volume changes (Post and Dawson, 1992). Other possibilities include changes in intracellular composition that lead, for example, to changes in intracellular calcium.

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