J. Membrane Biol. 112, 59–66 (1989)

The Journal of
Membrane Biology
© Springer-Verlag New York Inc. 1989

Activation of Cl^-/OH^- Exchange by Parachloromercuribenzoic Acid in Rabbit Renal Brush-Border Membranes

Lawrence P. Karniski
Laboratory of Epithelial Transport, Department of Internal Medicine, University of Iowa, Iowa City, Iowa 52242, and Veterans Administration Medical Center, Iowa City, Iowa 52242

Summary. The effect of the sulfhydryl reagent parachloromercuribenzoic acid (PCMB) on chloride transport was examined in rabbit renal brush-border membrane vesicles (BBMV). PCMB had no effect on the chloride conductive pathway. In the presence of an inside-alkaline pH gradient and a K^+/valinomycin voltage clamp, the addition of PCMB stimulated ^{36}Cl uptake and induced a threefold overshoot above the equilibrium value, indicating Cl/OH exchange. The effect of PCMB was reversed by dithiothreitol. Cl/OH exchange was not observed in the absence of PCMB. PCMB-activated Cl/OH exchange persisted even when the membrane potential was made inside-negative relative to the controls, thus, demonstrating that PCMB's effect on ^{36}Cl uptake under pH-gradient conditions is not mediated by parallel Cl^- and H^+ conductive pathways. PCMB-activated Cl/OH exchange was inhibited by 4,4′-diisothiocyanostilbene -2,2′-disulfonic acid (DIDS) and 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) with IC_{50} values of 290 and 80 μM, respectively. These results demonstrate that modification of sulfhydryl groups by PCMB activates Cl/OH exchange in BBMV.

Key Words parachloromercuribenzoic acid · Cl/OH exchange · sulfhydryl reagents · chloride transport · DIDS · brush-border membranes

Introduction

In the mammalian proximal tubule, filtered chloride is reabsorbed by paracellular and transcellular processes [2, 3, 6, 11]. Microperfusion studies of rat and rabbit proximal tubules suggest that the majority of the active chloride entry across the apical membrane is via electroneutral Cl/formate exchange [1, 5, 25]. These same studies also show that a small component of Cl/base exchange remains in the absence of added formate. It has been proposed that this component of Cl/base exchange represents either Cl/HCO_3 exchange or Cl/formate exchange driven by the endogenous production of formate in proximal tubule cells. Attempts to confirm the presence of apical membrane Cl/HCO_3 (OH) exchange using brush-border membrane vesicles (BBMV) have yielded conflicting results, with some investigators supporting [28, 31] and others refuting [10, 16, 17, 27] its existence. Furthermore, BBMV studies have consistently identified a significant chloride conductive pathway that is not apparent in perfused proximal tubules [12, 16, 27, 28, 31].

The reason for the conflicting data regarding proximal tubule chloride transport is unclear. One possible explanation is that the various techniques or experimental conditions modify the transport proteins in such a way that transport activity is altered. Such an effect has been observed in certain mammalian cell systems where chloride transport activity can be altered by modification of sulfhydryl groups. For example, in Ehrlich ascites tumor cells and some species of erythrocytes, sulfhydryl reagents activate KCl cotransport [19, 21], while sulfhydryl reagents stimulate or inhibit chloride transport in the hamster thin ascending limb of Henle's loop [15]. Based on these observations, I examined the effects of sulfhydryl reagents on the functional expression of chloride transport in rabbit BBMV. The results demonstrate that the sulfhydryl reagent, parachloromercuribenzoic acid (PCMB), activates a significant DIDS-sensitive electroneutral Cl/OH exchanger in these membranes.

Materials and Methods

Methods

Membrane Isolation and Preparation

Brush-border membrane vesicles were isolated from renal cortices of male New Zealand White rabbits (2–3 kg) using the Mg-aggregation method described previously [4, 18]. Homogenization buffer contained 80 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 40 mM tetramethylammonium

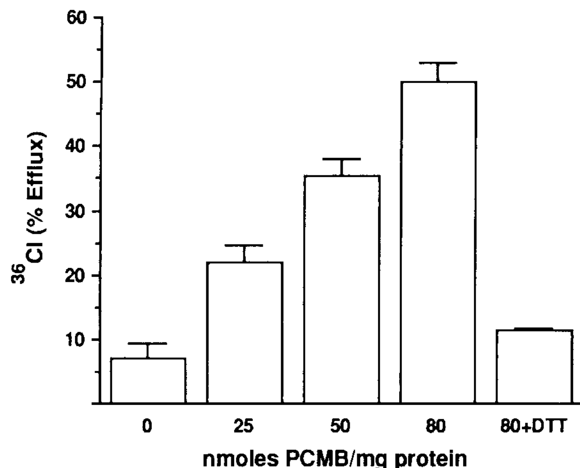

Fig. 1. Effect of PCMB on chloride efflux. Vesicles were pre-equilibrated with 146 mM mannitol, 78 mM HEPES, 39 mM TMA-OH, 10 mM [^{36}Cl]TMA-Cl, pH 7.5, plus or minus 10 mM DTT. The 3-sec efflux of ^{36}Cl was determined following a 1:25 dilution of vesicles into a medium containing TMA-H-M buffer with various concentrations of PCMB. Each datum represents the mean ± SE of three separate experiments

hydroxide (TMA-OH), 150 mM mannitol at pH 7.5 (TMA-H-M buffer). The membranes were kept at 3–5°C at all times during the isolation procedure. The final pellet was washed in excess TMA-H-M buffer plus 1 mM EDTA to remove the magnesium and stored at −70°C. No difference in baseline Cl^- conductive transport or Cl/OH exchange was noted using frozen membranes when compared to those freshly prepared. Protein concentration was determined by the method of Lowry et al. [22] as modified by Peterson [24].

Transport Assays

Vesicles were pre-equilibrated with the desired salts by incubating for 2–3 hr at 20°C. The composition of the vesicles and uptake media are given in the appropriate figure legends. Uptakes of radiolabeled isotopes were measured using the rapid filtration technique as described previously [18]. Ice-cold stop solution for the termination of uptake consisted of 80 mM mannitol, 177 mM K, 95 mM PO_4, 2 mM probenecid, pH 7.6. Efflux experiments were performed by pre-equilibrating the vesicles with isotope then diluting them into nonisotopic efflux medium for various lengths of time. The rate of efflux was calculated by subtracting the remaining intravesicular isotope content from the initial concentration, which was determined by rapid filtration of pre-equilibrated vesicles added directly into stop solution. In experiments where [^{3}H]-glucose and ^{36}Cl were used simultaneously, a correction was made for crossover from one channel to the other.

Stock solutions of 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) and 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) were made by dissolving the compounds in DMSO. Final concentrations of DMSO never exceeded 3.6% (vol/vol) and equal volumes of DMSO were always added to the control solutions. In experiments using valinomycin, the ionophore was added from a stock solution in ethanol directly to the membrane vesicles with 30 min remaining in the pre-equilibration period. Ethanol was added in equal amounts to the appropriate controls and never exceeded a concentration of 1% (vol/vol). The data are expressed either as representative experiments with each point performed in triplicate, or as the mean ± SE of at least three experiments using different membrane preparations.

MATERIALS

PCMB, parachloromercuribenzenesulfonic acid (PCMBS), DIDS, probenecid, and valinomycin were purchased from Sigma. From ICN we obtained D-[6-^{3}H]-glucose (18 Ci/mmol in 90% ethanol) and [^{36}Cl]HCl (13.1 mCi/g Cl) neutralized with TMA-OH. NPPB was obtained from Dr. Rainer Greger.

Results

In order to examine the effects of sulfhydryl reagents on chloride transport in BBMV, the rate of ^{36}Cl efflux was determined in the presence of increasing amounts of PCMB. As seen in Fig. 1, PCMB markedly stimulated the 3-sec efflux of chloride. This effect is probably the result of sulfhydryl group modification since the effect of PCMB was not observed in vesicles exposed to the thiol-reducing agent dithiothreitol (DTT). A similar effect was observed with increasing concentrations of N-ethylmaleimide (NEM), but only when it was added to the vesicles during the preincubation period (*data not shown*). Because the immediate effect seen with PCMB makes this compound easier to study, it was used in the following experiments.

To be certain that the results shown in Fig. 1 are not the result of alterations in either vesicular volume or membrane integrity, vesicles were first preloaded with ^{36}Cl and [^{3}H]-glucose, then exposed for 5 sec to efflux media with or without PCMB. As can be seen in Fig. 2, PCMB stimulated ^{36}Cl efflux almost fourfold while it had no effect on the rate of [^{3}H]-glucose efflux. These results demonstrate that PCMB-induced chloride flux is not simply the result of a generalized increase in membrane permeability.

It was next determined if the PCMB-induced chloride transport in BBMV is the result of an increase in chloride flux through the conductive pathway. To test this, PCMB-stimulated ^{36}Cl flux in the presence of an inside-positive membrane potential ($K_o > K_i$ + valinomycin) was compared to PCMB-stimulated chloride transport under voltage-clamped conditions ($K_o = K_i$ + valinomycin). As seen in the Table, in the presence of an inside-positive membrane potential, PCMB-stimulated ^{36}Cl uptake is increased 0.76 nmol · mg protein^{-1} compared to voltage-clamped conditions. However, rabbit renal BBMV have an endogenous chloride conductance and in the absence of PCMB chloride uptake is stimulated by a similar amount (0.84 nmol · mg

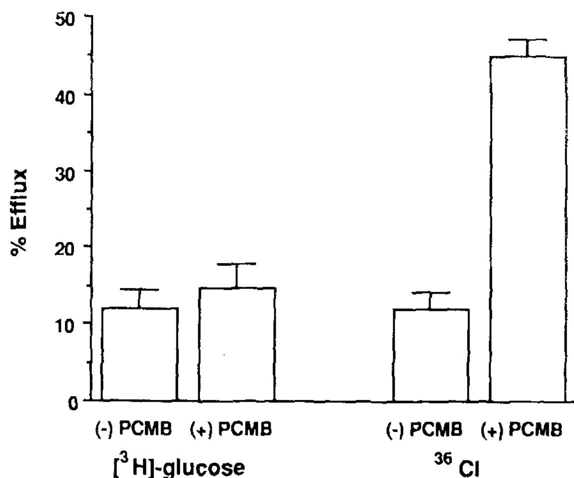

Fig. 2. Effect of PCMB on chloride and glucose efflux. Vesicles were pre-equilibrated with 146 mM mannitol, 78 mM HEPES, 39 mM TMA-OH, 10 mM [^{36}Cl]TMA-Cl, 0.28 μM [^{3}H]-glucose, pH 7.5. The 5-sec efflux of isotope was determined after a 1 : 9 dilution of vesicles into a medium containing TMA-H-M plus or minus 80 nmol PCMB/mg protein. Each datum represents the mean ± SE of three separate experiments

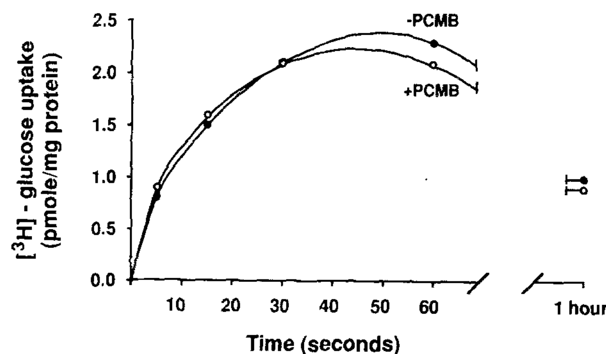

Fig. 3. Effect of PCMB on Na-glucose cotransport. Vesicles were pre-equilibrated with 105 mM mannitol, 56 mM HEPES, 120 mM gluconate, 80 mM K, 68 mM TMA, pH 7.5. The uptake of 0.38 μM [^{3}H]-glucose was determined in the presence of 103 mM mannitol, 55 mM HEPES, 120 mM gluconate, 36 mM Na, 80 mM K, 31 mM TMA plus (○) or minus (●) 80 nmol PCMB/mg protein

Table. Effect of the membrane potential on chloride transport in the presence and absence of PCMB

	5-sec uptake of ^{36}Cl (nmol · mg protein^{-1})	
	−PCMB	+PCMB
$K_i = K_o$	0.72 ± 0.21	3.06 ± 0.37
$K_o > K_i$	1.56 ± 0.36[a]	3.82 ± 0.39[a]
Absolute increase	0.84	0.76

The effect of changes in the membrane potential on ^{36}Cl uptake was studied in vesicles pre-equilibrated in 113 mM mannitol, 60 mM HEPES, 30 mM TMA, 100 mM K, 100 mM gluconate, pH 7.5 ($K_i = K_o$), or with isosmotic replacement of 90 mM K with TMA ($K_o > K_i$). The 5-sec uptake of chloride was assayed following a 1 : 9 dilution of vesicles into a medium containing 111 mM mannitol, 59 mM HEPES, 100 mM K, 100 mM gluconate, 4 mM [^{36}Cl] TMA Cl, pH 7.5, plus or minus 80 nmol PCMB/mg protein. Valinomycin (20–24 μg/mg protein) was added to the membranes during incubation. The results are expressed as means ± SE of five separate experiments.

[a] $P < 0.05$ ($K_o > K_i$ compared to $K_o = K_i$ conditions) as determined by the Student's paired t test.

protein^{-1}). Without an absolute increase in the voltage-sensitive component, it would appear that PCMB is not increasing chloride transport through the conductive pathway. To test for an effect of PCMB on chloride conductance another way, changes in the membrane potential were measured in the presence of an inwardly directed chloride gradient with or without the addition of PCMB using the electrogenic Na-glucose cotransporter as a probe of the membrane potential. As shown in Fig. 3, Na-stimulated [^{3}H]-glucose uptake is unaffected by PCMB, confirming its suitability for the following experiments.

The rate of electrogenic Na-glucose cotransport is increased in the presence of an inside-negative membrane potential and decreased in the presence of an inside-positive membrane potential; therefore, in the absence of a permeable counter ion, an inwardly directed sodium gradient will generate an inside-positive membrane potential and inhibit the rate of Na-glucose cotransport. On the other hand, the presence of an inwardly directed gradient of a permeable anion or an outwardly directed gradient of a permeable cation, will tend to collapse the sodium-generated membrane potential. As the membrane permeability of the counter ion increases, the sodium-generated membrane potential will be reduced and the rate of electrogenic, Na-glucose cotransport will also increase [7, 8, 14]. This effect is shown in Fig. 4. In the absence of PCMB (unhatched bars), an inwardly directed chloride gradient stimulates Na-glucose cotransport compared to the relatively impermeant gluconate anion, while an outwardly directed K^{+} gradient plus the ionophore valinomycin increases the rate of transport further. If the conductive chloride flux is increased by PCMB, then in the presence of an inwardly directed Cl^{-} gradient Na-glucose cotransport should be stimulated further when PCMB is added. However, as seen in Fig. 4, the addition of PCMB had no effect on electrogenic Na-glucose cotransport in the presence of chloride, suggesting that chloride conductance is not increased. This finding is not the result of an alteration in the voltage sensitivity of Na-glu-

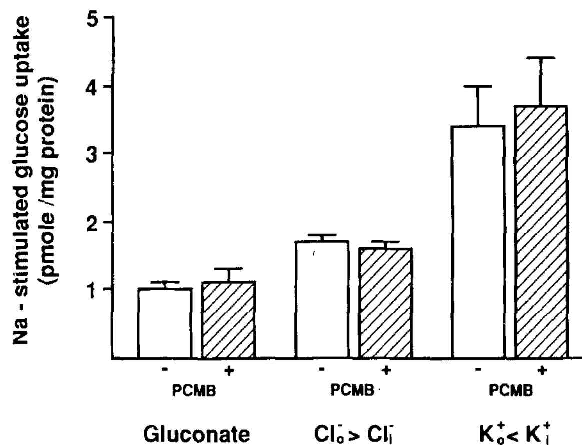

Fig. 4. Effect of PCMB on the chloride conductive pathway. Vesicles were pre-equilibrated in 105 mannitol, 56 mM HEPES, 120 mM gluconate, 148 mM TMA, pH 7.5, or with isosmotic replacement of TMA with 64 mM K ($K_o^+ < K_i^+$). The 5-sec uptake of 0.38 μM [^{3}H]-glucose was determined in the presence of 103 mM mannitol, 55 mM HEPES, 120 mM gluconate, 50 mM Na, 97 mM TMA, pH 7.5 (gluconate), or with isosmotic replacement of either gluconate by 50 mM Cl ($Cl_o^- > Cl_i^-$) or TMA by 6 mM K ($K_o^+ < K_i^+$). Valinomycin (22 μg/mg protein) was added during preincubation of the vesicles containing potassium. Each datum represents the mean ± SE for at least three separate experiments

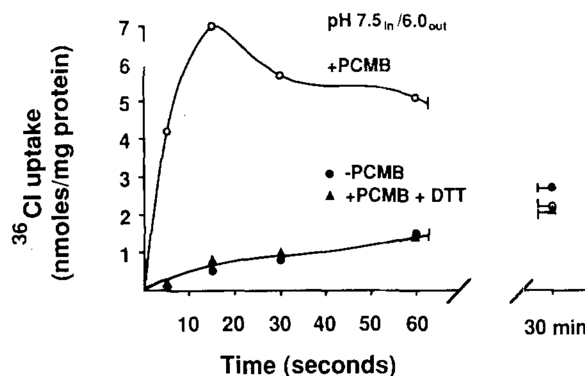

Fig. 5. Effect of PCMB on chloride uptake in the presence of an inside-alkaline pH gradient. Vesicles were pre-equilibrated with 113 mM mannitol, 60 mM HEPES, 30 mM TMA, 100 mM K, 100 mM gluconate, pH 7.5, plus (▲) or minus (○, ●) 10 mM DTT. The uptake of chloride was assayed following a 1 : 9 dilution of vesicles into uptake medium containing 112 mM mannitol, 60 mM MES, 25 mM TMA, 100 mM K, 100 mM gluconate, 2 mM [^{36}Cl]-TMA Cl, pH 6.0, plus (○, ▲) or minus (●) 40 nmol PCMB/mg protein. Valinomycin (22 μg/mg protein) was added to the membrane vesicles during preincubation

cose cotransport since the increased rate of [^{3}H]-glucose uptake in the presence of potassium plus valinomycin persists following the addition of PCMB.

Taken together, the results in the Table and Fig. 4 demonstrate that PCMB-induced chloride transport does not proceed through the conductive pathway but may instead represent an electroneutral transport process. This is possibly the result of Cl/OH exchange since PCMB-stimulated chloride transport is observed when OH^- is the only permeable anion present. To test for Cl/OH exchange, ^{36}Cl uptake was measured in the presence of an inside-alkaline pH gradient with and without the addition of PCMB. A potassium/valinomycin voltage clamp was employed to shunt the membrane potential to zero ($K_i = K_o$). As seen in Fig. 5, in the absence of PCMB a chloride overshoot is not observed. This is in agreement with results obtained by some investigators in that significant Cl/OH exchange is not found in rabbit renal BBMV under conditions where the membrane potential is adequately voltaged clamped [16, 17, 27]. When PCMB is added, ^{36}Cl uptake is stimulated several-fold and a significant overshoot is seen. This effect is abolished by DTT. These results suggest that PCMB-induced chloride transport in BBMV is the result of activation of Cl/OH exchange; however, an alternate explanation for these results is that PCMB increased the membrane $H^+(OH^-)$ permeability generating a voltage gradient despite the presence of the K^+/valinomycin voltage clamp. The subsequent inside-positive membrane potential would in turn serve as the driving force for the uphill accumulation of chloride through its conductive pathway.

To prove that PCMB-induced, OH-stimulated ^{36}Cl uptake represents electroneutral Cl/OH exchange, the following experiments were performed. These experiments were designed to use the Na-glucose cotransporter to detect relative changes in voltage gradients and to eliminate problems associated with confirming the presence or absence of a membrane potential. All experiments were performed in the presence of an inside-alkaline pH gradient and the uptakes of [^{3}H]-glucose and ^{36}Cl were measured simultaneously by double-labeling the uptake medium. The membrane potential was altered by varying the potassium gradients in the presence of valinomycin, and three conditions were employed: $K_o > K_i$, $K_o < K_i$ and $K_o < K_i$ (+PCMB).

As seen in Fig. 6*A*, when $K_o < K_i$, the rate of electrogenic Na-glucose cotransport in the presence and absence of PCMB is identical for the first 10 sec, suggesting that there is little difference in the membrane potential between these two conditions at early time points. More importantly, when $K_o > K_i$, the rate of Na-stimulated glucose uptake is markedly decreased as we would expect when an inside-positive membrane potential is generated. Even though the absolute value of the membrane potential cannot be determined, these results dem-

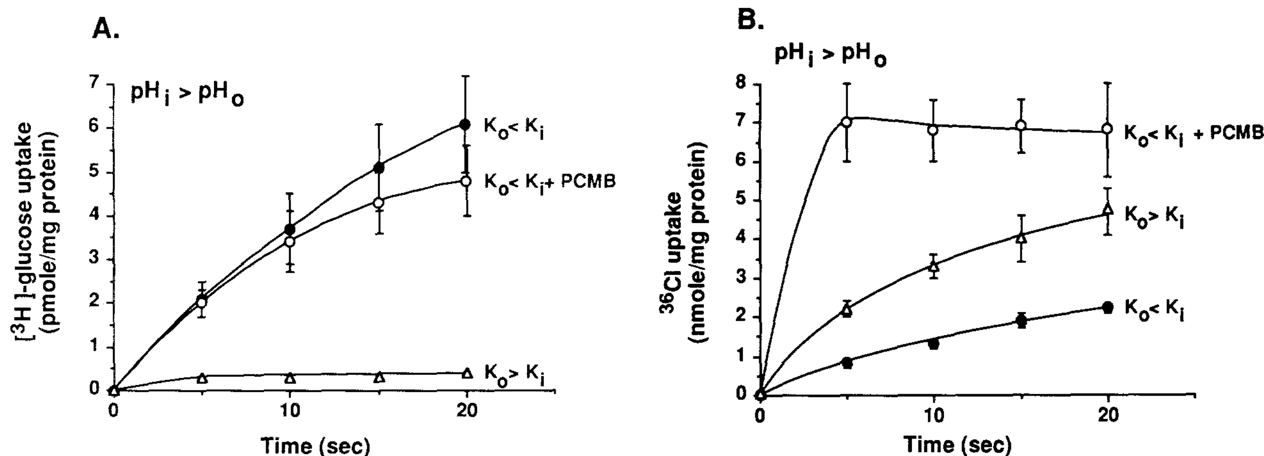

Fig. 6. Effect of membrane potential on Na-glucose cotransport and Cl/OH exchange. Vesicles were pre-equilibrated with 105 mM mannitol, 56 mM HEPES, 169 mM TMA, 1 mM K, 120 mM gluconate, pH 8.5 ($K_o > K_i$ [△]), or with isosmotic replacement of TMA with 80 mM K ($K_o < K_i$ [●] and $K_o < K_i$ + PCMB [○]). The uptake of chloride and glucose was assayed following a 1 : 9 dilution of vesicles into a medium containing 101 mM mannitol, 54 mM HEPES, 90 mM TMA, 120 mM gluconate, 40 mM Na, 0.42 μM [^{3}H]-glucose, 4 mM [^{36}Cl]TMA Cl, pH 6.9, with (○) or without (●, △) the addition of 80 nmol PCMB/mg protein. For $K_o > K_i$ conditions, TMA was replaced with 80 mM K. Valinomycin (27–32 μg/mg protein) was added to the membrane vesicles during incubation. Each datum represents the mean ± SE of at least three separate experiments

onstrate that there is an inside-negative membrane potential when $K_o < K_i$, with or without PCMB, compared to $K_o > K_i$ conditions. When chloride transport is now exmained under these same voltage and pH gradient conditions (Fig. 6*B*), ^{36}Cl uptake is markedly stimulated in the presence of PCMB and an outward potassium gradient ($K_o < K_i$ + PCMB). Since this condition represents an inside-negative membrane potential relative to $K_o > K_i$, if transport was through the conductive pathway, the rate of ^{36}Cl uptake should have been reduced, not stimulated, by the outward K^+ gradient as was observed in the absence of PCMB when $K_o < K_i$. These results demonstrate that PCMB-induced ^{36}Cl uptake cannot be attributed to a voltage-dependent conductive pathway but instead represents activation of electroneutral Cl/OH exchange.

The anion specificity of the PCMB-activated Cl/OH exchanger was tested by examining the *cis*-inhibitory effect of various anions on ^{36}Cl uptake in the presence of an inside-alkaline pH gradient plus PCMB. As shown in Fig. 7, the halide selectivity sequence is $I^- > Br^- \geq Cl^- > F^-$. Interestingly formate inhibited PCMB-activated Cl/OH exchange as well as chloride while NO_3^- had little effect.

Dose response curves demonstrating the ability of DIDS, NPPB and probenecid to inhibit PCMB-activated Cl/OH exchange are shown in Fig. 8. NPPB, a chloride channel blocker and anion exchange inhibitor [9, 30], was found to be the most effective inhibitor tested. In data not shown here, furosemide was without an effect at a concentration of 5 mM.

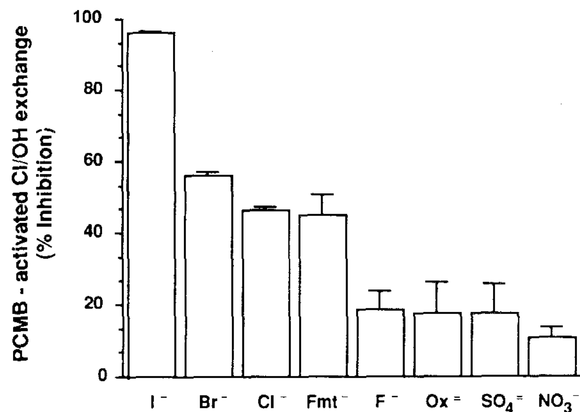

Fig. 7. Inhibitory effect of various anions on PCMB-activated Cl/OH exchange. Vesicles were pre-equilibrated with 113 mM mannitol, 60 mM HEPES, 30 mM TMA, 100 mM K, 100 mM gluconate, pH 7.5. The 5-sec uptake of chloride was assayed following a 1 : 9 dilution of vesicles in a solution containing 111 mM mannitol, 59 mM MES, 24 mM TMA, 100 mM K, 100 mM gluconate, 2 mM [^{36}Cl]-TMA Cl, 80 nmol PCMB/mg protein, pH 6.0 (control), or with gluconate replaced by 10 mM of various anions. Valinomycin (22 μg/mg protein) was added to the vesicles during incubation. Each datum represents the mean ± SE of three separate experiments

Finally, the effects of the organomercurial parachloromercuribenzenesulfonic acid (PCMBS) was examined. This compound is less lipid soluble than is PCMB and has been used to study outwardly

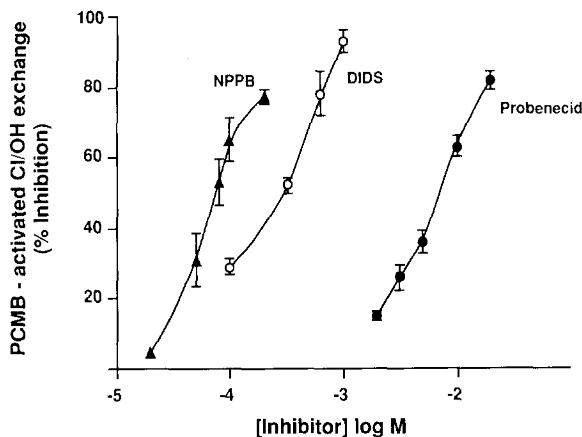

Fig. 8. Effect of inhibitors on PCMB-activated Cl/OH exchange. Vesicles were pre-equilibrated with 113 mM mannitol, 60 mM HEPES, 30 mM TMA, 100 mM K, 100 mM gluconate, pH 7.5. The 5-sec uptake of chloride was assayed following a 1 : 9 dilution of vesicles in uptake medium containing 105 mM gluconate, 2.8 mM [^{36}Cl]-TMA Cl, pH 6.0 (control). Stock solutions of probenecid (●), DIDS (○), and NPPB (▲) were added at various dilutions. Valinomycin (22–25 μg/mg protein) was added to the membrane vesicles during incubation. Each datum represents the mean ± SE of three separate experiments

facing SH groups located on the cell surface [29]. Concentrations of 800 nmol PCMBS/mg protein did not activate Cl/OH exchange (*data not shown*). This represents a concentration of PCMBS more than 30-fold greater than an effecitve dose of PCMB (Fig. 1). The lack of an effect by PCMBS suggests that the sulfhydryl groups involved in the activation of Cl/OH exchange in BBMV are either embedded in the membrane or are located within the cytoplasmic space.

Discussion

The results of the present study confirm the presence of a chloride conductive pathway in BBMV. Furthermore, they support the conclusion reached by some investigators that in the presence of valinomycin and high potassium concentrations ($K_i = K_o$), an inside-alkaline pH gradient is unable to stimulate the uphill accumulation of chloride in BBMV under standard conditions [17, 27]. However, these studies also show that modification of sulfhydryl groups with PCMB leads to a rapid activation of Cl/OH exchange. This transporter is sensitive to known inhibitors of anion exchange and does not require the presence of Na^+ or K^+.

Because of the significant chloride and H^+ (OH^-) conductive pathways in BBMV, it is necessary to demonstrate that the ^{36}Cl uptake in the presence of an inside-alkaline pH gradient is not due to electrical coupling of transport via parallel conductive pathways. Previous studies have addressed this problem by employing a K^+/valinomycin voltage clamp; however, because of the probes used to measure membrane voltage in BBMV, it is difficult to prove the adequacy of the clamp. These probes include the electrogenic Na-glucose cotransporter, the lipophilic cation tetraphenylphosphonium and the fluorescent dyes such as $DiSC_3$ (5). Each of these probes is inadequate to accurately measure the absolute value of the membrane potential at a given time, instead they have the capacity to detect only relative changes in voltage gradients. Subsequently, it is difficult to use these probes to "prove" that the voltage was clamped to zero in the presence of a pH gradient. In the present study, I took advantage of the ability of the Na-glucose cotransporter to detect a relative change in membrane potential and demonstrated that in the presence of an inside-alkaline pH gradient, the addition of PCMB does not result in a more favorable electrical driving force for ^{36}Cl uptake. This was accomplished by measuring PCMB-activated Cl/OH exchange under conditions intentionally made unfavorable for chloride conductive transport by generating an inside-negative membrane potential relative to controls. Therefore, regardless of the absolute value of the membrane potential in the experiments shown in Fig. 6, the fact that PCMB stimulates ^{36}Cl uptake in the presence of an inside-negative membrane potential relative to controls, demonstrates that chloride flux is not mediated by the conductive pathway but most likely represents electroneutral Cl/OH exchange. In fact, while these conditions were employed to inhibit ^{36}Cl conductive transport, they also underestimate the rate of electroneutral Cl/OH exchange and reduce the apparent overshoot (*compare* Figs. 5 and 6). This results from the inside-negative membrane potential favoring conductive H^+ influx leading to a more rapid collapse of the pH gradient. Furthermore, the inside-negative membrane potential will favor ^{36}Cl backleak via its conductive pathway.

While the PCMB-activated Cl/OH exchanger shares several characteristics with other anion exchangers identified in rabbit renal BBMV, it probably represents a separate transport system. For example, both Cl/oxalate exchange and PCMB-activated Cl/OH exchange accept chloride, OH^- and formate as substrates [18, 20]; however, PCMB-activated Cl/OH exchange has a much lower affinity for oxalate and DIDS. On the other hand, both the Cl/formate exchanger and the PCMB-activated Cl/OH exchanger accept chloride and formate as substrates and are inhibited by simi-

lar concentrations of DIDS and NPPB [9, 18]; however, in contrast to the Cl/formate exchanger, the Cl/OH exchanger has very little affinity for NO_3^- or furosemide.

It has been suggested that Cl/HCO_3 (or OH) exchange is located on the apical membrane of the mammalian proximal tubule but direct evidence confirming its existance in intact tubules has been lacking [26]. Recently, two studies examined the effect of luminal chloride on proximal tubule cell pH in the presence and absence of formate. In the rat, Alpern [1] observed cell acidification of 0.02 pH units when chloride was added to the lumen suggesting Cl/HCO_3 exchange; however, when chloride was added in the presence of 1 mM formate a 0.06 pH unit change was seen. Similarly, in studies by Baum [5], on isolated perfused rabbit proximal tubules, the addition of luminal chloride resulted in a small degree of cell acidification (0.03 pH units) that was much more pronounced in the presence of formate (0.11 pH units). Both these studies demonstrate that the majority of apical Cl/base exchange proceeds via Cl/formate exchange with Cl/HCO_3 exchange accounting for only a small component. This contrasts with the rate of transport observed in the present study. While a direct comparison with the chloride/formate exchange was not performed in this study, the magnitude of the ^{36}Cl overshoot observed with PCMB-activated Cl/OH exchange is at least as great as previously observed with chloride/formate exchange [18]. Furthermore, the rate of ^{36}Cl efflux in the presence of 80 nmol PCMB/mg protein is extremely rapid, with 50% of intravesicular chloride (10 mM) effluxing in the first 3 sec (Fig. 1). Compared to the small component of Cl/HCO_3 exchange seen in intact tubules, this raises the possibility that under normal conditions this transporter functions below its capacity. Because it is difficult to extrapolate transport rates from vesicles to in vivo conditions, additional studies will be required to determine if PCMB stimulates Cl/HCO_3 exchange in intact proximal tubules or if modification of sulfhydryl groups simply reactivates a transporter inactivated during the preparation of BBMV.

If Cl/OH exchange can be activated in vivo, what is its physiologic role? One possibility is that normally quiesent Cl/OH exchange is activated in response to certain environmental conditions. Such an activation would proceed through the alteration of critical sulfhydryl groups. Modification of SH groups is known to activate chloride transport processes in other biological systems. For example, the sulfhydryl reagent NEM activates KCl cotransport in certain species of red blood cells and in Ehrlich ascites tumor cells [19, 21]. Interestingly, NEM-activated KCl cotransport in these cells mimics KCl cotransport activated by exposure to hypotonic medium. It has been postulated that modification of sulfhydryl groups plays a critical role in regulating intracellular volume in these cell systems.

Given the nature of the ion gradients in the proximal tubule, apical membrane Cl/OH exchange could result in intracellular acidification. Interestingly, when Ehrlich ascites tumor cells and rat kidney cortical cells are incubated in an acidic medium, cell death from anoxia is delayed (23). Furthermore, the onset of cell death can be delayed by conditions that lower cell pH in hepatocytes that have been chemically depleted of ATP to mimic hypoxia (13). It is interesting to speculate that an alteration in the thiol/disulfide redox state of the cell during conditions that favor oxidation, such as hypoxia, could result in modification of critical SH groups. Subsequent activation of Cl/OH exchange would lead to protective acidosis. Further studies on the regulation of Cl/OH exchange will be required to address the role of this transport system in the proximal tubule.

The excellent technical assistance of Randy Manning is appreciated. I would like to thank Cheryl Pieper for preparing the manuscript and Drs. J. Stokes and M. Welsh for their critical comments. This work was supported by grants from the American Heart Association and the Veterans Administration.

References

1. Alpern, R.J. 1987. Apical membrane chloride/base exchange in the rat proximal convoluted tubule. *J. Clin. Invest.* **79:**1026–1030
2. Alpern, R.J., Howlin, K.J., Preisig, P.A. 1985. Active and passive components of chloride transport in the rat proximal convoluted tubule. *J. Clin. Invest.* **76:**1360–1366
3. Andreoli, T.E., Schafer, J.A., Troutman, S.L., Watkins, M.C. 1979. Solvent drag component of Cl^{-1} flux in superficial proximal straight tubules: Evidence for a paracellular component of isotonic fluid absorption. *Am. J. Physiol.* **237:**455–462
4. Aronson, P.S. 1978. Energy-dependence of phlorizin binding to isolated microvillus membranes. *J. Membrane Biol.* **42:**81–98
5. Baum, M. 1988. Effect of luminal chloride on cell pH in rabbit proximal tubule. *Am. J. Physiol.* **254:**F677–F683
6. Baum, M., Berry, C.A. 1984. Evidence for neutral trancellular NaCl transport and neutral basolateral chloride exit in the rabbit proximal convoluted tubule. *J. Clin. Invest.* **74:**205–211
7. Beck, J.C., Sacktor, B. 1975. Energetics of the Na^+-dependent transport of D-glucose in renal brush border membrane vesicles. *J. Biol. Chem.* **250:**8674–8680
8. Beck, J.C., Sacktor, B. 1978. The sodium electrochemical potential-mediated uphill transport of D-glucose in renal brush border membrane vesicles. *J. Biol. Chem.* **253:**5531–5535
9. Boelkins, M.R., Karniski, L.P. 1988. Inhibitory effect of a

new chloride channel blocker on Cl transport in rabbit renal microvillus membranes. *Kidney Int.* **33:**416 (*Abstr.*)

10. Cassano, G., Stieger, B., Murer, H. 1984. Na/H and Cl/OH exchange in rat jejunal and rat proximal tubular brush border membrane vesicles. *Pfluegers Arch.* **400:**309–317
11. Cassola, A.C., Mollenhauer, M., Fromter, E. 1983. The intracellular chloride activity of rat kidney proximal tubular cells. *Pfluegers Arch.* **399:**259–265
12. Chen, P.-Y., Illsley, N.P., Verkman, A.S. 1988. Renal brush-border chloride transport mechanisms characterized using a flourescent indicator. *Am. J. Physiol.* **254:**F114–F120
13. Gores, G.J., Nieminen, A.-L., Wray, B.E., Herman, B., Lemasters, J.J. 1989. Intracellular pH during "chemical hypoxia" in cultured rat hepatocyte. Protection by intracellular acidosis against the onset of cell death. *J. Clin. Invest.* **83:**386–396
14. Guggino, S.E., Martin, G.J., Aronson, P.S. 1983. Specificity and modes of the anion exchanger in dog renal microvillus membranes. *Am. J. Physiol.* **244:**F612–F621
15. Imai, M., Kondo, Y., Koseki, C., Yoshitomi, K. 1988. Dual effect of N-ethylmaleimide on Cl^- transport across the thin ascending limb of Henle's loop. *Pfluegers Arch.* **411:**520–528
16. Ives, H.E., Chen, P.-Y., Verkman, A.S. 1986. Mechanism of coupling between Cl^- and OH^- transport in renal brush border membranes. *Biochim. Biophys. Acta* **863:**91–100
17. Karniski, L.P., Aronson, P.S. 1985. Chloride-formate exchange with formic acid recycling: A mechanism of active Cl transport across epithelial membranes. *Proc. Natl. Acad. Sci. USA* **82:**6362–6365
18. Karniski, L.P., Aronson, P.S. 1987. Anion exchange pathways for Cl^- transport in rabbit renal microvillus membranes. *Am. J. Physiol.* **253:**F513–F521
19. Kramhoft, B., Lambert, I.H., Hoffmann, E.K., Jorgensen, F. 1986. Activation of Cl^- dependent K transport in Ehrlich ascites tumor cells. *Am. J. Physiol.* **251:**C369–C379
20. Kuo, S.-M., Aronson, P.S. 1988. Oxalate-OH exchange in rabbit renal microvillus membrane vesicles *FASEB J.* **2:**A753 (*Abstr.*)
21. Lauf, P.K. 1985. $K^+:Cl^-$ cotransport: Sulfhydryls, divalent cations, and the mechanism of volume activation in a red cell. *J. Membrane Biol.* **88:**1–13
22. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265–275
23. Pentilla, A., Trump, B.F. 1974. Extracellular acidosis protects Ehrlich tumor cells and rat renal cortex against anoxic injury. *Science* **185:**277–278
24. Peterson, G.L. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal. Biochem.* **83:**346–356
25. Schild, L., Geibisch, G., Karniski, L.P., Aronson, P.S. 1987. Effect of formate on volume reabsorption in the rabbit proximal tubule. *J. Clin. Invest.* **79:**32–38
26. Schwartz, G.J. 1983. Absence of Cl^--OH^- or Cl^--HCO_3^- exchange in the rabbit renal proximal tubule. *Am. J. Physiol.* **245:**F462–F469
27. Seifter, J.L., Knickelbein, R., Aronson, P.S. 1984. Absence of Cl-OH exchange and NaCl cotransport in rabbit renal microvillus membrane vesicles. *Am. J. Physiol.* **247:**F753–F759
28. Shiuan, D., Weinstein, S.W. 1984. Evidence for electroneutral chloride transport in rabbit renal cortical brush border membrane vesicles. *Am. J. Physiol.* **247:**F837–F847
29. Vansteveninck, J., Weed, R.I., Rothstein, A. 1965. Localization of erythrocyte membrane sulfhydryl groups essential for glucose transport. *J. Gen. Physiol.* **48:**617–632
30. Wangemann, P., Wittner, M., DiStefano, A., Englert, H.C., Lang, H.J., Schlatter, E., Greger, R. 1986. Cl^- channel blockers in the thick ascending limb of the loop of Henle. Structure activity relationship. *Pfluegers Arch.* **407:**S128–S141
31. Warnock, D.G., Yee, V.J. 1981. Chloride uptake by brush border membrane vesicles isolated from rabbit renal cortex. *J. Clin. Invest.* **67:**103–115

Received 9 March 1989