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Transepithelial electrical responses to Cl^- of nonsensory region of gerbil utricle

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Sheets of utricular epithelium from gerbil were mounted in a micro-Ussing chamber in order to identify and localize chloride conductances. The $[Cl^-]$ was rapidly reduced (substituted with isethionate) in the apical or basolateral perfusate and the transepithelial potential difference (V_t) and transepithelial resistance (R_t) were monitored continuously. In addition, agents known to inhibit anion transport in other epithelia were applied. The direction of all initial changes in V_t and R_t due to Cl^- substitutions were consistent with the presence of ionic conductances for Cl^- on both sides of the epithelium. The time-courses and magnitudes of the fall in V_t and increase in R_t during apical $[Cl^-]$ steps in the presence and absence of basolateral bumetanide were monophasic and identical in the two cases. The response of V_t to basolateral $[Cl^-]$ steps was biphasic and the initial response was greatly attenuated by bumetanide. These findings demonstrate that the largest conductance for Cl^- is in the basolateral cell membrane, but that the paracellular and/or apical pathway also possess a finite Cl^- conductance. All three agents tested, 3',5-dichlorodiphenylamine-2-carboxylic acid (DCDPC), 5-nitro-2(3-phenylpropylamino)benzoic acid (NPPB) and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), caused an increase in V_t. NPPB and DIDS were more effective from the apical side. DCDPC and DIDS administered from the apical side led to a decrease in R_t. These results suggest that these agents act in this tissue by enhancing a conductive pathway on the apical membrane rather than blocking the basolateral Cl^- conductance.

Introduction

The region of the gerbil utricle which contains predominantly dark cells and is free of sensory cells produces a K^+-rich, low-Na^+ luminal fluid, the endolymph, by means of electrogenic secretion of K^+ and electroneutral absorption of Na^+ [1]. In other species there is evidence that part of the Na^+ absorption of Na^+ [1]. In other species there is evidence that part of the Na^+ absorption may also partly be via an electrodiffusive pathway [2]. Transepithelial transport of these ions is dependent upon activity of Na^+/K^+-ATPase and of Na^+-Cl^--K^+ cotransport and can be inhibited by basolateral application of ouabain and furosemide-type loop diuretics [1–4].

Chloride is the major extracellular anion and is present in approximately identical concentration on both sides of this tissue in vivo. Since the loop diuretic-sensitive cotransporter presumably is involved in the movement of Cl^-, it was of interest to search for other Cl^- transport pathways in this tissue. A previous study employing measurement of transepithelial potential difference (V_t) during $[Cl^-]$ steps on the basolateral side in a 'split oil drop' preparation suggested the presence of a cellular Cl^- conductance, but the lack of an independently perfused lumen precluded definitive interpretation of the relaxation phenomena observed [3]. Attempts have also been made to identify a Cl^- conductance on the basolateral side by application of the anion transport inhibitors anthracene-9-carboxylic acid (9AC), diphenylamine-2-carboxylic acid (DPC), and 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS). These agents had no effect on V_t from the basolateral side [3].

Abbreviations: V_t, transepithelial potential difference; R_t, transepithelial resistance; DCDPC, 3',5-dichlorodiphenylamine-2-carboxylic acid; NPPB, 5-nitro-2(3-phenylpropylamino)benzoic acid; DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; CDPC, 5-chlorophenylamine-2-carboxylic acid; DPC, diphenylamine-2-carboxylic acid.

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In the present study, we tried to identify and localize conductive permeabilities for Cl^- in the K^+-secreting region of the utricle. A micro-Ussing chamber was used to measure changes in V_t and transepithelial resistance (R_t) during rapid changes of the perfusate of the apical (luminal) or basolateral (abluminal) sides of the tissue. Responses to [Cl^-] steps were measured in the presence and absence of bumetanide. Agents known to block anion transport processes in other epithelial preparations were also used: DCDPC, NPPB, CDPC and DIDS. A preliminary report has been made of some of the results [5].

Methods

Gerbils 6–10 weeks old were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and decapitated. The temporal bones were removed and the dark cell region of the utricle (identified by the melanocytes present in the underlying connective tissue) was dissected at 4°C. The epithelium was transferred to an Ussing chamber which had an aperture of 140 μm diameter [4]. Both sides were perfused at a flow rate of about 500–700 μl/min with Control solution (see below) at 37°C. Solution changes were 90% complete at the aperture within about 3 s, as judged by the time-course of liquid junction potential measurements in the chamber.

In order to use only preparations which sealed well to the chamber and which displayed responses to experimental manipulations large enough to readily quantify, preparations which developed a V_t less than +2 mV, apical side positive, were discarded. Since this criterion excluded only 8% of the samples expected in a normally distributed population [6], the statistical tests for significance of the experimental changes in V_t and R_t were not seriously compromised. Negative potentials were never observed under control conditions.

V_t was measured via symmetric agar bridges made from Control solution and Ag/AgCl electrodes. Corrections were made for liquid junction potentials. R_t was obtained from the voltage response to current pulses passed across the tissue (50 nA, 300 ms duration at 1–10 s intervals). R_t was either derived by sample-and-hold circuitry in the current clamp unit (Frankenberger, Munich, F.R.G.) or was derived from the recorded pulse height. Due to the geometry of the chamber, series resistance from control solution represented less than 5% of typical tissue resistance (100 kΩ). Changes due to altered solution composition were considered negligible. V_t and R_t were monitored on a strip chart recorder.

The Control solution consisted of (in mM): 150 NaCl; 5 KCl; 1.6 Na_2HPO_4; 0.4 NaH_2PO_4; 1 $MgCl_2$; 5 glucose; 0.75 $CaCl_2$ (pH 7.4). In low-Cl^- solution, 100 mM sodium isethionate, sodium cyclamate or sodium sulfate (made isotonic with mannitol) substituted for an equimolar amount of NaCl and $CaCl_2$ was raised to 3.3 mM to compensate for binding of Ca to the anion substitutes. CDPC, DCDPC and NPPB (substances No. 130, 131 and 144 in Ref. 7) were produced by Hoechst Pharmaceutical (Frankfurt, F.R.G.) and were the generous gift of Dr. Rainer Greger. DIDS was purchased from Sigma Chemical Co. (St. Louis, MO) and bumetanide was a gift of Hoffmann-La Roche (Nutley, NJ). All drugs were dissolved in DMSO to a final concentration of 0.1% DMSO in control solution. DMSO at this concentration had no effect on V_t or R_t.

Data are given as mean ± S.E. (n = number of utricles). Statistical significance was determined by use of the Student's t-test for paired samples, with $P < 0.05$ considered as a significant change.

Results

In 42 utricles, the initial V_t was +5.6 ± 0.4 mV and R_t was 30 ± 3 $\Omega \cdot cm^2$. These values compare well with measurements of +5.6 mV, 24 $\Omega \cdot cm^2$ [4] and +5.2 mV, 30 $\Omega \cdot cm^2$ [8].

Ion substitutions

In Fig. 1 are shown recordings of V_t during changes of [Cl^-] from 150 to 50 mM on the apical or basolateral side. Reducing [Cl^-] in the apical perfusate led to a mean decrease of V_t by 4.2 mV (Table I). Reduction of [Cl^-] in the basolateral perfusate led to an initial increase of V_t by 12.6 mV and a secondary relaxation phase settled to a final value statistically indistinguisha-

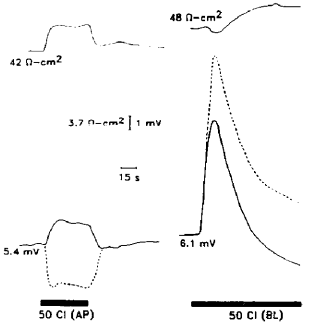

Fig. 1. Representative traces of R_t (upper panel) and V_t (lower panel) during perfusion of control solution and low-Cl^- solution in one utricle. Recordings of V_t have been approximately corrected for liquid junction potentials (broken lines). The times of low-Cl^- perfusions are indicated under the trace by the filled bars. Numbers at the beginning of each trace indicate initial values. Apical (AP), Basolateral (BL).

TABLE I

Response of V_t and R_t to [Cl⁻] steps from 150 to 50 mM

Mean ± S.E. (N). All values in 50 mM Cl^- were corrected for liquid junction potentials. Peak = greatest initial value; S-S = final steady-state value (see Fig. 1).

	V_t (mV)			R_t ($\Omega \cdot cm^2$)	
[Cl⁻] (mM):	150	50 (peak)	50 (S-S)	150	50 (S-S)
Apical					
Control (17)	5.7 ± 0.6	– [a]	1.5 ± 0.6	30.4 ± 3.4	34.3 ± 3.7
Bumetanide (5)	−0.4 ± 0.2	– [a]	−5.3 ± 0.1	42.7 ± 9.0	48.1 ± 10.2
Basolateral					
Control (16)	5.6 ± 0.5	18.2 ± 1.2	5.6 ± 0.3	30.7 ± 3.4	35.5 ± 4.5
Bumetanide (5)	−0.4 ± 0.3	5.8 ± 1.1	3.9 ± 0.8	42.9 ± 8.7	48.1 ± 9.9
AP and BL					
Control (9)	5.7 ± 0.8	14.4 ± 1.2	1.7 ± 0.4	33.0 ± 5.0	42.5 ± 7.6
Bumetanide (7)	−0.5 ± 0.2	0.5 ± 0.2	−0.5 ± 0.2	34.1 ± 7.7	43.8 ± 10.4

[a] The response was monophasic.

ble from the value before the solution change. Simultaneous perfusion of apical and basolateral sides with low-Cl^- solution increased V_t initially by 8.7 mV, but the secondary relaxation led to a V_t which was 4.0 mV lower than the V_t in control solution. R_t rapidly increased by 12.8% during apical [Cl^-] reduction, but increased slowly by 15.7% when [Cl^-] was reduced in the basolateral perfusion. During simultaneous [Cl^-] steps, R_t increased by 28.6%, a number nearly identical to the sum of the values from the unilateral [Cl^-] steps.

Several different anion substitutes for Cl^- were used, since it was possible that some of the observed effects might be due to unspecific actions on the tissue. The biphasic response to basolateral low-Cl^- solution prepared with isethionate, cyclamate or sulfate were similar to each other in shape and magnitude (data not shown).

A comparison was made between the response to [Cl^-] steps in the presence and absence of basolateral 10^{-4} M bumetanide (Table I). In the presence of bumetanide, a [Cl^-] step on the apical side lowered V_t from −0.4 mV to −5.3 mV. Paired experiments revealed no significant effect of bumetanide on the response of V_t to a change in [Cl^-] (Fig. 2). The initial peak response of V_t to basolateral low-Cl^- solution was markedly reduced in the presence of bumetanide. Bumetanide had no apparent effect on the increase in R_t caused by low-Cl^- perfusions on each side (Table I, Fig. 2). Simultaneous apical and basolateral perfusion with low-Cl^- solution in the presence of bumetanide led to peak values of V_t which were close to the algebraic sum of those for the unilateral perfusions.

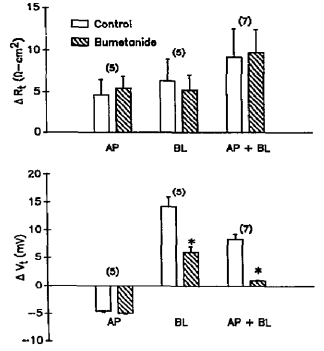

Fig. 2. Bar graph comparing ΔR_t (upper panel) and ΔV_t (lower panel) due to [Cl^-] steps in the absence ('control') and presence of 10^{-4} M bumetanide (5–7 paired experiments each). Initial peak responses caused by [Cl^-] steps on the basolateral (BL) and apical (AP) sides and by bilateral (AP + BL) [Cl^-] steps. Number of utricles is by each bar. Statistical significance is indicated by *.

Inhibitors

We were interested in demonstrating effects of substances reported to block Cl^- channels in other cells. Three agents (CDPC, DCDPC and NPPB) were chosen which belong to a family of recently developed compounds with structural similarity to DPC, which have been shown to block Cl^- channels in different epithelia. A compound known to block a variety of Cl^- transport processes including Cl^- channels, DIDS, was also tested. NPPB (10^{-4} M) caused a transient increase in V_t of 2.6 ± 0.5 mV ($n = 5$) when applied from the apical side and of 0.7 ± 0.1 mV ($n = 5$) when added to the basolateral side (Fig. 3). The initial response was larger and the time course shorter when NPPB was added to the apical perfusate. No significant changes in R_t due to NPPB were observed. In Fig. 4 are shown the dose-response curves of the initial responses from paired experiments. The response of V_t was significantly greater for apical perfusion at 50 and 100 μM.

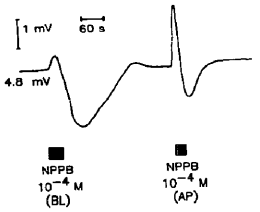

Fig. 3. Representative trace of V_t during apical (AP) or basolateral (BL) perfusion of $1 \cdot 10^{-4}$ M NPPB in one utricle. The times of perfusions are indicated under the trace by the filled bars. Numbers at the beginning of each trace indicate initial values.

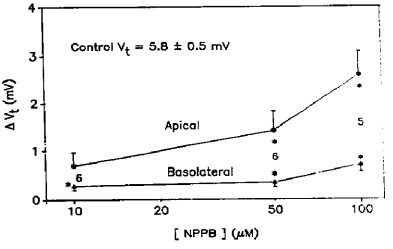

Fig. 4. Summary of results of changes in V_t during apical or basolateral perfusion of NPPB as in Fig. 3 at concentrations of $1 \cdot 10^{-5}$, $5 \cdot 10^{-5}$ and $1 \cdot 10^{-4}$ M.

The biphasic responses of V_t to DCDPC added to the apical perfusate were similar ($P > 0.05$) to those caused by NPPB (Fig. 5). In addition, R_t decreased

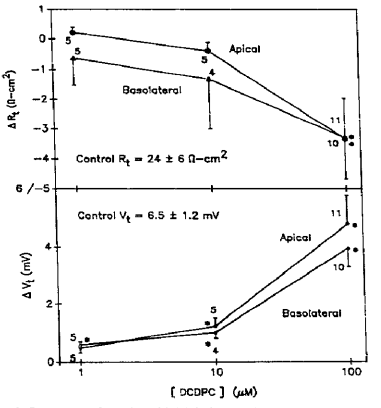

Fig. 6. Summary of results of initial changes in R_t (upper panel) and V_t (lower panel) during apical or basolateral perfusion of DCDPC as shown in Fig. 5. Statistical significance of difference from 0 indicated by *.

during the increase of V_t. Addition of DCDPC to the basolateral perfusate led to similar changes in V_t and R_t, although a subsequent overshoot of R_t was consistently observed during recovery. Dose-response curves for the initial peak action of DCDPC on V_t and R_t are shown in Fig. 6. DCDPC was effective in the same concentration range as NPPB, but there were no statistical differences between the effects on V_t and R_t from the apical and basolateral sides.

In one experiment, another agent of the DPC family, CDPC, was tested at 10^{-5} M. CDPC produced results

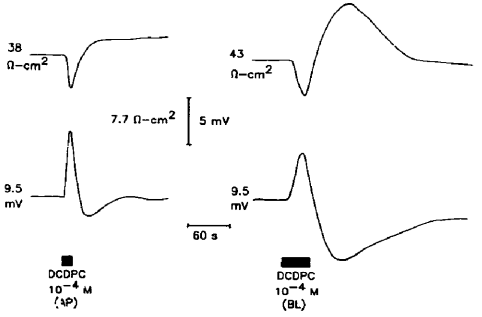

Fig. 5. Representative trace of the change in R_t (upper) and V_t (lower) during apical (AP) and basolateral (BL) perfusion of DCDPC at 10^{-4} M in one utricle. The times of perfusions are indicated under the trace by the filled bars. Numbers at the beginning of each trace indicate initial values.

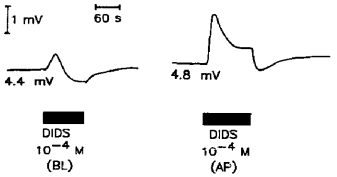

Fig. 7. Trace of the changes in V_t during basolateral (BL) or apical (AP) perfusion of DIDS at 10^{-4} M in one utricle. The times of perfusion are indicated under the trace by the filled bars.

similar to the other DPC derivatives: an increase in V_t of 1.5 mV when added to the apical side and an increase of about half that when added to the basolateral side ($n = 1$).

In Fig. 7 is shown a recording of the effect on V_t of 10^{-4} M DIDS. DIDS transiently elevated V_t when added to the apical perfusate (initial increase: 1.1 ± 0.2 mV ($n = 11$)) and decreased R_t (-1.1 ± 0.4 $\Omega \cdot cm^2$ ($n = 11$)). In five samples there was no observable change in R_t and in the six others the change ranged from -0.4 to -3.0 $\Omega \cdot cm^2$. No statistically significant effect on V_t or R_t was observed when DIDS was added to the basolateral side (0.2 ± 0.1 mV ($n = 7$)). Although the effects from the apical side on V_t were similar to those of DCDPC and NPPB, the secondary phase of the V_t response to DIDS was much smaller and V_t remained elevated during application of the agent.

Discussion

We have measured the responses of V_t and R_t of the Dark-cell region of the utricle to step changes in $[Cl^-]$ and to several putative Cl^- transport inhibitors. Results obtained can be discussed in terms of action on known and postulated ion transport pathways in the paracellular shunt, apical and basolateral membranes.

A sudden reduction of basolateral $[Cl^-]$ led to a striking biphasic response of V_t. This type of response is well known in cells which have a membrane potential influenced substantially by a Cl^- permeability in parallel with another processes which can make a larger negative contribution to the potential. In the present case, the change in V_t would reflect a fraction of the change in the basolateral membrane voltage. The rapid, initial phase would then be due to a reduction in the cell-negative basolateral electromotive force (EMF) for Cl^-, intrinsic to a basolateral Cl^- permeability, as a result of the extracellular drop in $[Cl^-]$. The slower, secondary decline of V_t would be due to repolarization of the EMF for Cl^- as a result of a presumed decline in intracellular $[Cl^-]$ ($[Cl^-]_i$). This decline in $[Cl^-]_i$ would be caused by the membrane potential being held more negative than the Cl^- equilibrium potential as a result of the above-mentioned parallel transport process.

In most cells, such as muscle, the process in parallel with the Cl^- permeability is a K^+ permeability [9]. Measurements of intracellular potential in vestibular dark cells indeed reveal a large Cl^- conductance as demonstrated by Cl^- step experiments [10]. However, it also appears that these cells have little K^+ conductance when perfused on both sides with control solution (unpublished observations). If this interpretation is correct, the above description of events (leading to the observed changes of V_t during basolateral perfusion of low-Cl^- solution) would still hold, except that the secondary decline of $[Cl^-]_i$ could be produced by the as-yet-unidentified electrogenic K^+-secretory process in the apical membrane.

Another possible contributing factor to the secondary decline of V_t is a partial inhibition of Na^+-Cl^--K^+ cotransport activity. Inhibition of this transporter by loop diuretics is known to reduce V_t (vide infra, Refs. 3, 4). The K_m of this process for Cl^- has been found in other cells [11] to be near 50 mM, the concentration which was used in the present experiments. In addition, it may be that the net driving force for inward Na^+-Cl^--K^+ cotransport drops sufficiently to decrease transport activity *.

In spite of the likely retarded activity of this transporter, the average final value of V_t during basolateral low-Cl^- perfusion was not significantly different from that before the experiment (Table I). On the other hand, V_t was reduced during bilateral low-Cl^- perfusion. Under this condition, there was no paracellular diffusion potential. The final value of V_t during basolateral low-Cl^- perfusion may therefore be a composite of a reduced electrogenic secretion plus a positive diffusion potential across the basolateral membrane and/or paracellular pathway. This could be tested in the future by looking for an effect of 50 mM Cl^- on the rate of K^+ secretion.

The biphasic response of V_t to basolateral Cl^- steps was observed earlier in a split oil drop preparation of utricle nonsensory tissue, but there was some question of the origin of the secondary phase since in this preparation there may have been slow changes in luminal composition in the absence of luminal perfusion [3].

* Although the stoichiometry for the cotransporter in the utricle has not yet been demonstrated, we can calculate a range of driving forces for assumed stoichiometries of 1 or 2 Cl^-, 1 Na^+ and 1 K^+ using reasonable values of intracellular ion concentration. Assuming intracellular ion concentrations of Na^+ 10 to 20 mM, K^+ 140 to 130 mM and Cl^- 70 mM, the net driving force has a range of -13 to $+14$ mV ($+$ = net force directed inward). Intracellular $[Cl^-] = 70$ mM was chosen because cells with a cotransporter typically have a high $[Cl^-]$ and because preliminary measurements of intracellular dark cell potential have shown an average value of about -20 mV which seems to be determined primarily by a large Cl^- conductance [10].

The present experiments, which utilized a rapid individual perfusion on both sides of the epithelium, show that the secondary phase is not dependent on an unstirred apical compartment.

Further evidence that the observed effect of basolateral Cl^- steps was on a cellular pathway was derived from the bumetanide series. It was clearly seen that the amplitude of the initial change in V_t was attenuated in the presence of bumetanide. Such a response can be expected if a Na^+-Cl^--K^+ cotransporter normally keeps intracellular [Cl^-] above equilibrium. Blocking of this transporter with bumetanide would reduce intracellular [Cl^-], thereby reducing the conductance of this membrane for Cl^-. This same effect of bumetanide on the response of V_t to basolateral Cl^- steps was also observed in a split oil drop preparation of the same tissue [3].

The rise in R_t could be due to two effects. The first is the presumed decline in intracellular [Cl^-] which would lower the conductance of the basolateral membrane. The second may be a paracellular Cl^- conductance. If the latter contributed significantly, however, it is not clear why the rise in R was delayed compared to that for the apical Cl^- step (see below). One must then assume that the lateral infoldings of the cells present a rather significant unstirred layer to the basolateral solution, thereby delaying access of the low-Cl^- basolateral solution to the junctional complexes.

The apical [Cl^-] steps, by contrast, produced rapid, monophasic responses of V_t with a concurrent increase in R_t. One would expect this response if (1) the paracellular pathway were permeable to Cl^- and/or (2) the apical cell membrane contains Cl^- channels but there is no transport process driving the cell membrane more negative than the EMF for Cl^-, thereby precluding a secondary relaxation phase. The present experiments do not allow us to distinguish between these possibilities.

In these [Cl] step experiments we observed strikingly different responses to unilateral low-Cl^- perfusions on each side of the tissue. Although symmetric biionic and dilution potentials were found in another K^+-secretory inner ear preparation, the semicircular canal duct of the dogfish, those measurements were made 10 min after perfusion, making comparison to the present results (in which transient responses to basolateral perfusion were observed) difficult [12]. Nonetheless, our steady-state measurements (about 2 min) still show a marked difference in V_t between apical and basolateral low-Cl^- perfusion (Table I).

In view of the above results which suggested the presence of basolateral Cl^- channels, we were interested in testing the effect of some of the newly developed Cl^- channel blockers of the DPC family [7]. DPC itself and the even less-potent agent anthracene-9-carboxylic acid had no effect on the split oil drop preparation [3].

Several of the newly developed blockers were found to produce dramatic effects on V_t. NPPB has been found to strongly block chloride channels at micromolar concentrations in renal cells [7], colonic cells [13] and shark rectal gland [14]. By contrast, NPPB had little effect at 10 μM on V_t of the utricle, although at higher doses it produced rapid, large transient increases in V_t from the apical side and smaller responses from the basolateral side. The smaller responses seen from the basolateral side can be attributed to cross-over to the apical membrane because the drugs in this series are highly lipophilic [7].

It has been found that some epithelial tissues which contain a Cl^- conductance have different sensitivities to the DPC analogs [15]; e.g., the sweat duct has been found to be relatively insensitive to NPPB [16]. In sweat duct however, it was found that another blocker from this family, DCDPC, was active in reducing Cl^- conductance, although at a relatively high concentration [16]. DCDPC acted on V_t of the dark cell region of utricle also at high concentrations and in a similar way as NPPB. Although the mean responses to DCDPC were slightly higher for apical perfusion, there was no statistically significant difference in paired experiments. The same relative amount of apparent membrane cross-over (application at one membrane, action at the other) for NPPB and DCDPC was seen in rabbit cortical thick ascending limb kidney tubules; i.e., very strong cross-over for DCDPC and less for NPPB [7]. The experiment with CDPC showed consistent results: an increase in V_t which was greater from the apical side than from the basolateral side.

Stilbene disulfonates, such as DIDS, comprise a class of amino-reactive reagents which are known to inhibit several anion transport systems. They were initially used to block anion transport systems other than Cl^- channels such as Cl^-–HCO_3^- exchange [17,18]. It was found in the present study that DIDS produced an increase in V_t from the apical side, similar to the DPC analogs, although the secondary decline was not nearly as strong. In fact, when DIDS perfusion was sustained, V_t remained elevated (e.g., Fig. 7). DIDS had no significant effect on V_t from the basolateral side, as found earlier with another stilbene disulfonate, SITS [3].

It is known from studies on other tissues that all of these agents, including DIDS, have actions on transporters other than those of their customary or intended use. Gögelein and Pfannmüller [19] have found that NPPB and DCDPC inhibit a non-selective cation channel in pancreas and that DIDS activates this channel from the cytosolic side. DIDS has also been found to block Cl^- conductances in some tissues but not others [20,21]. The present findings point to the possibility that the drugs tested here may be especially useful tools for exploring the conductive properties of utricular dark cells with additional techniques, such as the patch clamp technique.

One possible explanation for the observed results would be that DIDS and the DPC derivatives were inhibiting an apical Cl^-–HCO_3^- exchanger. It has been found that DPC, as well as DIDS, inhibits Cl^-–HCO_3^- exchange in red blood cells and *Necturus* gallbladder [22,23]. Since Cl^-–HCO_3^- exchange is electroneutral, the effect on V_t and R_t would then be indirect; i.e., via changes in intracellular pH. One might then interpret the effect of the apical Cl^- steps as a partial inhibition of this exchanger due to reduction of the chemical driving force.

At least two reasons argue against this interpretation. (1) The decrease in V_t and rise in R_t due to the putative partial inhibition of Cl^--HCO_3^- exchange by low-Cl^- perfusion is not consistent with the large increases in V_t and fall in R_t by the putative blocking of the same mechanism by DIDS and the DPC derivatives. (2) The direction of change of V_t with apical low-Cl^- perfusion was the same as found for apically applied amiloride [8]. If the amiloride was acting on an Na^+–H^+ exchanger, it is not to be expected that intracellular acidification by blockage with amiloride would lead to the same changes in V_t as alkalinization by inhibition of Cl^-–HCO_3^- exchange.

Two questions arise: (1) Are these agents all acting on the same transport pathway? and (2) are any or all of them blocking Cl^- channels and, if not, what processes are they affecting? Although the present data are not sufficient to answer those questions with certainty, several points can be made. It appears likely that the primary effect of each agent tested is on the same transport process and that the locus is the apical membrane. This interpretation is based on the structural similarity amongst the DPC analogs, the similarity of the transient positive response of V_t to each, and the predominance of the responses to apical perfusion for the less lipophilic substances.

If this interpretation holds, the second question reduces to: Can blocking of an apical Cl^- conductance explain the observations? On the one hand, if the apical membrane contains both Cl^- channels and an electrogenic K^+ transporter, blocking of the Cl^- channels would be expected to enhance the effect of electrogenic K^+ secretion which would result in an increase in V_t as observed. However, two points argue against this interpretation. First, it was found that apical DCDPC and DIDS each significantly reduced R_t, rather than increasing it as one might expect for a membrane with blocked ion channels. Second, the results of the Cl^- step experiments were more in line with the idea that basolateral Cl^- conductance influences V_t far more than an apical Cl^- conductance.

Our results are therefore more consistent with the presence of a large basolateral Cl^- conductance and action of the DPC analogs plus DIDS on an apical transporter. The drug action appears to initially enhance a conductive process rather than inhibit it. The transport process initially affected remains unidentified. The secondary collapse of V_t from the DPC derivatives is most likely due to their interference with metabolism.

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