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Na^+ and Cl^- Conductances Are Controlled by Cytosolic Cl^- Concentration in the Intralobular Duct Cells of Mouse Mandibular Glands

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Abstract. Our previously published whole-cell patch-clamp studies on the cells of the intralobular (granular) ducts of the mandibular glands of male mice revealed the presence of an amiloride-sensitive Na^+ conductance in the plasma membrane. In this study we demonstrate the presence also of a Cl^- conductance and we show that the sizes of both conductances vary with the Cl^- concentration of the fluid bathing the cytosolic surface of the plasma membrane. As the cytosolic Cl^- concentration rises from 5 to 150 mmol/liter, the size of the inward Na^+ current declines, the decline being half-maximal when the Cl^- concentration is approximately 50 mmol/liter. In contrast, as cytosolic Cl^- concentration increases, the inward Cl^- current remains at a constant low level until the Cl^- concentration exceeds 80 mmol/liter, when it begins to increase. Studies in which Cl^- in the pipette solution was replaced by other anions indicate that the Na^+ current is suppressed by intracellular Br^-, Cl^- and NO_3^- but not by intracellular I^-, glutamate or gluconate. Our studies also show that the Cl^- conductance allows passage of Cl^- and Br^- equally well, I^- less well, and NO_3^-, glutamate and gluconate poorly, if at all. The findings with NO_3^- are of particular interest because they show that suppression of the Na^+ current by a high intracellular concentration of a particular anion does not depend on actual passage of that anion through the Cl^- conductance. In mouse granular duct cells there is, thus, a reciprocal regulation of Na^+ and Cl^- conductances by the cytosolic Cl^- concentration. Since the cytosolic Cl^- concentration is closely correlated with cell volume in many epithelia, this reciprocal regulation of Na^+ and Cl^- conductances may provide a mechanism by which ductal Na^+ and Cl^- transport rates are adjusted so as to maintain a stable cell volume.

Key words: Na^+ and Cl^- conductance — Amiloride — Intralobular ducts — Mouse mandibular glands

Introduction

The primary saliva produced by the secretory end-pieces of the mandibular glands in mouse, rat and rabbit is modified by electrolyte transport processes in the excurrent duct system, resulting in the formation of a hypotonic end-saliva that is poor in NaCl [23]. Perfusion studies carried out on the main excretory ducts of the mandibular glands of the rat and rabbit suggest that ductal NaCl absorption, at least in the extralobular ducts, depends on the presence of Cl^- channels [2, 3] and amiloride-sensitive Na^+ channels in the luminal membranes of the duct cells [6, 14, 15, 19], although, until recently [8], these predictions had not been tested by the use of patch-clamp techniques. Furthermore, there has only been indirect evidence to suggest that the transport model developed for the extralobular ducts applies also to the intralobular ducts [16, 23].

The intralobular (striated) ducts of many rodent mandibular glands are greatly modified by the presence of secretion granules in sufficiently large numbers to justify their being named granular ducts [22]. This modification is especially conspicuous in male mice, although it is also quite marked in male rats; in females of both species the granules are relatively inconspicuous [22]. The presence of the granules in male mice is so marked that they can readily be discerned in isolated duct cells viewed under Differential Interference Contrast (DIC) optics. This

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makes it possible to distinguish them from the cells of the secretory endpieces so that the properties of the two cell types in a dispersed mixed cell preparation can be studied separately. Taking advantage of this property, we recently performed experiments in the male mouse in which we demonstrated that the granular duct cells, but not the cells of the secretory endpieces, contain an amiloride-inhibitable Na^+ conductance, probably localized to the luminal plasma membrane [8]. In the present study, we show that these cells also contain a Cl^- conductance and that the activities of both the Na^+ and Cl^- conductances are controlled by the intracellular Cl^- concentration.

Materials and Methods

Mice were killed by cervical dislocation. The mandibular glands were removed and incubated in a physiological salt solution containing 50 U/ml collagenase (Worthington type IV, Freehold, New Jersey) for 60 min with intermittent trituration until single cells were liberated. These were then washed and suspended in a Na^+-rich solution having the following composition (in mmol/liter): NaCl (150), $CaCl_2$ (1), $MgCl_2$ (1.2), NaH_2PO_4 (1.2), Na-HEPES (7.5), H-HEPES (7.5) and glucose (10); the pH was adjusted to 7.4 with NaOH. The pipettes were filled with a K^+-rich solution containing (in mmol/liter): K-glutamate and/or KCl (150), $MgCl_2$ (1), H-HEPES (10), glucose (10) and EGTA (5); the pH was adjusted to 7.2 with KOH. Na-HEPES, H-HEPES, EGTA and amiloride were obtained from Sigma (St Louis, MO).

Standard patch-clamp techniques were used [13]. Patch-clamp pipettes were pulled from borosilicate microhematocrit tubes (Modulohm, Hevik, Denmark) so as to have resistances of 1–3 MΩ for whole-cell recordings. The reference electrode was a Ag/AgCl electrode which was connected to the bath by a bridge filled with the control bath solution and agar (10 mg/ml). An Axopatch-1D patch-clamp amplifier (Axon Instruments, Foster City, CA) was used to measure whole-cell currents. Command voltages were generated by a PGEN function generator (NBD, Stony Brook, NY) and the whole-cell currents were sampled with a MacLab-4 data acquisition interface (ADInstruments, Sydney, Australia) attached to a Macintosh-IIci computer. All experiments were performed at about 20°C. Pipette potentials were corrected for the junction potential between the various pipette solutions used and the NaCl-rich bath solution [4]. The junction potentials measured between the pipette solutions and the NaCl-rich bath solution are given in the Table; all lie within 3 mV of the values calculated using the Henderson equation [4]. The flowing junction technique used to measure the liquid junction potential was similar to that described by Neher [17], except that we used a 3 mol/liter KCl agar bridge rather than a pipette filled with a 3 mol/liter KCl solution as the reference electrode in the bath. The potential differences we recorded were corrected for the junction potentials between the bridge and the bath solutions so as to yield the liquid junction potential between the NaCl solution and the pipette solution. The junction potentials between the agar bridge and the bath solutions were calculated from the Henderson equation [4].

Results

Figure 1*A* shows the currents elicited in response to voltage steps from a potential of 0 mV in a granular duct cell held in the whole-cell configuration with the KCl solution in the pipette and the NaCl solution in the bath. The currents showed marked activation at negative potentials with a half-time of about 100 ms. Replacement of bath Na^+ with the larger cation, N-methyl-D-glucamine ($NMDG^+$), had little effect on the currents (Fig. 1*B*), indicating that the inward current demonstrated in Fig. 1*A* was not carried by Na^+. Figure 1*C* shows the averaged current-voltage (*I-V*) relation obtained from eight experiments of this type. The *I-V* relation was inwardly rectifying with a maximum slope conductance at negative pipette potentials of 16.1 nS ± 2.3 ($n = 5$) and a reversal potential of 12.1 mV ± 5.1 ($n = 5$). Since replacement of extracellular Na^+ with $NMDG^+$ had no effect on the inwardly rectifying current (Fig. 1*C*), it follows that the inward current must have been due to the efflux of Cl^- ions from the cell (*but see below*).

These results appear at first sight to be in conflict with our previously reported studies conducted with a K-glutamate solution in the pipette, which demonstrated that the inward current in granular duct cells was largely carried by Na^+ ions through an amiloride-inhibitable conductance [8]. This problem is illustrated in Fig. 2 in which we show an experiment similar to those depicted in Fig. 1 but with K-glutamate in the pipette. Figure 2*A* shows that under these circumstances the activation of the inward current at negative pipette potentials no longer has the slow time course seen in Fig. 1*A* and that replacement of bath Na^+ with $NMDG^+$ reduces the inward current (Fig. 2*B*). The *I-V* relation with NaCl solution in the bath was linear or slightly inwardly rectifying with a slope conductance of 1.9 nS at negative pipette potentials (Fig. 2*C*); replacement of the Na^+ in the bath solution by $NMDG^+$ reduced the slope conductance to 0.6 nS and shifted the cell potential by −10 mV (Fig. 2*C*). As we concluded in our earlier study [8], it is evident that the inward current seen with K-glutamate in the pipette is largely due to the influx of Na^+ ions into the cell.

It thus appears that when glutamate is the major anion in the pipette solution, an inward Na^+ current is activated which is not present when Cl^- is the predominant anion in the pipette solution. To study this phenomenon further, we used a protocol in which we repeatedly voltage-clamped the cell alternately at −4 mV for 600 ms, −84 mV for 800 ms, −4 mV for 400 ms and +56 mV for 200 ms. The long duration of the clamp at −84 mV was necessary to ensure that steady-state was reached, whereas at −4 and +56 mV, steady-state was rapidly reached so that shorter clamp durations were possible. After obtaining current records using this protocol with NaCl in the bath, we changed the bath solution from NaCl to NMDG-Cl so as to eliminate inward cur-

Table. Comparison of junction potentials (J_p) between K^+-rich pipette solutions containing various anions and the NaCl-rich bath solution[a]

Pipette solution		Junction potential (J_p)	
Cl^- (mmol/liter)	Replacement anion (mmol/liter)	(Measured) (mV)	(Calculated) (mV)
152	0	−3.9 ± 0.2 (7)	−3.8
7	145 (glutamate)	−14.0 ± 0.5 (7)	−16.8
22	130 (glutamate)	−11.7 ± 0.4 (7)	−15.2
52	100 (glutamate)	−9.8 ± 0.3 (7)	−12.1
82	70 (glutamate)	−8.4 ± 0.4 (7)	−9.3
122	30 (glutamate)	−5.1 ± 0.3 (4)	−6.1
7	145 (NO_3^-)	−5.8 ± 0.8 (5)	−4.7
7	145 (Br^-)	−3.1 ± 0.2 (7)	−3.5
7	145 (I^-)	−2.5 ± 0.4 (6)	−3.7
7	145 (gluconate)	−14.2 ± 0.5 (6)	−15.9

[a] Measured by the flowing junction technique [17] and calculated from the Henderson equation [4]. Potential differences have been expressed as the pipette potential with respect to bath. The measured values of J_p are means ± SEM with the number of observations given in parentheses.

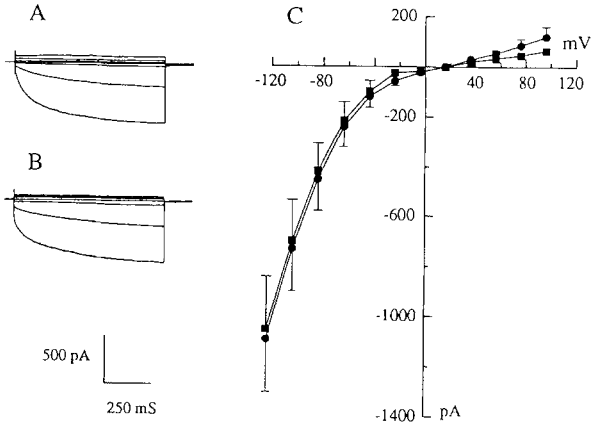

Fig. 1. (*A*) Voltage-clamp records from a granular duct cell recorded in the whole-cell mode with a KCl solution in the pipette and the standard NaCl solution in the bath. (*B*) Voltage-clamp records from the cell in *A* following replacement of Na^+ in the bath with $NMDG^+$. (*C*) Mean *I-V* relations from four experiments (mean ± SEM) of this type with the standard NaCl solution in the bath (filled circles) and with the NMDG-Cl solution in the bath (filled squares). The holding potential was −4 mV. In *A* and *B*, test potentials ranged from −124 to +76 mV in 40 mV steps.

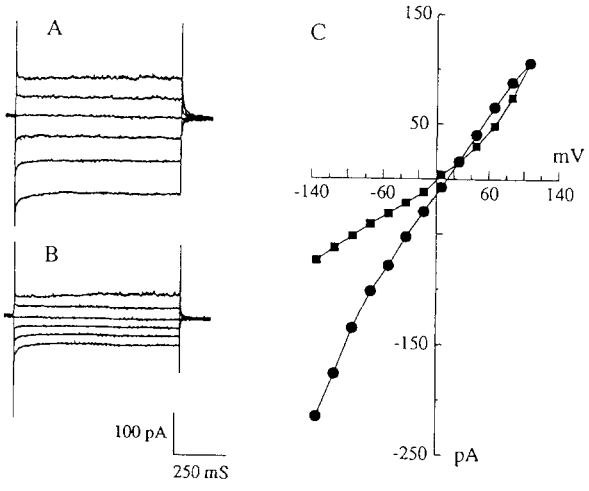

Fig. 2. (*A*) Voltage-clamp records from a granular duct cell recorded in the whole-cell mode with a K-glutamate solution in the pipette and the standard NaCl solution in the bath. (*B*) Voltage-clamp records from the cell in *A* following replacement of Na^+ in the bath with $NMDG^+$. (*C*) Steady-state *I-V* relations from the same experiment with the standard NaCl solution in the bath (filled circles) and with the NMDG-Cl solution in the bath (filled squares). The holding potential was −4 mV. In *A* and *B*, test potentials ranged from −124 to +76 mV in 40 mV steps.

rents carried by Na^+. Figure 3 shows three experiments of this type, one with a pipette solution containing KCl (5 mmol/liter) and K-glutamate (145 mmol/liter), one with a pipette solution containing KCl (50 mmol/liter) and K-glutamate (100 mmol/liter), and one with a pipette solution containing only KCl (150 mmol/liter). The Na^+ current, i.e., that component of the inward current seen at −84 mV which was sensitive to $NMDG^+$ substitution, declined as the pipette Cl^- concentration increased. Conversely, that component of the inward current which was not inhibited by replacement of bath Na^+ by $NMDG^+$ (the Na^+-insensitive current) increased as the pipette Cl^- increased.

The dependency of the inward Na^+ current on pipette Cl^- is more clearly illustrated in Fig. 4 in which the size of the inward Na^+ current is plotted as a function of the Cl^- concentration in the pipette solution. The Na^+ current declined monotonically as the pipette Cl^- concentration increased, with a

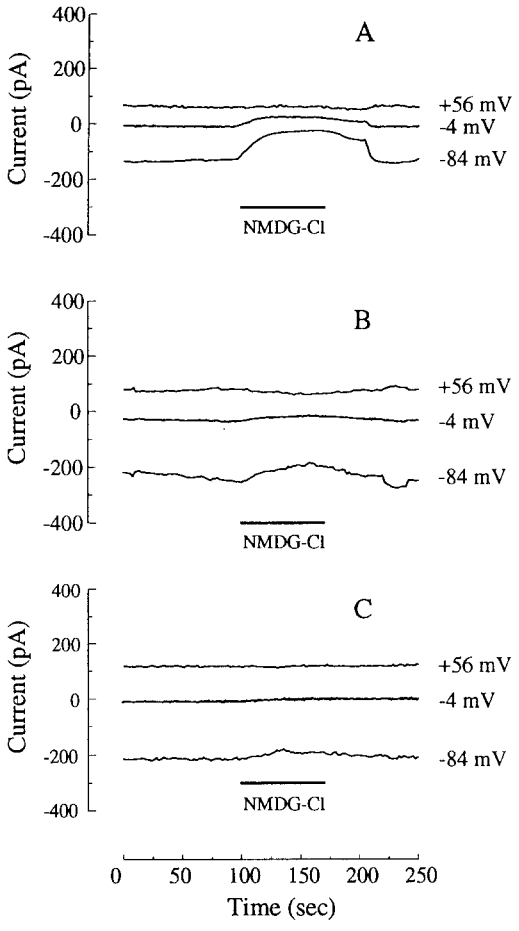

Fig. 3. Voltage-clamp records from three granular duct cells in which the cells were repetitively clamped at two-second intervals by a protocol in which they were held at −4 mV for 600 ms, −84 mV for 800 ms, −4 mV for 400 ms and +56 mV for 200 ms. The traces show the time course of the steady-state currents at each clamp voltage. Each trace is a series of data points representing the steady-state current reached at the end of each voltage pulse. The steady-state current of each −84 mV pulse was calculated by averaging between 700 and 800 ms following the voltage jump and for each −4 mV and each +56 mV pulse the steady-state currents were calculated by averaging once the capacitive transients had died out. In each experiment, Na^+ in the bath was replaced by $NMDG^+$ during the periods indicated. The pipette solutions were: *A*, KCl (5 mmol/liter) and K-glutamate (145 mmol/liter); *B*, KCl (50 mmol/liter) and K-glutamate (100 mmol/liter); and *C*, KCl (150 mmol/liter).

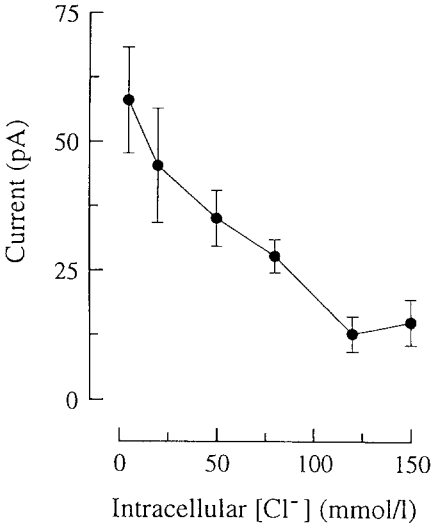

Fig. 4. The size of the inward Na^+ current in granular duct cells clamped at −84 mV as a function of the pipette Cl^- concentration. The data are from experiments of the type illustrated in Fig. 3. The Na^+ current was calculated by subtracting the current at −84 mV with an $NMDG^+$ solution in the bath from the current observed at −84 mV with a Na^+ solution in the bath. Each point represents the mean of at least four experiments ± SEM.

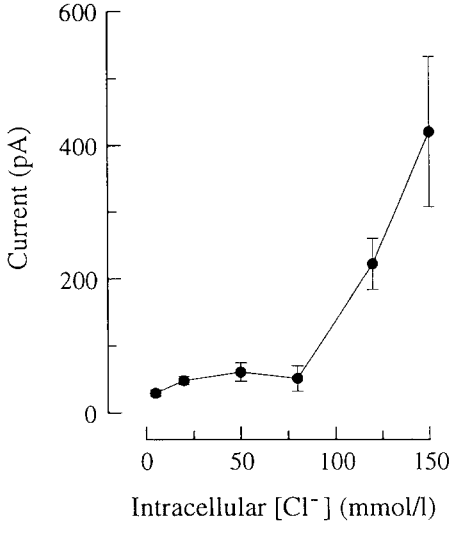

Fig. 5. The size of the inward current in granular duct cells clamped at −84 mV with the $NMDG^+$ solution in the bath (i.e., current carried mainly by Cl^-) as a function of the pipette Cl^- concentration. The data are from experiments of the type illustrated in Fig. 3. Each point represents the mean of at least four experiments ± SEM.

half-maximal inhibitory effect at a Cl^- concentration of approximately 50 mmol/liter.

The dependency of the Na^+-insensitive inward current on pipette Cl^- is illustrated in Fig. 5. The Na^+-insensitive inward current at −84 mV was 29.6 pA ± 5.2 (n = 9) when the pipette solution contained 5 mmol/liter KCl and 145 mmol/liter K-glutamate, and remained at this level as the Cl^- concentration increased until the pipette Cl^- exceeded 80 mmol/liter. At Cl^- concentrations above 80 mmol/liter, the Na^+-insensitive inward current increased, reaching 420.7 pA ± 112.3 (n = 8) when the pipette solution contained 150 mmol/liter KCl (Fig. 5). With the Na^+ in the bath replaced by $NMDG^+$, the ionic basis of

the inward current at −84 mV must either have been an efflux of Cl^- ions or an influx of Ca^{2+} or Mg^{2+} ions. Since removal of all extracellular Ca^{2+} and Mg^{2+} together with the addition of EGTA (5 mmol/liter) to the bath had no effect on the inward current (two experiments, *data not shown*), whereas replacement of all but 7 mmol/liter of the pipette Cl^- with glutamate inhibited the Na^+-insensitive inward current by 93% (Fig. 5), we conclude that the Na^+-insensitive inward current was largely carried by the efflux of Cl^- ions.

To determine whether the activation of the Na^+ current was attributable to the presence of glutamate or to the absence of Cl^-, we performed experiments in which glutamate in the pipette solution was replaced instead with another large anion, gluconate (Fig. 6*A*). The current at −84 mV with gluconate in the pipette solution was not significantly different from that with glutamate in the pipette solution, indicating that the Na^+ current was influenced by the absence of Cl^- from the pipette solution rather than by the presence of glutamate.

We then attempted to determine the anion specificity of the mechanism responsible for suppression of the Na^+ current (Fig. 6*A*). Br^- and NO_3^- (150 mmol/liter) suppressed the Na^+ current at −84 mV to an extent comparable to that produced by the same concentration of Cl^-, whereas I^- (150 mmol/liter) failed to suppress the Na^+ current at all. Figure 6*B* shows the dependence on the intracellular anion composition of that component of the inward current seen at −84 mV which remains after $NMDG^+$ substitution (i.e., the Na^+-insensitive inward current): this component can be attributed to anion efflux from the cell because it is abolished by replacement of pipette Cl^- by the impermeant anions glutamate and gluconate (Fig. 6*B*). Intracellular Br^- and Cl^- permitted large inward currents, but NO_3^- and I^- did not.

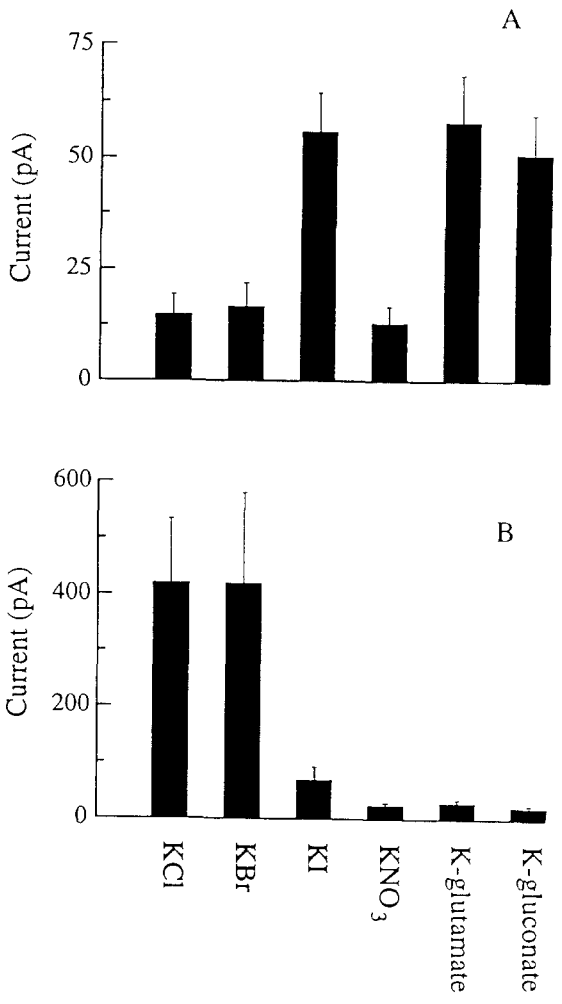

Fig. 6. (*A*) The size of the inward Na^+ current in granular duct cells clamped at −84 mV (calculated as described in the legend to Fig. 4) with various potassium salts in the pipette solution and the standard Na^+ solution in the bath. (*B*) The size of the residual (anion) current in granular duct cells clamped at −84 mV with various potassium salts in the pipette solution and an $NMDG^+$-containing solution in the bath. The data are from experiments of the type illustrated in Fig. 3. Each bar represents the mean of at least four experiments ± SEM.

Discussion

As indicated above, we have shown previously that granular duct cells contain an amiloride-sensitive Na^+ conductance that is demonstrable when the pipette solution contains K-glutamate [8]. In the present paper, we show that the Na^+ current is inhibited by high intracellular Cl^- concentrations and that this effect is mimicked by Br^- and NO_3^- but not I^-, glutamate or gluconate. The inhibition cannot be due directly to the presence of an anion current, however, because NO_3^- inhibited the Na^+ current although it was not able to carry any significant inward current.

We also show in this study that a Cl^- conductance dominates the whole-cell *I-V* relation when the pipette solution has a Cl^- concentration greater than 80 mmol/liter. The Cl^- conductance is inwardly rectifying (Fig. 1*C*), is activated at negative pipette potentials (Fig. 1*A*), conducts Cl^- and Br^- equally well (Fig. 6*B*), conducts I^- less well (Fig. 6*B*) and NO_3^-, glutamate and gluconate poorly, if at all (Fig. 6*B*). Its anion selectivity is similar to that reported for the Cl^- conductance in the rabbit mandibular main excretory duct ($Cl^- >= Br^- > I^-$) [10]. Its selectivity also recalls the properties described for the CFTR Cl^- channel [1], although, unlike the con-

ductance described in the present study, the CFTR channel is not activated by hyperpolarization of the membrane potential nor is its steady-state *I-V* relation inwardly rectifying when it is bathed in symmetrical Cl^- solutions [1].

The observation that the Na^+ conductance in granular duct cells is regulated by intracellular Cl^- is novel. There have been reports in a variety of Na^+-transporting epithelia that extracellular replacement of Cl^- by other anions leads to changes in apical Na^+ permeability (*reviewed in* [20]) which, depending on the tissue studied and the anion used, are sometimes stimulatory and sometimes inhibitory. These changes have been attributed to changes in cell pH, Na^+, Ca^{2+} or volume, or to changes in surface charge consequent on the anion substitution [20]. Our experiments make it appear unlikely that changes in intracellular Ca^{2+}, pH or Na^+ could account for our observations.

In salivary glands in some species, there is experimental evidence to suggest that the Cl^- conductance in duct cells is controlled by intracellular Cl^-. Thus, in the rabbit mandibular main excretory duct, addition of amiloride to the luminal fluid causes a biphasic change in transepithelial conductance: the initial phase has been attributed to amiloride block of apical Na^+ channels, and the latter phase to changes in Cl^- conductance secondary to changes in cytosolic composition, possibly of the Cl^- concentration [5, 6]. Furthermore, inhibition of the Na^+-K^+-ATPase by ouabain in this duct is accompanied by a decrease in transepithelial resistance [3, 5] which has been attributed to an increase in intracellular Cl^-.

Epithelial Na^+ channels have previously been shown to be regulated by intracellular Na^+ [21], pH [11], Ca^{2+} [12] and by G proteins [7], and both cytosolic Na^+ and cytosolic Ca^{2+} have been postulated to play a role in matching Na^+ influx across the apical membrane to the rate of Na^+ extrusion across the basolateral membrane [18]. The present study suggests that cytosolic Cl^- may also play a role in linking Na^+ influx across the apical membrane to the transport state of the epithelium. Given that cytosolic Cl^- in epithelia is closely correlated with cell volume [9], it is tempting to speculate that the regulation of apical Na^+ conductance by cytosolic Cl^- provides a mechanism by which epithelial Na^+ transport is modulated by cell volume.

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