Control of the amiloride-sensitive Na^+ current in mouse salivary ducts by intracellular anions is mediated by a G protein

A. Dinudom, P. Komwatana, J. A. Young and D. I. Cook*

Department of Physiology, University of Sydney, NSW 2006, Australia

1. We have previously reported that the Na^+ conductance in mouse intralobular salivary duct cells is controlled by cytosolic anions, being inhibited by high cytosolic concentrations of Cl^- and NO_3^- but not of glutamate. In the present paper, we use whole-cell patch-clamp methods to investigate whether this anion effect is mediated by a G protein.

2. Inclusion of 100 μmol l^{-1} GTP-γ-S, a non-hydrolysable GTP analogue, in the glutamate-containing pipette solution, i.e. when the Na^+ conductance is active, reduced the size of the Na^+ conductance whereas inclusion of 100 μmol l^{-1} GDP-β-S, a non-hydrolysable GDP analogue, had no effect.

3. Inclusion of 100 μmol l^{-1} GDP-β-S in the NO_3^--containing pipette solution, i.e. when the Na^+ conductance is inhibited, reactivated the conductance. Inclusion of 500 ng ml^{-1} activated pertussis toxin in the NO_3^--containing pipette solution had a similar effect on the Na^+ conductance.

4. We conclude that the inhibitory effect of intracellular anions such as NO_3^- and Cl^- on the amiloride-sensitive Na^+ conductance in mouse mandibular intralobular duct cells is mediated by a G protein sensitive to pertussis toxin.

The salivary duct tree absorbs Na^+ and Cl^- from an isotonic, NaCl-rich primary fluid secreted by the endpieces, to form a final saliva that is hypotonic as it enters the mouth (reviewed in Cook, Van Lennep, Roberts & Young, 1994). Microperfusion studies performed on isolated segments of the main excretory ducts of salivary glands suggest that much of this transepithelial Na^+ absorption takes place via an amiloride-sensitive Na^+ conductance in the apical membranes of the duct cells (Cook *et al.* 1994), and recent patch-clamp (Dinudom, Young & Cook, 1993*b*) and membrane vesicle (Moran, Davis & Turner, 1995) studies on duct cells have confirmed the presence of this Na^+ conductance in the apical membranes. This apical Na^+ conductance is relatively insensitive to amiloride, half-maximal inhibition being observed with 2 μmol l^{-1} amiloride (Bijman, Cook & Van Os, 1983; Dinudom *et al.* 1993*b*). Nevertheless, for the following reasons, the conductance seems likely to be closely related to the amiloride-sensitive Na^+ channels found in cells of the renal collecting duct and other tight Na^+-absorbing epithelia: (i) it is selective for Na^+ over K^+ (Dinudom *et al.* 1993*b*); (ii) the conductance of the underlying channels is 4 pS when the membrane is bathed symmetrically in 150 mmol l^{-1} Na^+ solutions (P. Komwatana, A. Dinudom, J. A. Young & D. I. Cook, unpublished results); (iii) ductal Na^+ reabsorption is responsive to aldosterone (Gruber, Knauf & Frömter, 1973); and (iv) intralobular salivary duct cells contain mRNA for all three of the known subunits of the epithelial Na^+ channel (Duc, Farman, Canessa, Bonvalet & Rossier, 1994).

The electrogenic, amiloride-sensitive movement of Na^+ out of the duct lumen across the epithelium is accompanied by the passive flow of transcellular Cl^- down the electrochemical gradient established by the transport of Na^+ (Cook *et al.* 1994). Na^+ and Cl^- transport by duct cells is thus coupled electrically and, since the Cl^- transport is transcellular (Cook *et al.* 1994), cytosolic Cl^- concentration can be expected to vary with the rate of Na^+ transport (Lau, Evans & Case, 1994). We have previously reported that the amiloride-sensitive Na^+ conductance in mouse mandibular intralobular duct cells is inhibited by cytosolic Cl^- (Dinudom, Young & Cook, 1993*a*) in the concentration range (40–70 mmol l^{-1}) actually observed in duct cells (Lau *et al.* 1994). Given the close relationship between Na^+ transport rate and cytosolic

* To whom correspondence should be addressed.

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Cl^- in these cells, this action of intracellular Cl^- on the apical Na^+ conductance might well form part of a physiological mechanism for matching the rate of Na^+ entry to the rate of Cl^- entry. Since intracellular Cl^- is correlated with cell volume at constant extracellular osmolality (Robertson & Foskett, 1994), cytosolic Cl^- might also regulate Na^+ influx in response to changes in cell volume.

It has been reported that changes in ambient Cl^- concentration regulate the activity of G proteins (Higashijima, Ferguson & Sternweis, 1987; Nakajima, Sugimoto & Kurachi, 1992). Since other epithelial Na^+ channels are known to be regulated by G proteins (Ohara, Matsunaga & Eaton, 1993), we wondered whether in duct cells, intracellular Cl^- might exert its action on the Na^+ conductance by influencing the activity of a G protein. In this study, we have used whole-cell patch-clamp methods to investigate this possibility.

METHODS

Male mice were killed by cervical dislocation. The mandibular glands were removed, finely chopped and incubated in a physiological salt solution containing 50 units ml^{-1} collagenase (Worthington Type IV, Freehold, NJ, USA) for 40 min with intermittent trituration, until single cells were liberated. These were then washed and suspended in a Na^+-rich solution having the following composition (mM): NaCl, 145; KCl, 5·5; $CaCl_2$, 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. In Na^+-free bath solutions, Na^+ was replaced in equimolar amounts with *N*-methyl-D-glucamine ($NMDG^+$) and the pH was adjusted with Tris base. The pipettes were filled with $NMDG^+$-rich solutions containing (mM): NMDG glutamate or NMDG nitrate, 150; $MgCl_2$, 1; H-Hepes, 10; glucose, 10; and EGTA, 5; the pH was adjusted to 7·2 with Tris base. Differential interference contrast (DIC) microscopy was used to identify the isolated granular duct cells on the basis of the large number of secretory granules in this cell type (Dinudom *et al.* 1993*a*).

Patch-clamp techniques were used as previously described (Dinudom *et al.* 1993*a*). Patch-clamp pipettes were pulled from borosilicate microhaematocrit tubes (Modulohm, Hevik, Denmark) so as to have resistances of 1–3 MΩ. The reference electrode was a Ag–AgCl pellet. All potential differences were corrected for junction potentials as appropriate (Dinudom *et al.* 1993*a*).

An Axopatch-1D patch-clamp amplifier (Axon Instruments) was used to measure whole-cell currents. To determine *I–V* relations, a MacLab 4 data acquisition interface (ADInstruments, Sydney, Australia) attached to a Macintosh IIci computer was used to generate command voltages and to sample the whole-cell currents.

Hepes, EGTA, NMDG, amiloride, dithiothreitol and nicotinamide adenine dinucleotide (NAD) were obtained from Sigma. GTP-γ-S and GDP-β-S were obtained from Boehringer Mannheim Australia and pertussis toxin from Calbiochem. Pertussis toxin was stored as a 111 μg ml^{-1} stock solution and was activated prior to use by incubation with 5 mmol l^{-1} dithiothreitol for 15 min at 35 °C. It was then diluted to 500 ng ml^{-1} in samples of pipette solution to which 1 mmol l^{-1} NAD had been added (Zong & Lux, 1994).

In all figures, outward current, defined as positive charge leaving the pipette, is depicted as an upward deflection, and potential difference is expressed as the pipette potential with respect to the bath. *I–V* relations were measured with 800 ms voltage pulses delivered at levels 20 mV apart over the range +100 to −120 mV. The voltage pulses were separated by 5 s intervals during which the cell potential was held at 0 mV. Steady-state currents were calculated by averaging the whole-cell current between 70 and 170 ms from the start of each voltage pulse. The chord conductance was measured between −120 mV and the zero-current potential of the Na^+ current, and the limiting conductance was measured between −40 and −120 mV. All experiments were performed at room temperature (20–22 °C). Results are presented as means ± S.E.M. with the number of cells tested in parentheses. Unless otherwise indicated, statistical significance was assessed using Student's unpaired *t* tests. Probability levels (*P*) were calculated to three decimal places and a value of $P < 0{\cdot}050$ was regarded as statistically significant.

RESULTS

We first investigated whether activation of G proteins with GTP-γ-S suppresses the Na^+ conductance. For these experiments we used an NMDG glutamate pipette solution, since the glutamate anion does not suppress the Na^+ conductance (Dinudom *et al.* 1993*a*), and measured the Na^+ current by changing the bath solution from the Na^+-rich solution to a solution in which Na^+ had been replaced by equimolar amounts of the impermeant $NMDG^+$ cation (Fig. 1*A*). The *I–V* relation of the Na^+ current under these conditions was inwardly rectifying (Fig. 1*B*) with a slope conductance at negative potentials of 0·92 ± 0·33 nS ($n = 4$) and a zero-current potential of +40·1 ± 13·5 mV ($n = 4$). In earlier studies, we showed that this current was Na^+ selective and could be blocked by amiloride (Dinudom *et al.* 1993*b*). When we repeated the present experiment using the NMDG glutamate pipette solution to which 100 μmol l^{-1} GTP-γ-S had been added (so as to activate G proteins; Fig. 1*C*), the Na^+ conductance (Fig. 1*D*) was only 0·07 ± 0·09 nS ($n = 4$), a value not significantly different from zero, and significantly ($P < 0{\cdot}05$) less than that observed in the control experiments (Fig. 1*B*).

We then determined whether inhibition of G proteins with GDP-β-S prevents suppression of the Na^+ conductance by anions such as NO_3^- or Cl^-. For these experiments we used a pipette solution in which the major anion was NO_3^- so as to eliminate the hyperpolarization-activated Cl^- current that dominates the *I–V* relation when Cl^--rich or Br^--rich pipette solutions are used (Dinudom *et al.* 1993*a*; Komwatana, Dinudom, Young & Cook, 1994). In Fig. 2 we show the details of studies in which we derived the Na^+ conductance by calculating the component of the *I–V* relation that was sensitive to amiloride. We obtained similar results in other studies in which we used Na^+ substitution (using the protocol in Fig. 1) to measure the Na^+ conductance (Fig. 3*E*).

In the control experiments, with an NMDG nitrate pipette solution and Na^+-rich bath solution (Fig. 2*A*), the addition of 100 μmol l^{-1} amiloride to the bath had no effect on the inward conductance or the zero-current potential of the steady-state *I–V* relation (Fig. 2*A*). Consistent with this, the conductance of the amiloride-sensitive current at negative potentials was only $0{\cdot}08 \pm 0{\cdot}31$ nS ($n = 4$; Fig. 2*B*). When we repeated these experiments with an NMDG nitrate pipette solution to which 100 μmol l^{-1} GDP-β-S had been added (Fig. 2*C*), we found that the addition of amiloride reduced the inward conductance of the steady-state *I–V* relation from $2{\cdot}47 \pm 0{\cdot}58$ nS ($n = 4$) to $1{\cdot}11 \pm 0{\cdot}43$ nS ($n = 4$) and shifted the zero current potential from $7{\cdot}28 \pm 9{\cdot}55$ mV ($n = 4$) to $-6{\cdot}61 \pm 7{\cdot}02$ mV ($n = 4$) (Fig. 2*C*). The *I–V* relation of the amiloride-sensitive current (Fig. 2*D*) had a zero-current potential of $+36{\cdot}03 \pm 11{\cdot}63$ mV ($n = 4$) and a limiting conductance of $1{\cdot}36 \pm 0{\cdot}40$ nS ($n = 4$), a value significantly ($P < 0{\cdot}05$) greater than that observed with the control NMDG nitrate pipette solution (Fig. 2*B*). Thus, the addition of GDP-β-S to the NMDG nitrate pipette solution activated the amiloride-sensitive Na^+ conductance.

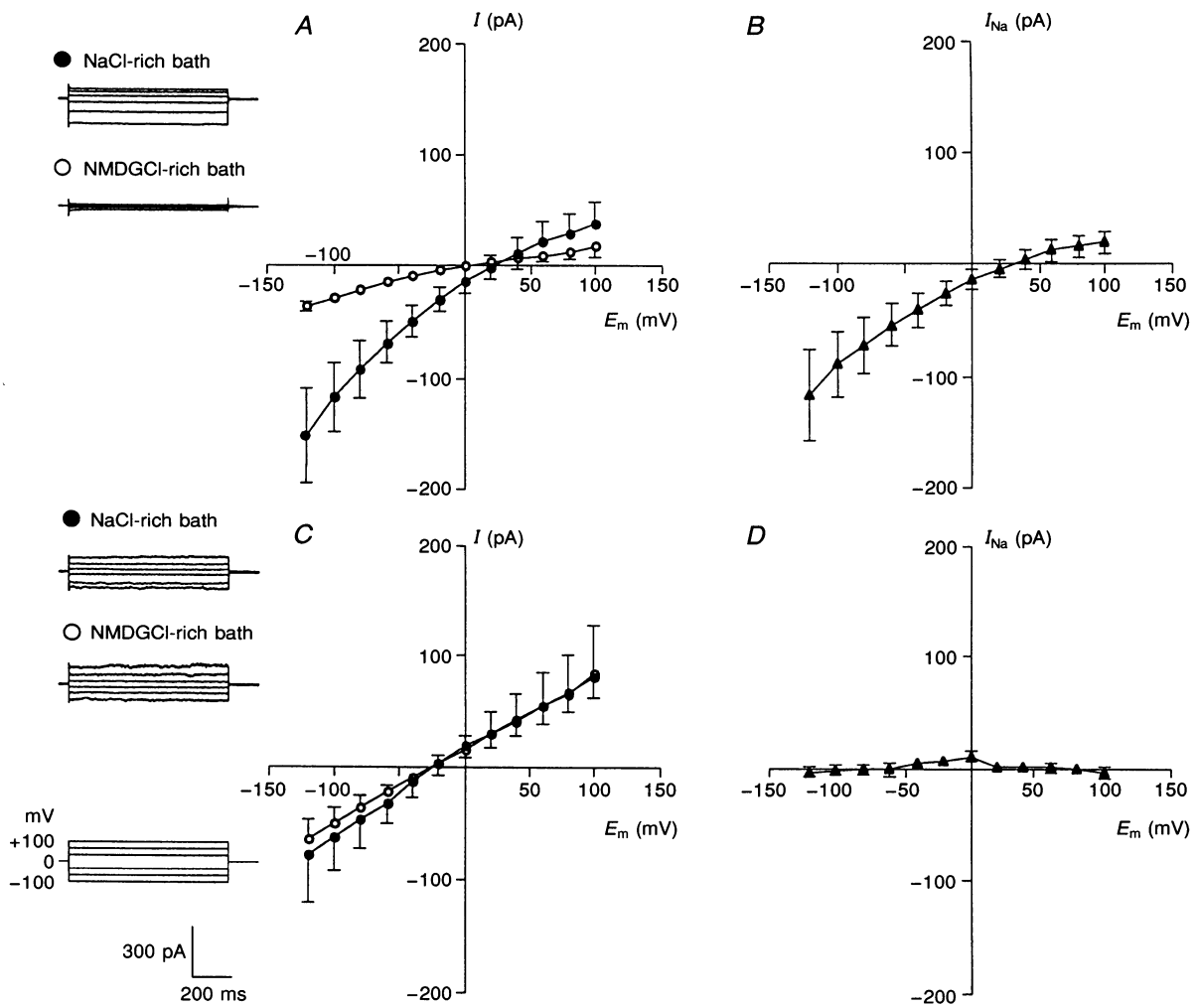

Figure 1. Inhibition of the Na^+ current by GTP-γ-S

The whole-cell Na^+ currents in salivary duct cells studied with an NMDG glutamate pipette solution (panels *A* and *B*) or an NMDG glutamate pipette solution containing 100 μmol l^{-1} GTP-γ-S (panels *C* and *D*) are shown. For each set of experiments we show representative whole-cell recordings taken prior to and following replacement of the Na^+-rich bath solution with the $NMDG^+$-rich bath solution, together with the corresponding steady-state *I–V* relations (panels *A* and *C*). The steady-state *I–V* relation of the Na^+ current, calculated by subtraction of the *I–V* relation when the bath solution was $NMDG^+$-rich from the *I–V* relation when the bath solution was Na^+-rich, is shown in panels *B* and *D*. Each point in panels *B* and *D* is the mean ± S.E.M. of 4 separate experiments.

The size of the Na^+ conductance activated by the addition of GDP-β-S to the NMDG nitrate pipette solution was not significantly different from that of the Na^+ conductance observed with the control NMDG glutamate pipette solution (Fig. 3). Furthermore, we found that the addition of 100 μmol l^{-1} GDP-β-S to NMDG glutamate pipette solution had no effect on the size of Na^+ conductance (Fig. 3). Thus, GDP-β-S appeared to act by blocking the inhibitory action of cytosolic NO_3^- on the Na^+ conductance, rather than by producing a generalized activation of the Na^+ conductance.

Finally, we examined whether the suppression of Na^+ conductance by NO_3^- is inhibited by pertussis toxin. We did this by using an NMDG nitrate pipette solution to which 500 ng ml^{-1} activated pertussis toxin had been added. The conductance was measured by replacement of bath Na^+ with $NMDG^+$ following the protocol shown in Fig. 1. We found that inward Na^+ conductance in the presence of pertussis toxin was significantly ($P < 0.05$) greater than was observed with the control NMDG nitrate pipette solution (Fig. 3). It was not significantly different from that observed with the NMDG nitrate pipette solution to which GDP-β-S had been added (Fig. 3), or that observed with the NMDG glutamate pipette solution (Fig. 3). The zero-current potential of the pertussis toxin-activated Na^+ current was $+30.75 \pm 14.66$ mV ($n = 5$),

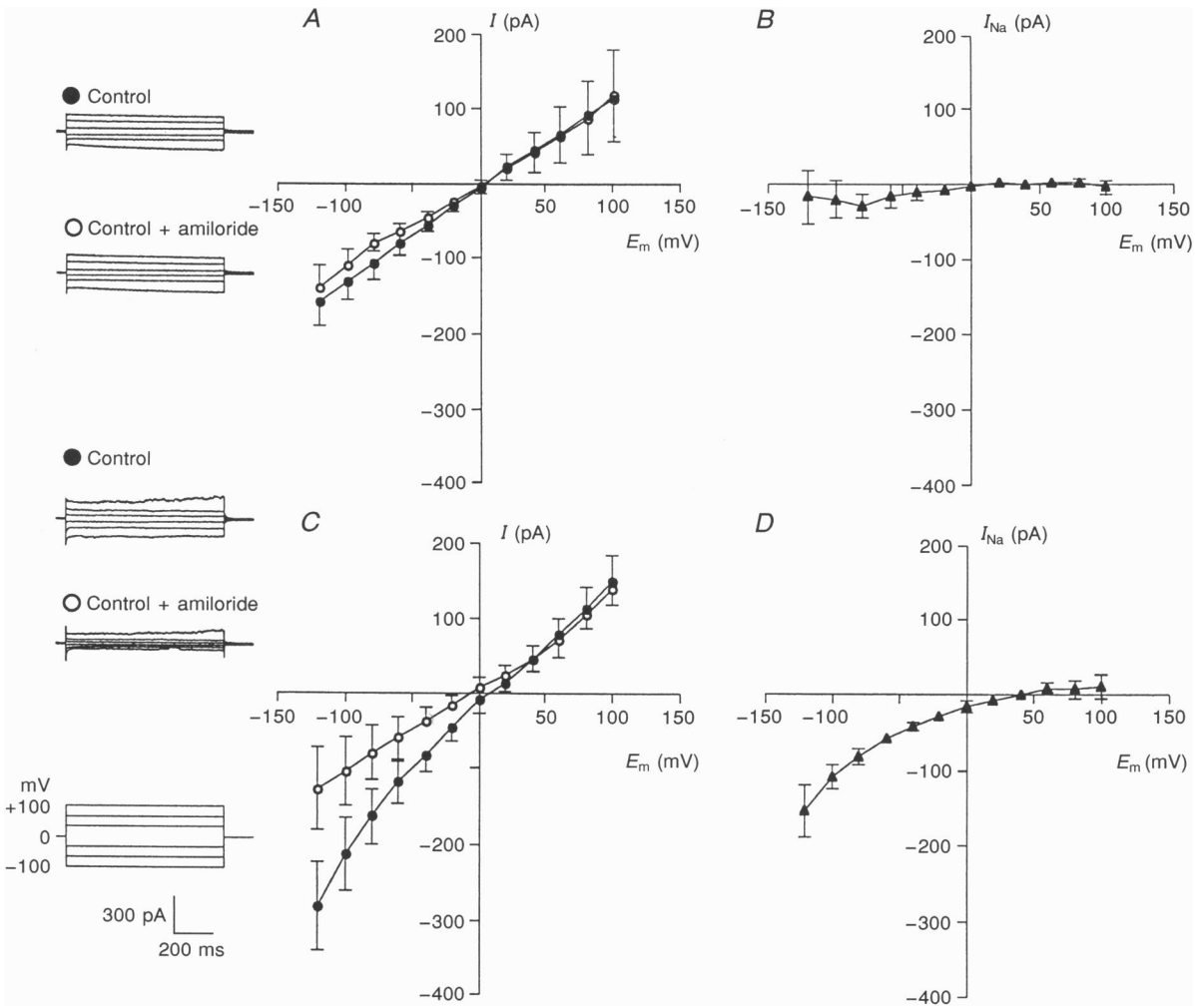

Figure 2. Activation of the Na^+ current by GDP-β-S

The whole-cell Na^+ currents in salivary duct cells studied with the NMDG nitrate pipette solution (panels *A* and *B*) or NMDG nitrate pipette solution with 100 μmol l^{-1} GDP-β-S (panels *C* and *D*) are shown. For each set of experiments we show representative whole-cell recordings taken prior to and following the addition of 100 μmol l^{-1} amiloride to the bath solution together with the corresponding steady-state *I–V* relations (panels *A* and *C*). The steady-state *I–V* relations of the Na^+ current calculated by subtraction of the *I–V* relation in the presence of amiloride from the *I–V* relation prior to the addition of amiloride are shown in panels *B* and *D*. The points in panels *B* and *D* are the means ± S.E.M. of 4 separate experiments.

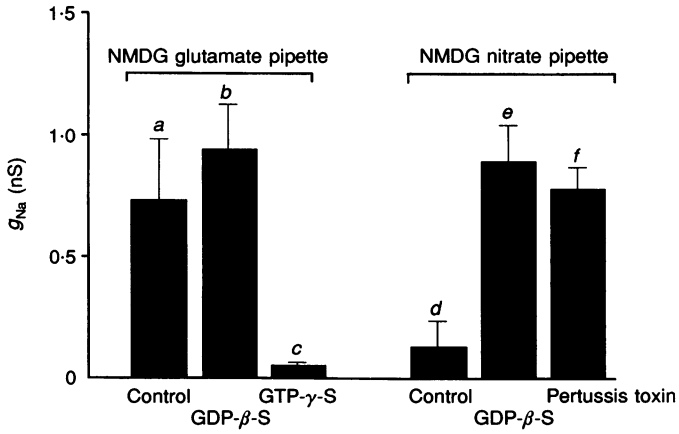

Figure 3. Effects of G protein modulators with various anions in the pipette solution

Histogram showing the chord conductance of the Na^+ current measured with a range of pipette solutions. In each case the Na^+ current was measured by replacing bath Na^+ with $NMDG^+$ as shown in Fig. 1. *a*, NMDG glutamate pipette solution ($n = 4$); *b*, NMDG glutamate pipette solution plus 100 μmol l^{-1} GDP-β-S ($n = 4$); *c*, NMDG glutamate pipette solution plus 100 μmol l^{-1} GTP-γ-S ($n = 4$); *d*, NMDG nitrate pipette solution ($n = 9$); *e*, NMDG nitrate pipette solution plus 100 μmol l^{-1} GDP-β-S ($n = 5$); *f*, NMDG nitrate pipette solution plus 500 ng ml^{-1} activated pertussis toxin ($n = 5$). Each bar represents the mean ± S.E.M.

which was not significantly different from the zero-current potential of the Na^+ current observed with NMDG glutamate pipette solution. In four experiments, addition to the NMDG nitrate pipette solution of 1 mmol l^{-1} NAD and 25 μmol l^{-1} dithiothreitol (which are present in the pertussis toxin-containing pipette solution), did not activate the Na^+ current. In three further experiments we confirmed that the pertussis toxin-activated Na^+ conductance was inhibited by the addition of 100 μmol l^{-1} amiloride to the bath (data not shown).

DISCUSSION

In the present paper we show that the effect of anions on the amiloride-sensitive Na^+ conductance in intralobular salivary ducts is mediated by a G protein which, when active, inhibits the Na^+ conductance. The evidence supporting this conclusion is as follows: (i) suppression of Na^+ conductance by NO_3^- is prevented by the inclusion of GDP-β-S in the pipette solution (Figs 2 and 3); (ii) this action of GDP-β-S is not due to a non-specific stimulatory effect because GDP-β-S has no effect on the Na^+ conductance when the pipette solution is glutamate-rich (Fig. 3); (iii) addition of GTP-γ-S to the glutamate-rich pipette solution inhibits the Na^+ conductance (Fig. 1); (iv) the selectivity pattern for the inhibitory effect of the anion on the Na^+ conductance, viz. Cl^-, Br^-, $NO_3^- \gg I^-$, glutamate, gluconate (Dinudom *et al.* 1993*a*), resembles that reported for anion activation of G proteins by Higashijima *et al.* (1987).

The intracellular concentration of Cl^- has been previously shown to influence the activity of cardiac K^+ channels (Nakajima *et al.* 1992) and the activation of neutrophils (Grinstein, Furuya & Downey, 1992) by stimulating G proteins. These effects on G protein activity are not thought to be due to a specific anion receptor, but have been attributed to the anion changing the rate at which the α-subunit of the G protein hydrolyses GTP (Higashijima *et al.* 1987; Nakajima *et al.* 1992). We have no direct evidence to show whether salivary ducts contain a receptor for intracellular anions linked to a G protein, but our finding that the anion sensitivity of the Na^+ conductance (Dinudom *et al.* 1993*a*) is identical to that of purified G_o (Higashijima *et al.* 1987) makes it unnecessary to postulate the existence of such a receptor.

We also show that the G protein controlling the Na^+ channel is sensitive to pertussis toxin. Since the epithelial Na^+ channel has been reported to contain a pertussis toxin-sensitive G protein (Ismailov, McDuffie & Benos, 1994) of the G_{i3} subtype, which, when activated, inhibits the Na^+ channel (Ismailov *et al.* 1994), it is tempting to suggest that this G protein mediates the anion effect. It should be remembered, however, that salivary duct cells contain numerous copies of G_o (Watson, Olver, D'Silva & Belton, 1994), a pertussis toxin-sensitive G protein that is known to be sensitive to ambient anions (Higashijima *et al.* 1987). Our data do not permit us to determine which of these G proteins might be involved.

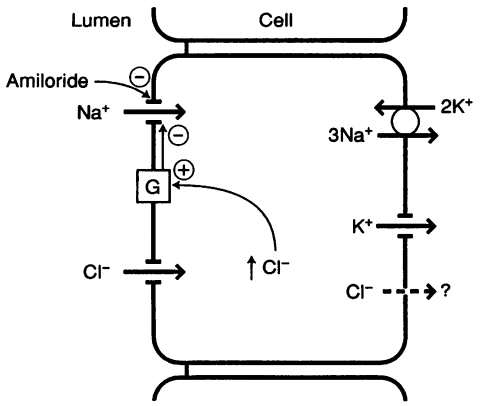

Figure 4. Duct transport model
Proposed model of the role of cytosolic Cl^- in regulating duct Na^+ transport.

As mentioned above in the introduction to this paper, the effects of intracellular Cl^- on the Na^+ conductance (Dinudom *et al.* 1993*a*) are seen in the physiological range of Cl^- concentrations (Lau *et al.* 1994) and the intracellular Cl^- concentration has been found to depend on the rate of Na^+ influx across the apical membranes of the duct cells (Lau *et al.* 1994). Thus, cytosolic Cl^- may be an important mediator of membrane 'cross-talk' whereby the apical influx of Na^+ is matched to the rate of Na^+ pumping across the basolateral membrane (Fig. 4). Since Cl^- transport is coupled electrically to Na^+ transport, apical Na^+ influx exceeding the rate of basolateral Na^+ pumping would lead to increased intracellular Na^+ and Cl^- concentrations and the increased cytosolic Cl^- would then inhibit further Na^+ influx. An apical Na^+ influx rate less than the rate of basolateral Na^+ pumping would lead to the converse sequence of events.

Cytosolic Cl^- has not been widely considered as a mediator of membrane cross-talk in absorptive epithelia, and most work on the topic has been concerned with the roles of cytosolic H^+, Na^+ or Ca^{2+} (Palmer, Frindt, Silver & Strieter, 1989; Turnheim, 1991). There have, however, been a sufficient number of reports of ambient anions influencing the apical Na^+ conductance of epithelia (see Turnheim, 1991) to suggest that the role for cytosolic Cl^- in mediating membrane cross-talk suggested here for salivary ducts may be applicable to absorptive epithelia generally. Furthermore, the recent reports that changes in cytosolic Cl^- mediate the muscarinic activation of the Na^+–K^+–$2Cl^-$ cotransporter and the Na^+–H^+ exchanger in the basolateral membranes of salivary endpiece cells (Robertson & Foskett, 1994) and mediate the β-adrenergic activation of a non-selective cation channel in pulmonary epithelial cells (Tohda, Foskett, O'Brodovich & Marunaka, 1994) suggest that intracellular Cl^- may be an important regulator of membrane transport in all epithelia.

Bijman, J., Cook, D. I. & Van Os, C. H. (1983). Effect of amiloride on electrolyte transport parameters of the main duct of the rabbit mandibular salivary gland. *Pflügers Archiv* **398**, 96–102.

Cook, D. I., Van Lennep, E. W., Roberts, M. L. & Young, J. A. (1994). Secretion by the major salivary glands. In *Physiology of the Gastrointestinal Tract*, 3rd edn, vol. 2, ed. Johnson, L., Christensen, J., Jackson, M., Jacobson, E. & Walsh, J., pp. 1061–1117. Raven Press, New York.

Dinudom, A., Young, J. A. & Cook, D. I. (1993*a*). Na^+ and Cl^- conductances are controlled by cytosolic Cl^- concentration in the intralobular duct cells of mouse mandibular glands. *Journal of Membrane Biology* **135**, 289–295.

Dinudom, A., Young, J. A. & Cook, D. I. (1993*b*). Amiloride-sensitive Na^+ current in the granular duct cells of mouse mandibular glands. *Pflügers Archiv* **423**, 164–166.

Duc, C., Farman, N., Canessa, C. M., Bonvalet, J. P. & Rossier, B. C. (1994). Cell-specific expression of epithelial sodium channel alpha, beta, and gamma subunits in aldosterone-responsive epithelia from the rat: localization by in situ hybridization and immunochemistry. *Journal of Cell Biology* **127**, 1907–1921.

Grinstein, S., Furuya, W. & Downey, G. P. (1992). Activation of permeabilized neutrophils: role of anions. *American Journal of Physiology* **263**, C78–85.

Gruber, W. D., Knauf, H. & Frömter, E. (1973). The action of aldosterone on Na^+ and K^+ transport in the rat submaxilliary main duct. *Pflügers Archiv* **344**, 33–49.

Higashijima, T., Ferguson, K. M. & Sternweis, P. C. (1987). Regulation of hormone-sensitive GTP-dependent regulatory proteins by chloride. *Journal of Biological Chemistry* **269**, 3597–3602.

Ismailov, I. I., McDuffie, J. H. & Benos, D. J. (1994). Protein kinase A phosphorylation and G protein regulation of purified renal Na^+ channels in planar bilayer membranes. *Journal of Biological Chemistry* **269**, 10235–10241.

Komwatana, P., Dinudom, A., Young, J. A. & Cook, D. I. (1994). Characterization of the chloride conductance in the granular duct cells of mouse mandibular glands. *Pflügers Archiv* **428**, 641–647.

Lau, K. R., Evans, R. L. & Case, R. M. (1994). Intracellular Cl^- concentration in striated intralobular ducts from rabbit mandibular salivary glands. *Pflügers Archiv* **427**, 24–32.

Moran, A., Davis, V. H. & Turner, R. J. (1995). Na^+ channels in membrane vesicles from intralobular salivary ducts. *American Journal of Physiology* **268**, C350–355.

NAKAJIMA, T., SUGIMOTO, T. & KURACHI, Y. (1992). Effects of anions on the G protein-mediated activation of the muscarinic K^+ channel in the cardiac atrial membrane. *Journal of General Physiology* **99**, 665–682.

OHARA, A., MATSUNAGA, H. & EATON, D. C. (1993). G protein activation inhibits amiloride-blockable highly selective sodium channels in A6 cells. *American Journal of Physiology* **264**, C352–360.

PALMER, L. G., FRINDT, G., SILVER, R. B. & STRIETER, J. (1989). Feedback regulation of epithelial sodium channels. In *Current Topics in Membranes and Transport*, vol. 34, *Cellular and Molecular Biology of Sodium Transport*, ed. SCHULTZ, S. G., pp. 45–60. Academic Press, San Diego.

ROBERTSON, M. A. & FOSKETT, J. K. (1994). Na^+ transport pathways in secretory acinar cells: membrane cross talk mediated by Cl^-. *American Journal of Physiology* **267**, C146–156.

TOHDA, H., FOSKETT, J. K., O'BRODOVICH, H. & MARUNAKA, Y. (1994). Cl^- regulation of a Ca^{2+}-activated nonselective cation channel in beta-agonist-treated fetal distal lung epithelium. *American Journal of Physiology* **266**, C104–109.

TURNHEIM, K. (1991). Intrinsic regulation of apical sodium entry in epithelia. *Physiological Reviews* **71**, 429–445.

WATSON, E. L., OLVER, C., D'SILVA, N. & BELTON, C. M. (1994). Localization of the G protein G_o in exocrine glands. *Journal of Histochemistry and Cytochemistry* **42**, 41–47.

ZONG, X. & LUX, H. D. (1994). Augmentation of calcium channel currents in response to G protein activation by GTPγS in chick sensory neurons. *Journal of Neuroscience* **14**, 4847–4853.

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