Volume-activated chloride channels in rat parotid acinar cells

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1. Rat parotid acinar cells undergo a regulatory volume decrease in response to hypotonically induced cell swelling that is sensitive to K^+ and Cl^- gradients. To investigate the potential mechanisms involved, the whole-cell patch-clamp technique was used to characterize a volume-sensitive Cl^- channel in rat parotid acinar cells.

2. Exposure of cells to a hyposmotic gradient induced large Cl^- currents that exhibited outward rectification and were not affected by membrane potential or the absence of intracellular Ca^{2+}. Low external pH increased the currents at all potentials without affecting current kinetics. These currents were nearly abolished when the cells were in hypertonic conditions. This decrease in the current amplitude was correlated with a decrease in the cell size.

3. The volume-sensitive currents displayed little or no time dependence, whereas Ca^{2+}-activated Cl^- channels, present in the same cells, displayed slow activation kinetics and large, time-dependent tail currents upon repolarization to the holding potential.

4. The reversal potential of the osmotically activated channels was close to the predicted chloride equilibrium potential and was sensitive to the physiological extracellular Cl^- concentration ($[Cl^-]_o$). The relationship between reversal potential and $[Cl^-]_o$ was fitted to a modified Nernst equation with a slope of 51 mV per decade, consistent with a Cl^- selective conductance.

5. The anion permeability sequence of the channel, obtained from the shifts of the reversal potentials of the volume-sensitive Cl^- current, was: $SCN^- > I^- > NO_3^- > Br^- > Cl^-$ > formate > propionate = methanesulphonate = acetate $\geqslant F^- \geqslant$ butyrate > valerate > gluconate = glucuronate = glutamate.

6. The current through the volume-sensitive channels was inhibited by the Cl^- channel blocker SITS (4-acetamido-4′-isothiocyanatostilbene-2,2′-disulphonic acid) in a voltage-dependent manner.

7. We conclude that rat parotid acinar cells express an outwardly rectifying Cl^- current that can be activated by swelling under hypotonic conditions. This Cl^- conductance may be an element of the cellular mechanisms of volume regulation in exocrine glands.

Many animal cells recover from hyposmotically induced swelling by increasing the permeability of the plasma membrane to ions. (For review see Hoffmann & Simonsen, 1989). The resulting regulatory volume decrease (RVD) is typically achieved by the loss of intracellular KCl to re-establish the original cell volume (Hoffmann & Simonsen, 1989). Loss of intracellular KCl during cell volume control is frequently associated with the opening of so-called hyposmotically activated channels, which are separate conductive pathways to K^+ and Cl^- (Sarkadi & Parker, 1991). Hyposmotic-activated Cl^- channels have been described in many cell types, including neutrophils, T lymphocytes, HL-60, human small intestinal, human colonic, HeLa, chick heart, *Xenopus* oocytes, rabbit osteoclasts, human endothelial, Ehrlich ascites tumour, and bovine chromaffin cells (Hoffmann, Simonsen & Lambert, 1984; Worrel, Butt, Cliff & Frizzell, 1989; Doroshenko & Neher, 1992; Kubo & Okada 1992; Stoddard, Steinbach & Simchowitz, 1993; Lewis, Ross & Cahalan, 1993; Diaz, Valverde, Higgins, Rucareanu & Sepulveda, 1993; Zhang, Rasmusson, Hall & Lieberman, 1993; Arreola, Hallows & Knauf, 1994*a*; Ackerman, Wickman & Clapham, 1994; Kelly, Dixon & Sims, 1994; Nilus, Oike, Zahradnik & Droogmans, 1994). In these cells volume-sensitive Cl^-

channels are presumably the pathway for Cl^- efflux during RVD. In this sense volume-sensitive Cl^- channels play an important role in cell volume regulation.

Rat parotid acinar cells are exocrine cells that normally secrete large amounts of fluid and electrolytes during muscarinic cholinergic stimulation (see Nauntofte, 1992; Turner, 1993 for reviews). Saliva secretion is under the strict control of apical Cl^- and basolateral K^+ effluxes through Ca^{2+}-sensitive Cl^- and K^+ channels, respectively. The large water and electrolyte fluxes associated with secretion subject these cells to osmotic stress. They exhibit RVD following hyposmotic shock (Foskett, Wong, Sue-A-Quan & Robertson, 1994) and this response resembles the volume regulation that also occurs during muscarinic stimulation (Nakahari, Murakami, Yoshida, Miyamoto, Sohma & Imai, 1990; Foskett *et al.* 1994)

Since it has been suggested that Cl^- efflux during RVD in most cell types studied occurs through volume-sensitive Cl^- channels, it was hypothesized that Cl^- efflux during cell volume changes in rat parotid acini are also mediated through a similar conductive pathway. In this paper we describe the properties of a volume-sensitive Cl^- channel in rat parotid acinar cells. This channel is anion selective, voltage-independent, sensitive to SITS (4-acetamido-4′-isothiocyanatostilbene-2,2′-disulphonic acid), and coexists with a Ca^{2+}-dependent Cl^- channel. The volume-sensitive Cl^- channel may play an important role in the regulation of acinar cell volume.

A preliminary report of this work has appeared in abstract form (Arreola, Melvin & Begenisich, 1994*b*).

METHODS

Cell dissociation

Single parotid acinar cells were prepared using a modified procedure as described by Foskett *et al.* (1994). Male 150–250 g Wistar strain rats (Charles River, Kingston Facility, NY, USA) were used in these experiments. After CO_2 anaesthesia, rats were killed by exsanguination and the parotid glands removed. Glands dissected free of lymph nodes, blood vessels and connective tissue were minced in Ca^{2+}-free minimum essential medium (MEM; Gibco, USA) + 1% bovine serum albumin (BSA; Fraction V, Sigma, USA). The minced tissue was treated for 20 min at 37 °C with a 0·02% trypsin solution (MEM Ca^{2+}-free + 1 mM EDTA + 2 mM glutamine + 1% BSA). After stopping the reaction with 2 mg ml^{-1} soybean trypsin inhibitor (Sigma), the tissue was dispersed with collagenase (100 units ml^{-1} of type CLSPA; Worthington Biochemical Corp., Freehold, NJ, USA) in Ca^{2+}-free MEM + 2 mM glutamine + 1% BSA and pipetted every 15 min with a 10 ml plastic pipette. After 60 min, the acinar suspension was centrifuged, the supernatant discarded and the pellet resuspended in fresh collagenase solution for an additional 60 min. This material was then centrifuged and washed with BSA-free basal medium Eagle (BME; Gibco). The 'single cell' pellet was resuspended in 2–5 ml BSA-free BME + 2 mM glutamine and plated onto poly-L-lysine-coated glass coverslips.

Whole-cell patch clamp

Electrophysiology. The whole-cell configuration of the patch-clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) was used to record macroscopic currents in dissociated parotid acinar cells. Cells plated on polylysine-coated coverslips were transferred to the recording chamber (volume 150–200 μl) on the stage of an inverted microscope and superfused with the external bath solution. Whole-cell currents were recorded during voltage pulses of 100 ms duration from a holding potential of −50 or 0 mV (unless otherwise noted). Connection to the external reference electrode was made with a 3 M CsCl–agar-filled electrode and so minimized changes of liquid junction potentials with the various external solutions. The junction potential between the standard pipette and bath solution was < 3 mV; the membrane potentials were not corrected for this small value.

Solutions. External and internal solutions were designed to enhance the recording of Cl^- currents. Cationic currents activated by either voltage or Ca^{2+} were minimized by utilizing tetraethylammonium (TEA) and tetramethylammonium (TMA) salts and by including 10 mM EGTA in the pipette solution. The extracellular [Ca^{2+}] was 0·5 mM to reduce Ca^{2+} influx. Except when noted, EGTA was included in the pipette solution to buffer changes in intracellular [Ca^{2+}] and thus any intracellular [Ca^{2+}]-dependent processes.

The osmolality of all solutions was determined with a vapour pressure osmometer (Wescor 5500, Logan, UT, USA). The standard internal (pipette) solution to record volume-sensitive Cl^- currents contained (mM): TEA-Cl, 120; TMA-F, 20; EGTA, 10; *N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid (Tes), 20; pH 7·3; 297 mosmol kg^{-1}. The internal solution to record volume-sensitive and Ca^{2+}-dependent Cl^- currents in the same cell contained (mM): 1,2-bis(*O*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), 0·6; $CaCl_2$, 0·1; TEA-Cl, 120; TMA-F, 20; Hepes, 20; pH 7·3; 295 mosmol kg^{-1}. The most frequently used external solution contained (mM): TEA-Cl, 149; $CaCl_2$, 0·5; Hepes, 20; pH 7·3. This solution had an osmolality of 284 mosmol kg^{-1}. Other external solutions of greater osmolality were made by the addition of D-mannitol. A reduced-osmolality external solution of 261 mosmol kg^{-1} was made by dilution of the standard solution.

External solutions with different levels of Cl^- were made by equimolar substitution of TEA-Cl with D-mannitol. For experiments on ion selectivity, the external TEA-Cl was replaced with an equal concentration of TEA-X (where $X = SCN^-$, I^-, NO_3^-, Br^-, formate, methanesulphonate, acetate, F^-, gluconate, glucuronate, glutamate, butyrate, propionate or valerate). To test the effect of low external pH on the Cl^- channels, 20 mM Mes (2-(*N*-morpholino)ethanesulphonic acid) was used to adjust the pH of the external isotonic solution to 6·0. SITS and ionomycin were added to the external solution to give the desired concentration.

Chemicals. TEA salts were made from TEA-OH and the corresponding acids, or obtained from Fluka (Buchs, Switzerland) or Sigma. BAPTA was from Fluka. EGTA, Hepes and Mes were obtained from Sigma. SITS was obtained from Pierce (Rockford, IL, USA). Ionomycin was purchased from Calbiochem (USA).

Analysis

Relative osmotic pressure is presented as tonicity of external solution minus tonicity of internal solution. Current–voltage relationships were constructed from steady-state currents.

Current reversal potentials were determined by interpolation from a fourth-order polynomial fit to the data. Permeability ratios (P_X/P_{Cl}) were calculated from the shifts of the reversal potentials using the modified Goldman–Hodgkin–Katz equation (Hille, 1992):

$$\Delta E_r = E_{r(X)} - E_{r(Cl)} = \frac{RT}{zF} \ln \frac{P_X[X]_e}{P_{Cl}[Cl]_e}, \quad (1)$$

where ΔE_r is the reversal potential shift induced by replacing external Cl^- by the anion X^-, $E_{r(X)}$ is the reversal potential in the presence of the anion X^-, $E_{r(Cl)}$ is the reversal potential in the presence of external Cl^-, R is the gas constant, T is the temperature, z is the valency of the ion (−1), and F is the Faraday constant.

We investigated cell and current block by using SITS and analysed the results in terms of a modified form of the Woodhull (1973) model for voltage-dependent block:

$$\frac{I_{SITS}}{I_{Control}} = \frac{1}{1 + \frac{[SITS]}{K_d^{(0)}} \exp[(-z\delta E_m) \frac{F}{RT}]}, \quad (2)$$

where $I_{Control}$ is the current in the absence of, and I_{SITS} is the current in the presence of, the applied concentration of external SITS ([SITS]); $K_d^{(0)}$ is the apparent dissociation constant at 0 mV; z is the SITS valency; δ represents the equivalent fraction of the membrane field that the blocking ion crosses to reach the binding site.

RESULTS

Changes in solution tonicity alter whole-cell currents and cell morphology

Figure 1 shows currents recorded from an isolated parotid acinar cell. The bath solution osmolality in the left panel of Fig. 1*A* was 359 mosmol kg^{-1} and the internal solution osmolality was 297 mosmol kg^{-1}. Thus, the bath was +62 mosmol kg^{-1} hypertonic. The left panel shows that the currents at all potentials were quite small when recorded under these hypertonic conditions. The middle panel of Fig. 1*A* demonstrates that a −13 mosmol kg^{-1} hypotonic gradient induced the appearance of much larger and sustained ionic currents. These currents increased even further, without a change in time course, with a −36 mosmol kg^{-1} hypotonic gradient as seen in the right panel of Fig. 1*A*.

The currents activated by hypotonicity exhibited outward rectification as seen in Fig. 1*B*. The current (from the experiment of Fig. 1*A*) at the end of each voltage-clamp pulse is plotted in hypertonic conditions (■) as well as with −13 (●) and −36 mosmol kg^{-1} (▲) hypotonic gradients. The currents in the hypotonic bath solutions were larger at positive than at negative potentials. The reversal potential for these current–voltage relationships was −2 mV, similar to the Cl^- equilibrium potential of −4 mV.

In most experiments, we patched cells with a small (−13 mosmol kg^{-1}) hypotonic gradient (bath solution, 284 mosmol kg^{-1} and pipette solution, 297 mosmol kg^{-1}). As illustrated in the left and middle panels of Fig. 2*A*, this had the effect of activating currents with characteristics similar to those seen with the hypotonic conditions of Fig. 1*A* (middle panel). The activation of current required a few minutes to reach steady levels, presumably due to the time required for pipette solution to exchange with the cell contents, and the time required for the cell swelling to stabilize. The currents in the left panel of Fig. 2*A* were recorded 1 min after achieving whole-cell mode. The currents in the centre panel were recorded after 3·5 min, in the same cell. These currents were inhibited by a subsequent change to hyperosmotic conditions (right panel of Fig. 2*A*). Except for some small, uncompensated capacity transients (cf. Fig. 1*A*, left panel and Fig. 2*A*, right panel), there was little or no time dependency of the osmotically activated currents (centre panel of Figs 1*A* and 2*A*). The time constant for the slow decay of the whole-cell current was 51 ± 9·7 s (mean ± S.E.M., $n = 4$) at +80 mV with pulses of 10 s duration.

The osmotic difference between the pipette and bath solutions induced a change in cell size as shown in the micrographs of Fig. 2*A*. We did not attempt to quantitate these volume changes. To do so accurately requires knowledge of the three-dimensional shape of the cell. An estimate can be made if a spherical geometry is assumed: there was an apparent change in diameter of about 1·4 for the experiment illustrated in Fig. 2*A* and, therefore, a 2·7-fold estimated change in volume. This value is much larger than the 1·3 ratio of solution osmolality. Essentially identical results have been observed in Jurkat T lymphocytes (Ross, Garber & Cahalan, 1994) and these authors have developed a model that tends to account for the observed large volume change.

A summary of many experiments like that shown in Fig. 2*A* is illustrated in Fig. 2*B*. Currents recorded within 0·5 min of achieving whole-cell mode (with a −13 mosmol kg^{-1} gradient) were small (■) but increased to a steady level 11- to 12-fold larger within 3–3·5 min (●). These currents displayed the same outward rectification and reversal potential seen in Fig. 1*B*. There is also a suggestion of additional rectification at the most negative potentials. Almost all the current recorded under these conditions was sensitive to osmolality. The currents recorded under hypertonic conditions (▲) were very small, and the current–voltage relationship was close to linear with a slope equivalent to a resistance of 7·7 GΩ – not much different from the average seal resistance of 9·5 GΩ.

It is known that parotid acinar cells have Ca^{2+}-dependent Cl^- channels (Iwatsuki, Maruyama, Matsumoto & Nishiyama, 1985; Gray, 1989; Shigetomi *et al.* 1991). To determine whether the volume-sensitive current is distinct from the Ca^{2+}-dependent current, experiments were designed to activate separately each conductance in the same cell. An example of this approach is shown in Fig. 3.

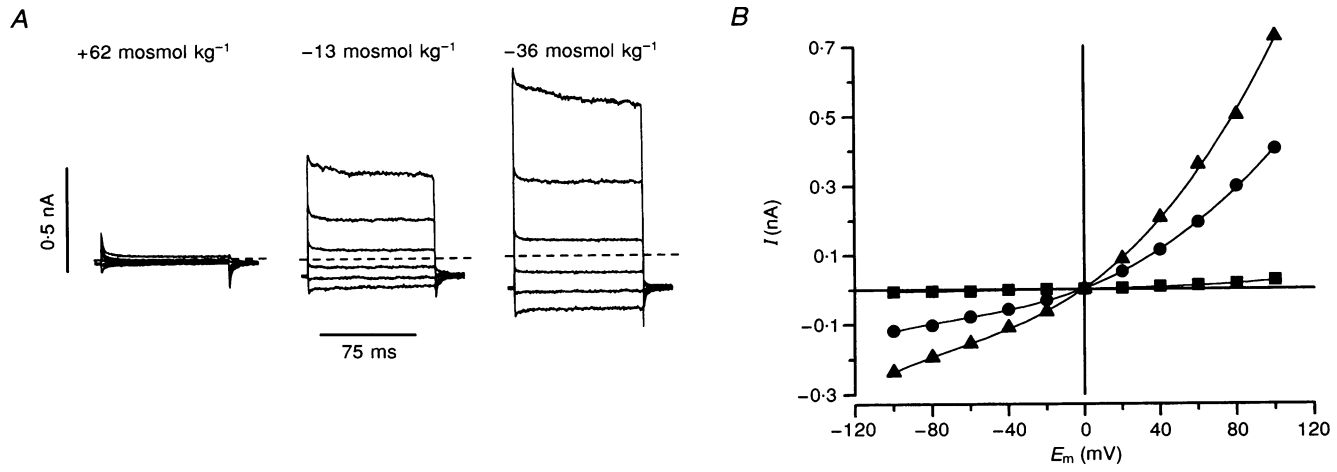

Figure 1. Activation of chloride channels by hyposmotic exposure of a parotid acinar cell

A, whole-cell Cl^- currents obtained from the same cell at the osmotic pressure indicated. Membrane potentials from −100 to +100 mV in 40 mV steps. *B*, current–voltage relationships from the raw data depicted in *A*. ■, +62 mosmol kg^{-1}; ●, −13 mosmol kg^{-1}; ▲, −36 mosmol kg^{-1}.

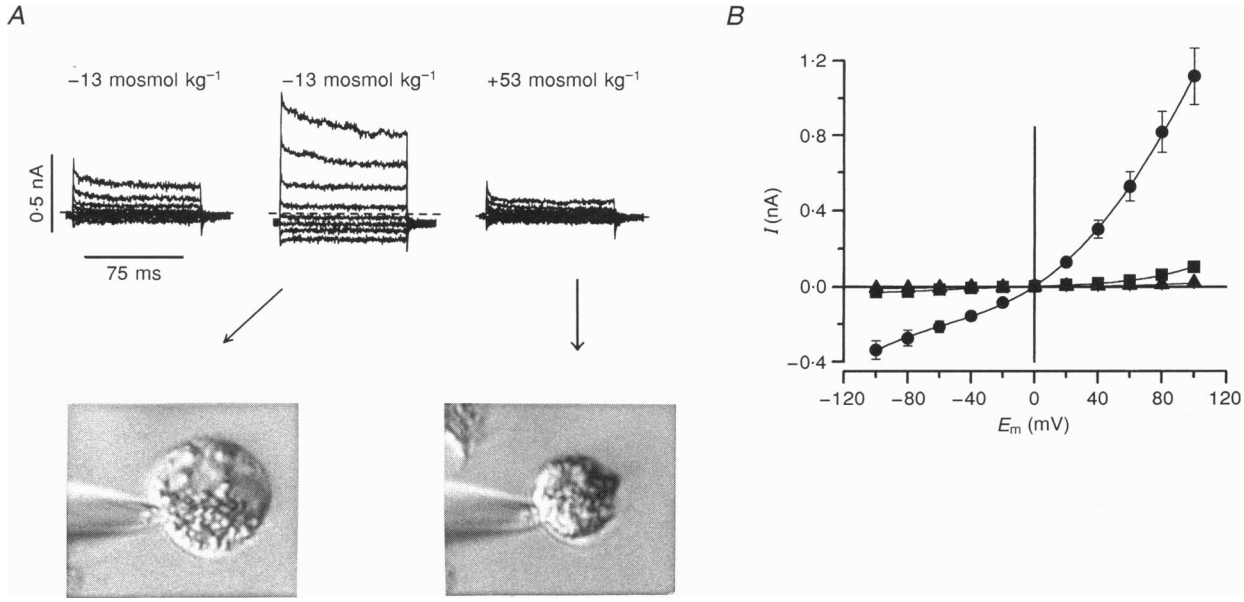

Figure 2. Spontaneous activation of chloride channels induced by −13 mosmol kg^{-1} osmotic pressure

A, whole-cell Cl^- currents from the same cell. Left and centre sets were obtained at 0·5 and 3·5 min after breakthrough. The right set depicts the current after exposure to a hypertonic medium. Membrane potentials from −140 to +140 mV in 40 mV steps from a holding potential of −50 mV. The lower panel shows the micrographs of the corresponding cells from which the ionic currents were recorded (as indicated by the arrows). Cell diameters were 15·5 and 11·1 μm for the left and right micrographs, respectively. *B*, current–voltage relationships of the spontaneously activated Cl^- channels. The steady-state current amplitudes in a hypotonic medium are shown 0–0·5 min (■, −13 mosmol kg^{-1}) and 3–3·5 min (●, −13 mosmol kg^{-1}) after breakthrough ($n = 9$). ▲, the steady-state current amplitude 3–3·5 min after breakthrough in a hypertonic (+62 mosmol kg^{-1}) medium ($n = 5$).

The left panel of Fig. 3*A* shows the spontaneously activated Cl^- current at different potentials after 3 min in hypotonic conditions. These currents are indistinguishable from those illustrated in Figs 1 and 2, and are not present in hypertonic conditions (centre panel). Treatment of cells in a hypertonic solution with the Ca^{2+} ionophore, ionomycin, resulted in the activation of currents (right panel) with quite different properties from those observed in hypotonic conditions.

Currents activated by ionomycin were time dependent, especially at potentials positive to +40 mV. These currents also displayed large tail currents upon repolarization. The steady-state current–voltage relationships of both the osmotically activated (■) and ionomycin-activated (▲) currents are shown in Fig. 3*B*. The ionomycin-activated currents exhibit even stronger outward rectification than the osmotically activated currents. The currents induced by ionomycin had properties quite similar to those of other Ca^{2+}-activated Cl^- channels previously described in rat and sheep parotid acinar cells, lachrymal cells and human colon carcinoma cells (Iwatsuki *et al.* 1985; Evans & Marty, 1986; Cliff & Frizzell, 1990; Ishikawa & Cook, 1993). Thus, rat parotid acinar cells express both Ca^{2+}-activated and osmotically activated currents that exhibit different kinetic properties.

The Cl^- channels activated by osmotic gradients were not sensitive to long-term changes in holding potential. Current–voltage relationships obtained from five cells at the normal holding voltage of −50 mV and with a holding potential of 0 mV were nearly identical. The close similarity of the current–voltage relationships demonstrates that the level of channel activation was osmotic but not voltage sensitive.

Chloride dependency and ion selectivity

The currents recorded in hypotonic conditions primarily reflect the movement of Cl^- ions as illustrated in Fig. 4. The ordinate of this figure is the shift of the current reversal potential that occurred when the external Cl^- concentration was reduced from the normal 150 mM to lower values. In order to prevent artifacts from potentially permeant replacement anions, TEA-Cl was replaced by an equivalent concentration of D-mannitol. As this replacement changes solution ionic strength, and therefore the chloride ion activity coefficient (Kielland, 1937), the ordinate is the Cl^- activity, not concentration.

The shift of the current reversal potential is clearly a function of Cl^- activity and over the concentration range of 150 to about 16 mM (activities of 122–15 mM) is close to that expected for perfect Cl^- selectivity (Fig. 4, dotted line). At lower concentrations the data deviate from this expectation, as has been noted in other work on volume-activated Cl^- channels (Zhang *et al.* 1993; Kelly *et al.* 1994).

We investigated the selectivity of the Cl^- channel activated by osmotic gradients by recording currents in normal, Cl^--containing external solutions, and in solutions with a test anion replacing Cl^-. As described in Methods, the relative permeability of the test anion to Cl^- was computed from the change in current reversal potential. The results of many such experiments with several different anions are summarized in Fig. 5. The

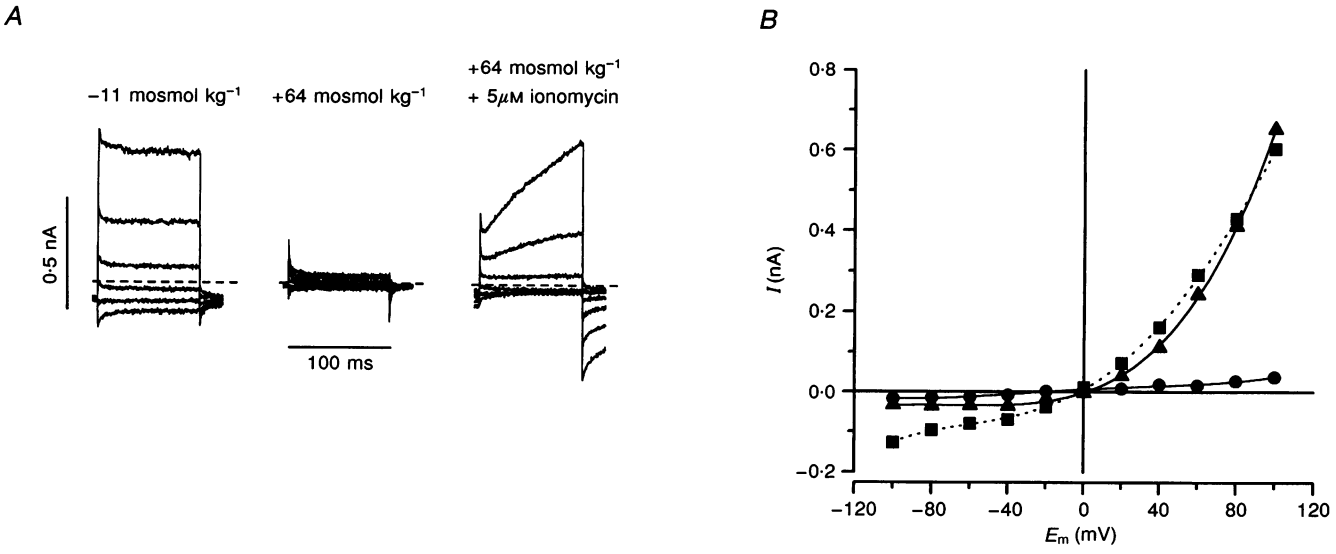

Figure 3. Volume-sensitive and Ca^{2+}-dependent chloride channels coexist within the same cell

A, whole-cell Cl^- currents were obtained from the same cell under the tonicity conditions indicated above each set. Membrane potentials were −100, −60, −20, 20, 60 and 100 mV. *B*, current–voltage relationship of data shown in *A*. Internal solution containing 0·6 mM BAPTA. ■, −11 mosmol kg^{-1}; ●, +64 mosmol kg^{-1}; ▲, +64 mosmol kg^{-1} + 5 μM ionomycin.

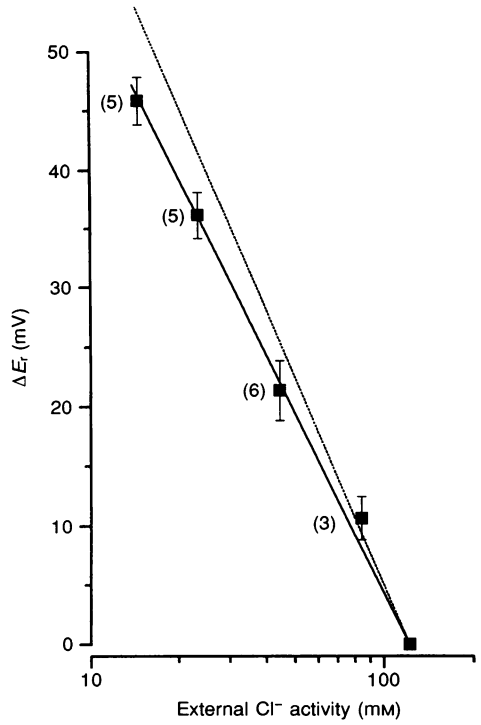

Figure 4. Dependency of the shift in reversal potential on external chloride concentration

The continuous line is the fit of eqn (1) to data with a slope of −51 mV per decade. The dotted line is the predicted behaviour for a Cl^- electrode with a slope of −58 mV per decade. The number of cells is indicated in parentheses.

apparent selectivity sequence for the osmotically activated channel was SCN^- (2·04 ± 0·28, $n = 4$) > I^- (1·46 ± 0·1, $n = 5$) > NO_3^- (1·23 ± 0·03, $n = 5$) > Br^- (1·19 ± 0·05, $n = 5$) > Cl^- (1) > formate (0·58 ± 0·06, $n = 4$) > propionate (0·48, $n = 2$) = methanesulphonate (0·47 ± 0·08, $n = 4$) = acetate (0·47 ± 0·1, $n = 3$) ≥ F^- (0·44 ± 0·02, $n = 4$) ≥ butyrate (0·41 ± 0·07, $n = 3$) > valerate (0·19 ± 0·03, $n = 4$) > gluconate (0·09 ± 0·01, $n = 5$) = glucuronate (0·08 ± 0·01, $n = 3$) = glutamate (0·07 ± 0·01, $n = 3$).

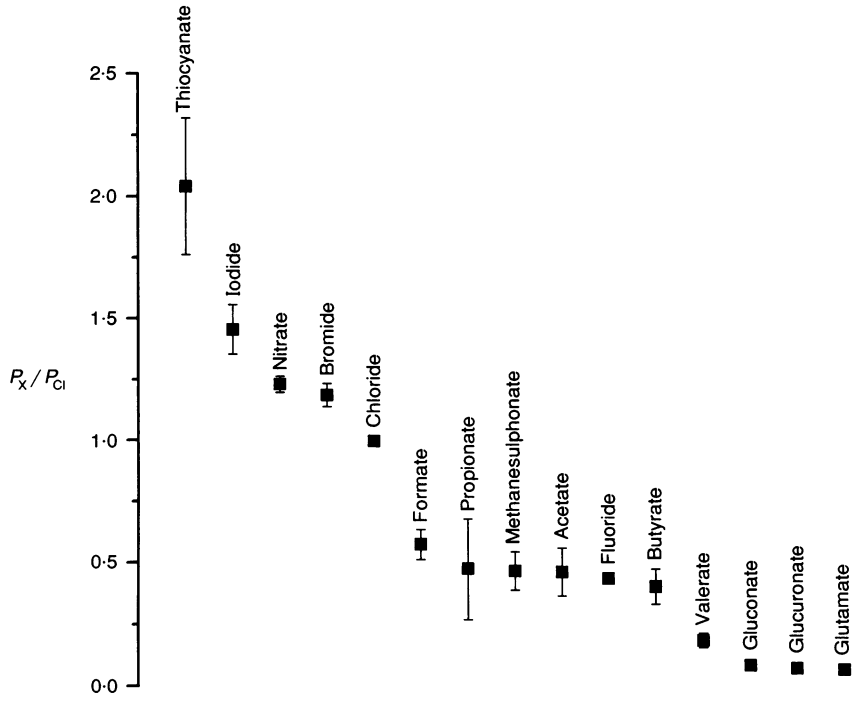

Figure 5. Selectivity sequence of the volume-sensitive chloride channels

The sequence is based on the permeability ratios relative to chloride, P_X/P_{Cl}, calculated from the shifts in the reversal potentials using eqn (1).

Effects of SITS and external pH on the volume-sensitive chloride channels

Volume-sensitive Cl^- channels in other cells are blocked by the distilbene SITS (Kubo & Okada, 1992; Lewis *et al.* 1993; Stoddard *et al.* 1993; Kelly *et al.* 1994; Ackerman *et al.* 1994). We examined the effect of 0·5 mM SITS on the volume-activated Cl^- channels in parotid acinar cells. Figure 6*A* shows an example of the effect of SITS on the whole-cell Cl^- currents. SITS reversibly decreased the current in a voltage-dependent manner, i.e. currents at positive potentials were reduced much more than currents at negative voltages. This effect can be clearly seen in the current–voltage relationships of Fig. 6*B*. At negative potentials, the magnitudes of the currents in the presence of SITS (●) were very similar to those obtained before (■) and after (□) SITS exposure. However, at positive potentials SITS produced a very large reduction of current.

A summary and quantitative analysis of the voltage dependency of SITS block is illustrated in Fig. 6*C*. The voltage dependence of the remaining fraction of current from four cells in the presence of 0·5 mM SITS is shown (■). The continuous line is a fit of eqn (2) to these data and is a reasonable description of the results at potentials negative to +50 mV. At positive potentials, a constant unblocked fraction of about 0·3 was observed which has also been noted in T lymphocytes (Lewis *et al.* 1993). The apparent dissociation constant at 0 mV ($K_d^{(0)}$) obtained from the fit was 0·68 mM. The voltage dependence of block was equivalent to a blocking site located from the external side approximately 31% within the membrane electric field.

The osmotically activated currents appeared to have a small, slow, time-dependent decay component as is apparent in previous figures. Ackerman *et al.* (1994) have recently shown that a similar time dependence occurs in an exogenous Cl^- channel in *Xenopus* oocytes and that the rate of current decline is enhanced in acidic external solutions. The osmotically activated Cl^- channels in acinar parotid cells did not exhibit this behaviour, as illustrated in Fig. 7.

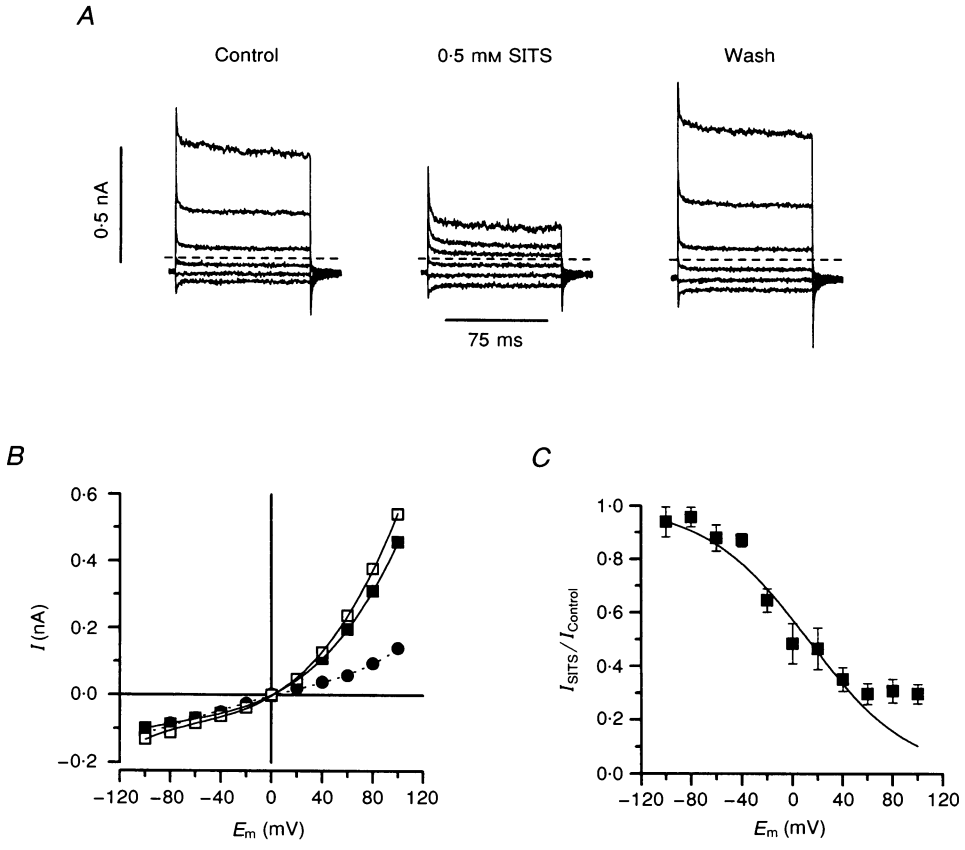

Figure 6. Voltage-dependent block of volume-sensitive chloride channels by SITS

A, whole-cell currents obtained before (Control), in the presence of 0·5 mM SITS (0·5 mM SITS), and after SITS wash (Wash). Membrane potentials were −100 to 100 mV in 40 mV steps. *B*, current–voltage relationships obtained from data shown in *A*. *C*, unblockade fraction of current ($I_{SITS}/I_{Control}$; see eqn (2)) by 0·5 mM SITS at different membrane potentials ($n = 5$). The continuous line is the fit of eqn (2) with the following parameters: $z = -2$, $\delta = 0{\cdot}308$ and $K_d^{(0)} = 0{\cdot}677$ mM.

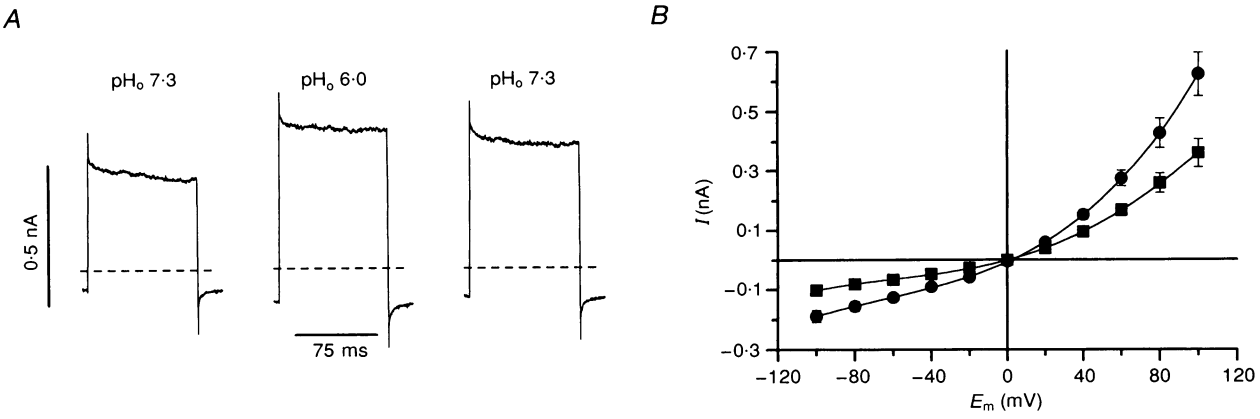

Figure 7. Effect of acidic external pH on volume-sensitive chloride channels

A, whole-cell Cl^- currents at +80 mV of membrane potential recorded from a cell bathed in media of pH$_o$ 7·3, 6 and 7·3. *B*, steady-state current–voltage relationships from data similar to those depicted in *A* at pH$_o$ of 7·3 (■) and 6·0 (●).

Figure 7*A* shows currents recorded at +80 mV at pH 7·3 (left panel), pH 6·0 (centre panel), and again at pH 7·3 (right panel). No change in the current time course is apparent in the low pH solution, but the magnitude of current was increased by the low pH solution and part of this increase was reversible in this example. A summary of the actions of low pH on steady-state currents is shown in Fig. 7*B*. Currents in low pH (●) were slightly larger than those recorded at pH 7·3 (■).

DISCUSSION

Summary of findings

We have described some of the electrophysiological characteristics of a volume-sensitive Cl^- channel in rat parotid acinar cells, the first observations of this channel type in exocrine glands. The magnitudes of the channel currents were related to the degree of cell swelling, and the currents were inhibited by hypertonic media that induced cell shrinkage. This volume-sensitive channel displayed outward rectification without voltage-dependent activation, and was insensitive to the chelation of intracellular Ca^{2+}. Parotid acinar cells also expressed a Ca^{2+}-dependent Cl^- current activated by ionomycin under hypertonic conditions. This Ca^{2+}-dependent current coexisted with the volume-sensitive current in the same cells, and had a different time course and voltage dependency. Cl^- ions passed freely through the volume-activated channel but its permeability sequence suggests a pore with little selectivity for different anions. The voltage-dependent blockade by SITS is similar to previous results on volume-sensitive Cl^- channels from other cells.

Comparison with other volume-activated chloride channels

In many ways, the volume-sensitive Cl^- channels in parotid acinar cells are similar to other volume-sensitive Cl^- channels previously described. For example, the voltage-dependent block by SITS is a common feature of Cl^- channels, including volume-sensitive Cl^- channels from human neutrophils (Stoddard *et al.* 1993), T lymphocytes (Lewis *et al.* 1993), human promyelocitic HL-60 cells (Arreola *et al.* 1994*a*), human small intestine epithelial cells (Kubo & Okada, 1992), *Xenopus* oocytes (Ackerman *et al.* 1994), and rabbit osteoclasts (Kelly *et al.* 1994). The weak voltage-dependent block found in the present experiments is comparable to the results seen in lymphocytes, neutrophils and HL-60 cells for similar SITS concentrations. The voltage-dependent block in lymphocytes, HL-60 cells and salivary glands suggests that the SITS binding site is located close to the external side. Voltage-dependent blockade of volume-sensitive Cl^- channels may be explained by changes in the dissociation constant of the SITS binding site at different membrane potentials.

From the permeability ratios calculated with the Goldman–Hodgkin–Katz equation, the selectivity sequence of volume-sensitive Cl^- channels from rat parotid acinar cells was the same as that found in other volume-sensitive Cl^- channels. Their permeability sequence ($SCN^- > I^- > NO_3^- > Br^- > Cl^-$ > formate = propionate = methanesulphonate = acetate $\geqslant F^- \geqslant$ butyrate > valerate > gluconate = glucuronate = glutamate) corresponds to sequence 1 of Wright & Diamond (1977), a prediction based on anion hydration

energies, binding site charge and binding site radii. Similar permeability sequences have been found for other volume-sensitive Cl^- channels in oocytes, human neutrophils, T lymphocytes, HL-60 cells, human intestinal cells, T84 cells, HeLa cells and chick heart. However, the cloned volume- and voltage-sensitive Cl^- channel clc-2 does not share this sequence. In this channel the selectivity sequence was $Cl^- > Br^- > I^-$ (Thiemann, Grunder, Pusch & Jentsch, 1992). Because the volume-sensitive Cl^- channel selectivity sequence corresponded to the predicted sequence 1, it suggests that the pore of this channel has a weak binding site for anions, as has been proposed for other Cl^- channels (Franciolini & Nonner, 1987; Bormann, Hamill & Sakmann, 1987; Kubo & Okada, 1992; Halm & Frizzell, 1992; Ackerman *et al.* 1994). The anion permeability diminished as the anion radii increased, with gluconate, glucuronate and glutamate being the least permeant anions, indicating that the pore has a diameter in the range of 0·58–0·64 nm. This pore size is comparable to the proposed pore size of GABA (0·56 nm), glycine (0·52 nm), T84 (0·55 nm), and rat hippocampal neuron (0·65 nm) Cl^- channels (Franciolini & Nonner, 1987; Bormann *et al.* 1987; Halm & Frizzell, 1992).

Protonation of a chemical group located close to the pore entrance may, in principle, increase the $[Cl^-]$ near the pore, thus enhancing the conductance. This hypothesis was proposed to explain the increased anionic conductance observed in muscle and epithelial cells at low pH (Hagiwara, Toyama & Hayashi, 1971; Halm & Frizzell, 1992). Such a mechanism predicts that outward currents (inward Cl^- movement) would increase with little or no effect on inward currents. This was clearly not the case in parotid acinar cells in which low external pH enhanced both inward and outward Cl^- currents. Alternatively, the neutralization of a repelling negative charge within the pore may account for the amplitude increase at all potentials of the volume-sensitive Cl^- current from parotid acinar cells.

In contrast to the above similarities, volume-sensitive Cl^- channels in parotid acinar cells also display kinetic properties that are distinct from volume-sensitive Cl^- channels in other cell types. For example, the time dependence of the endogenous volume-sensitive Cl^- channels in *Xenopus* oocytes is accelerated by external hydrogen ions (Ackerman *et al.* 1994). This effect was not present in volume-sensitive Cl^- channels in parotid acinar cells. Additionally, epithelial human colon carcinoma (T84) cells have a volume-sensitive Cl^- channel with voltage dependent activation and inactivation steps (Worrel *et al.* 1989). Unlike T84 cells, in parotid acinar cells there are no voltage-dependent steps in the activation or inactivation of the volume-sensitive Cl^- channels, and the currents at +80 mV decay with a time constant about 10 times slower than in T84 cells. It has also been proposed that the Ca^{2+}-dependent Cl^- channels are activated by a hyposmotic shock in lachrymal cells (Kotera & Brown, 1993). However, this was not the case in parotid acinar cells since volume-sensitive Cl^- channels were inhibited by hypertonic solutions and did not require Ca^{2+} to become, and stay, open; in contrast, Ca^{2+}-dependent Cl^- channels were activated under hypertonic conditions and needed Ca^{2+} to open (Fig. 3).

Taken together our results suggest that the volume-sensitive Cl^- channels in rat parotid acinar cells have a poorly selective anion conductance, a pore with a diameter of approximately 0·58–0·64 nm, a H^+ binding site that regulates conductance, and a stilbene binding site located close to the external face of the membrane. These properties are very similar to other volume-sensitive Cl^- channels previously described. However, in rat parotid acinar cells, the gating of volume-sensitive Cl^- channels is independent of the holding voltage and the channels are insensitive to external pH.

Physiological importance of the volume-sensitive chloride channels

During muscarinic-stimulated secretion from salivary acinar cells, dramatic changes in intracellular ion concentrations and cell volume occur concomitantly with transepithelial fluid and electrolyte movement. It is therefore likely that these cells have mechanisms in place to protect against excessive cell swelling. There are at least two physiological conditions that result in enhanced Cl^- uptake and cell swelling in parotid acinar cells which may activate the volume-sensitive Cl^- channels. First, muscarinic or α-adrenergic stimulated fluid secretion is associated with an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). This rise in $[Ca^{2+}]_i$ activates Ca^{2+}-dependent Cl^- and K^+ channels, resulting in KCl efflux and cell shrinkage (Foskett, 1990). At this stage in the secretion process the volume-sensitive Cl^- channels would be inactivated by the cell shrinkage. However, when stimulation ceases, $[Ca^{2+}]_i$ returns to the resting level, whereas the Cl^- content (Baum *et al.* 1990; J. E. Melvin, unpublished observations) and cell volume (Foskett & Melvin, 1989; Nakahari *et al.* 1990) increase beyond the original steady-state values by 10–20%. This increase in cell volume would be expected to activate the volume-sensitive Cl^- channel. Thus, activation of the volume-sensitive Cl^- channels following cessation of Ca^{2+}-stimulated secretion may be important in preventing excessive cell swelling by allowing the exit of Cl^- and the osmotically obligated water.

Second, β-adrenergic stimulation upregulates the Na^+–K^+–$2Cl^-$ cotransporter in salivary acinar cells about 5-fold via a phosphorylation mechanism (Paulais &

Turner, 1992). This results in an enhanced Cl^- uptake, increasing the intracellular Cl^- content by 20–30% (J. E. Melvin & R. J. Turner, unpublished observations; Martinez, Cassity & Reed, 1988). Since Cl^- content reflects cell volume (Foskett, 1990), the predicted consequence of this increased Cl^- content is an increase of about 10% in the cell volume. It is worth noting that salivary gland fluid secretion primarily arises from an increase in the cytosolic $[Ca^{2+}]$ in response to muscarinic or α-adrenergic stimulation. β-Adrenergic stimulation, at least in the physiological range, does not alter $[Ca^{2+}]_i$, rather it increases the intracellular cAMP concentration. If the volume-activated Cl^- channels are targeted to the apical membrane, the site required for transepithelial Cl^--dependent fluid secretion, then β-adrenergic stimulated fluid secretion might be driven by Cl^- efflux via volume-sensitive channels.

In summary, we have determined some of the basic properties of the volume-sensitive Cl^- channels in an exocrine cell type. These channels have properties that suggest that they play an important role in the regulation of parotid acinar cell volume under hypotonic conditions and during fluid and electrolyte secretion.

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