J. Membrane Biol. 135, 261–271 (1993)

The Journal of
Membrane
Biology

A Ca^{2+}-Activated Cl^- Current in Sheep Parotid Secretory Cells

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Received: 9 December 1992/Revised: 23 April 1993

Abstract. Previous studies have shown that the whole-cell current-voltage (*I-V*) relation of unstimulated sheep parotid cells is dominated by two K^+ conductances, one outwardly and the other inwardly rectifying. We now show that once these K^+ conductances are blocked by replacement of pipette K^+ with Na^+ and by the addition of 5 mmol/liter CsCl to the bath, there remains an outwardly rectifying conductance with a reversal potential of 0 mV. Replacement of 120 mmol/liter NaCl in the pipette solution with an equimolar amount of Na-glutamate shifted the reversal potential of this residual current to −55 mV, indicating that the conductance was Cl^- selective. The Cl^- current was activated by increasing the free Ca^{2+} in the pipette solution from 10 to 100 nmol/liter. When the Ca^{2+} concentration in the pipette solution was 10 nmol/liter, the relaxations observed in response to membrane depolarization could be fitted with a single exponential, whose time constant increased from 81 to 183 ms as the pipette potential was increased from −30 to +60 mV. Relaxation analysis showed that the current was activated by membrane depolarization. Reversal potential measurements in experiments in which external Cl^- was replaced with various anions, gave the following relative permeabilities: SCN^- (1.80) > I^- (1.09) > Cl^- (1) > NO_3^- (0.92) > Br^- (0.75). The relative conductances were: SCN^- (2.18) > I^- (1.07) > Cl^- (1.00) > Br^- (0.91) > NO_3^- (0.50). The Cl^- current was blocked by NPPB ($ID_{50} \approx 10$ μM), DIDS (10 or 30 μmol/liter) and furosemide (100 μmol/liter).

Key words: Anion current — NPPB — DIDS — Furosemide — Whole-cell patch clamp — Sheep parotid

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Introduction

The sheep parotid is of interest for two reasons. The first is that it secretes saliva in the absence of neurohumoral stimulation (spontaneous secretion) by a mechanism that is not blocked by pharmacological agents known to block secretion by other salivary glands [27]. The second is that, although it is widely accepted that the endpieces of salivary glands secrete a fluid with a composition similar to a plasma ultrafiltrate [28], in the sheep parotid, the phosphate concentration in its endpiece lumen is ten times the arterial plasma phosphate concentration, and the chloride concentration is approximately half [4].

In whole-cell patch-clamp studies, we have shown that sheep parotid secretory cells contain two types of K^+ conductance (one outwardly and the other inwardly rectifying) in the cell membrane [15, 16] and in single channel patch-clamp studies we have found that there are two distinct types of K^+ channel that are commonly observed in the basolateral membrane [25]. Taking the whole-cell and single channel patch-clamp studies together, we have suggested that the outwardly rectifying whole-cell K^+ current is carried by 250 pS, voltage- and Ca^{2+}-activated K^+ channels and that the inwardly rectifying whole-cell K^+ current is carried by 30 pS K^+ channels, which are slightly activated by membrane hyperpolarization and are insensitive to changes in cytosolic Ca^{2+}

In the present study we have used the whole-cell patch-clamp technique to demonstrate that a Cl^- conductance is present in sheep parotid secretory cells. We show that it is activated by internal Ca^{2+} and by depolarization of the cell membrane potential and that it can be blocked by NPPB, DIDS and furosemide.

Materials and Methods

Cross-bred sheep, fed on a lucerne-oaten chaff mix (30%:70%) with water *ad libitum*, were fasted for 24 hr before the start of the experiment. They were killed with a captive-bolt pistol and the parotid glands were excised, placed in a physiological saline solution, diced, and incubated in standard NaCl bathing solution containing collagenase (0.3 mg/ml, Worthington Type II, Freehold, New Jersey) for 10 min at 37°C in a shaking water bath. The medium was then replaced with a fresh solution and the tissue incubated once more for 25–30 min. In some experiments, to facilitate single cell preparation, the tissue was incubated for 10 min in standard NaCl bathing solution containing trypsin (1 mg/ml, Difco, Detroit) before a second incubation in the collagenase solution. The tissue was dissociated by trituration, and then centrifuged and washed with the standard NaCl bathing solution. Finally, the parotid cells were filtered through 200 and 75 μm mesh Nitex screens (Allied Screen Fabrics, Sydney, Australia) and suspended in the standard NaCl bath solution. The filtrate, which contained isolated cells and small clumps of cells, was plated out onto petri dishes. Only single cells were used for whole-cell experiments.

Patch-Clamp Methods

Standard patch-clamp techniques were used [14]. Patch-clamp pipettes were pulled from borosilicate microhematocrit tubes (Modulohm, Hevik, Denmark), so as to have resistances (when filled with the standard KCl solution) of 1–2 MΩ so as to ensure good dialysis of the cell interior. The reference electrode was a Ag/AgCl electrode which was connected to the bath via an agar bridge (100 mg/10 ml) filled with the 150 mmol/liter KCl solution.

An Axopatch 1C patch-clamp amplifier (Axon Instruments, Foster City, CA) was used to measure whole-cell currents. The command voltage was generated and the whole-cell current was sampled with a MacLab4 data acquisition interface (ADInstruments, Sydney, Australia) attached to a Mac IIci computer. Whole-cell currents were analyzed on a microVAX II computer and tracings for illustrative purposes were printed out with a laser printer. Steady-state whole-cell currents were estimated by averaging the currents between 550 and 750 ms after the onset of the voltage-pulse. This procedure has the effect of underestimating the true steady-state whole-cell currents in situations when the whole-cell current had not stabilized after 550 ms. Since this only occurred during the most extreme depolarizations, it has no effect on the estimates of reversal potentials but may produce underestimation of conductances at voltages more positive than +60 mV. The series resistance, which averaged 23.1 MΩ ± 1.0 (n = 66), was not electronically compensated during the experiments. Outward current, defined as positive charge leaving the pipette, has been indicated as an upward deflection in all traces, and potential differences are reported as pipette potential with respect to bath potential. The pipette potential was corrected for the liquid junction potentials between the pipette solution and the external solution, and between the external solution and different test solutions as described by Barry and Lynch [3]. All experiments were performed at about 20°C.

Two criteria were used to reject cells as having an abnormally large leakage conductance: (i) a sudden increase in the holding current and (ii) if the reversal potential of the current was not close to the Cl^- equilibrium potential, E_{Cl} (−50 mV), with standard pipette and bath solutions, i.e., in the range −70 to −30 mV. This last criterion did not result in the rejection of any whole cells which would not also have been rejected by the first criterion.

Except where otherwise indicated, the total pipette Cl^- concentration was 22 mmol/liter and the Cl^- concentration in the bath solution was 159.4 mmol/liter.

Analysis of Relaxation

Most relaxations were fitted with a single exponential using a least-squares method. In order to permit capacitive transients to die out, only data collected more than 20 ms after the potential change were used for fitting the exponentials. The amplitude of the relaxation was defined as the ratio of the steady-state current (estimated as the asymptote of the exponential) to the initial current immediately following the potential change (estimated by extrapolation of the exponential to the time of the potential change). We have assumed that the relaxation amplitude, as defined here, equals the ratio of the fraction of channels open at the test potential to the fraction of channels open at the holding potential (*cf.* ref. [9]) and hence that it is proportional to the channel open probability.

Solutions and Chemicals

The compositions of the standard bath and pipette solution were as follows. The pipette solution (pH 7.4) contained (in mmol/liter): NaCl (20), Na-glutamate (120), $MgCl_2$ (1.0), H-HEPES (10), EGTA (5) and glucose (10). The bathing solution (pH 7.4) contained (in mmol/liter); NaCl (145), KCl (5), CsCl (5), $CaCl_2$ (1), $MgCl_2$ (1.2), NaH_2PO_4 (1.2), Na-HEPES (7.5), H-HEPES (7.5) and glucose (10). Chemicals employed were of AR grade or higher. 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) was the gift of Professor R. Greger (Freiburg).

The free Ca^{2+} concentrations of the pipette solution were varied between 10^{-7} and less than 10^{-9} mol/liter using 5 mmol/liter EGTA as a Ca^{2+} buffer. The free concentrations of Ca^{2+} were calculated from an equation which takes into account the concentrations of Mg^{2+}, Ca^{2+}, EGTA (96% purity) and pH [20].

Statistics

Results are reported as the mean ± SEM, with n, the number of observations in parentheses.

Results

Effect of Replacement of K^+ with Na^+

We have shown previously that sheep parotid cell membranes contain two major K^+ conductances when the pipette contained an isotonic KCl solution and the bath contained an isotonic NaCl solution [16]. Thus, to isolate any Cl^- currents, we suppressed the K^+ currents by substituting Na^+ for K^+ in the pipette and adding 5 mmol/liter CsCl to the bath solution. The Cl^- concentrations in the bath and pipette solutions were 159 mmol/liter and 142

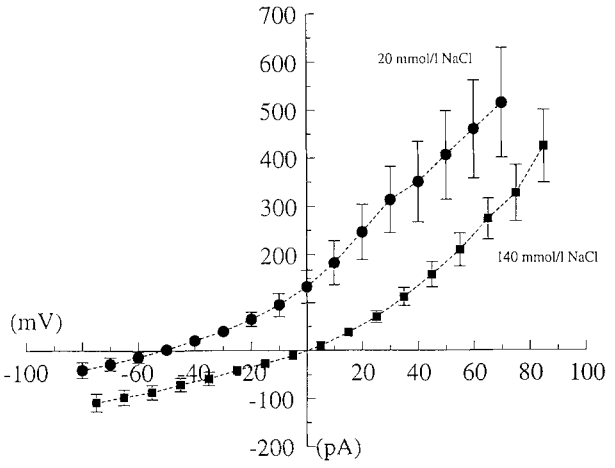

Fig. 1. Steady-state *I-V* relations of single sheep parotid secretory cells held in the whole-cell configuration with either the control Cl^--rich pipette solution (filled squares, mean of seven experiments) or a pipette solution in which all but 20 mmol/liter Cl^- was replaced with glutamate (filled circles, mean of nine experiments). In each case, the pipette solution contained Na^+ rather than K^+, and Cs^+ was present in the bath solution (*see* Materials and Methods). The cells were held at −60 mV between test voltage pulses. The voltage pulses were of 800 ms duration. Each bar represents the SEM but when this was so small as to lie within the symbols, it has been omitted.

mmol/liter, respectively. In cells studied in the whole-cell configuration under these conditions, an outwardly rectifying whole-cell current having a reversal potential close to 0 mV was observed (Fig. 1). The reversal potential of this current suggests it must have been carried by Na^+or Cl^- ions. In other experiments in which CsCl was substituted for KCl in the pipette solution, we obtained similar results [15]. To determine the ion selectivity of this current, we examined the effect of the replacement of NaCl in the pipette solution with Na-glutamate on the *I-V* relation. When 120 mmol/liter NaCl in the pipette solution was replaced by an equimolar amount of Na-glutamate, the reversal potential of the current shifted to −55.8 mV ± 5.1 (n = 9). Since the Nernst potential for Cl^- under these conditions was −50 mV, these results suggest that the current is selective for Cl^- and that there is no significant permeability to Na^+ ions (Fig. 1).

Effect of Different Ca^{2+} Concentrations on the Cl^- Currents

Figure 2 (*A*, *C* and *E*) shows typical recordings of the Cl^- currents evoked by voltage jumps when the pipette solution contained either 10^{-8} mol/liter, or 3×10^{-8} or 10^{-7} mol/liter Ca^{2+}, respectively. We were not able to test free Ca^{2+} higher than 10^{-7} mol/liter because the whole-cell voltage-clamp became unstable, presumably because the buffering capacity of the EGTA in the pipette solution was inadequate (*cf.* [9]). At each Ca^{2+} concentration tested, a slow outward relaxation was observed in response to positive voltage jumps. The amplitude of the current increased without any accompanying shift of the reversal potential as the free Ca^{2+} concentration in the pipette solution was increased. The *I-V* relations corresponding to the three different Ca^{2+} concentrations are shown in Fig. 2*B*, *D* and *F*. The whole-cell current was almost zero at E_{Cl} (−50 mV), suggesting that the time-dependent currents were mainly carried by Cl^- ions. When the pipette solution contained 10^{-7} or 3×10^{-8} mol/liter Ca^{2+}, the outward current evoked by membrane depolarization showed an almost linear increase with time. This linear increase in outward current is not attributable to the increased pipette Ca^{2+} because we also observed it in experiments in which extracellular Cl^- had been replaced with SCN^- when the pipette solution contained 10^{-8} mol/liter Ca^{2+} (*see* Fig. 6). Thus, the phenomenon is probably attributable to anions from the extracellular solution entering the cell in response to the depolarization and then interacting with the channel so as to alter its kinetics. A similar phenomenon has been observed in rat lacrimal secretory cells, where it has also been attributed to an interaction of anions with the cytosolic face of the channel [9]. Figure 3 shows the whole-cell *I-V* relations at the four different Ca^{2+} concentrations we used. In all subsequent experiments, we used pipette solutions containing 10^{-8} mol/liter free Ca^{2+}.

Kinetic Properties of the Ca^{2+}-Induced Cl^- Currents

Figure 4*A* shows the plot of the 'on' relaxation time constant (τ) as a function of membrane potential when the Ca^{2+} concentration in the pipette was 10^{-8} mol/liter: τ increased from 81.0 ms ± 22.6 (n = 3) to 183.4 ms ± 17.4 (n = 6) as the test potential was increased from −30 to +60 mV. A plot of the open probability of the channels carrying the Cl^- current, estimated from the amplitude of the relaxation (*see* Materials and Methods and [9]), as a function of membrane potential is shown in Fig. 4*B*. The estimate of open probability was normalized to a value of 1 at +40 mV. The relative open probability at −20 mV was 0.65 ± 0.03 (n = 6) and it increased to 0.94 ± 0.02 (n = 6) at +30 mV. The estimated open probability did not increase at voltages more positive than +40 mV. These results show that the open probability of the Cl^- current is increased by membrane depolarization. It was not possible to per-

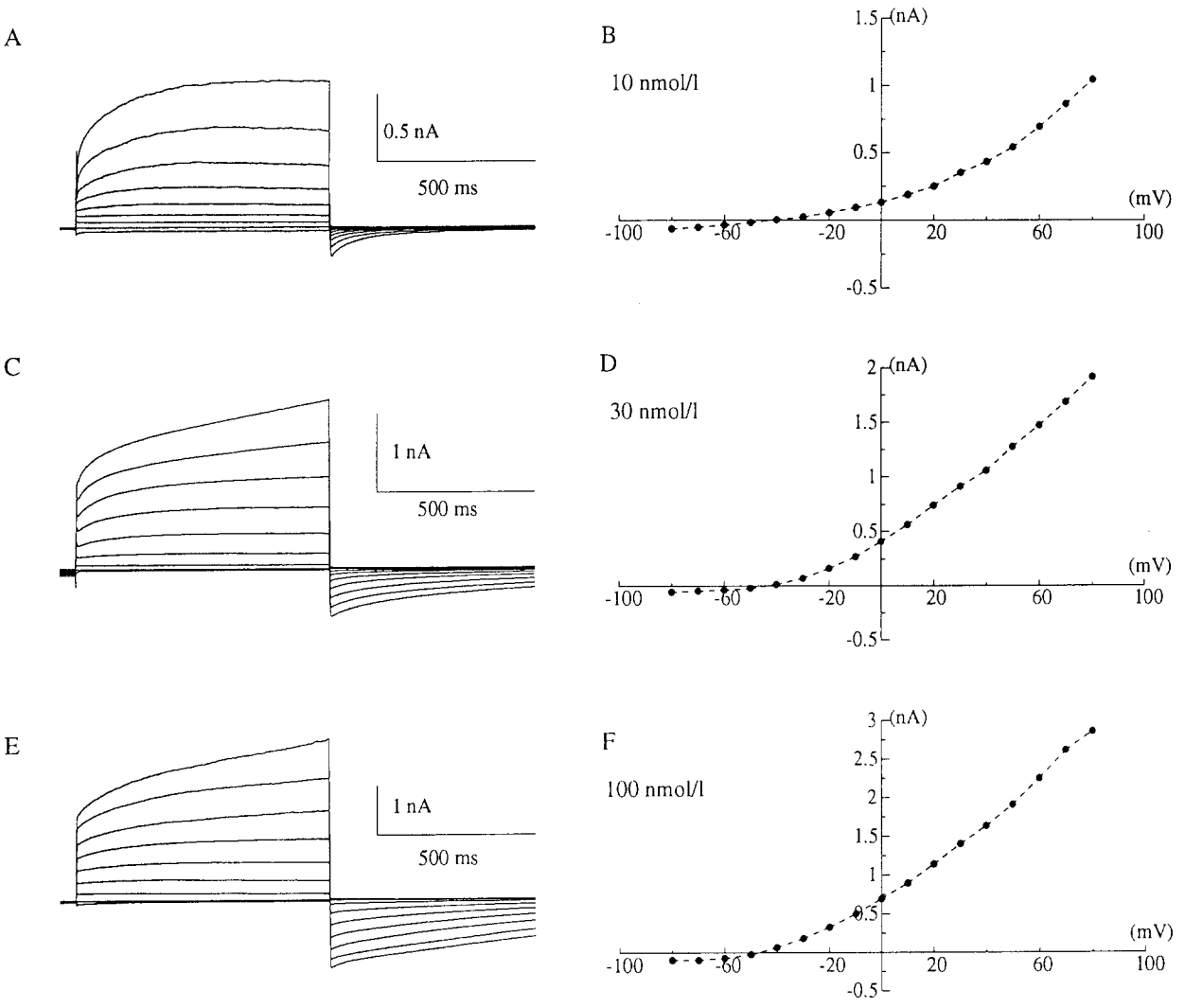

Fig. 2. Whole-cell current responses to voltage steps and the steady-state current-voltage relations of single sheep parotid secretory cells with free Ca^{2+} concentrations in the pipette solution of 10 nmol/liter (*A* and *B*), 30 nmol/liter (*C* and *D*) and 100 nmol/liter (*E* and *F*). The current responses are to voltage steps from the resting potential of −60 mV to test potentials of −80, −60, −40, −20, 0, 20, 40, 60, 80 and 100 mV (*C* only).

form this analysis at pipette free Ca^{2+} concentrations greater than 10^{-8} mol/liter because of the linear component to the relaxation response which was present (*see above*).

Ion Selectivity of the Cl^- Conductance

In order to determine the selectivity of the whole-cell Cl^- conductance for various anions, we substituted all but 14 mmol/liter of the Cl^- in the bathing solution with other monovalent anions. In experiments where NaCl in the bath solution was replaced with $NaNO_3$, scarcely any change in reversal potential was observed (Fig. 5*B*), but there was a marked reduction in both the outward current and the inward current (Fig. 5*A* and *B*). In four experiments, NO_3^- substitution reduced the outward current at +80 mV and the inward current at −80 mV to 55.5% ± 6.1 ($n = 4$) and 49.8% ± 8.0 ($n = 4$) of the control values, respectively. Replacement of NaCl in the bath solution by NaSCN shifted the reversal potential in the negative direction and increased both the outward and inward currents (Fig. 6). Replacement of NaCl in the bath solution by NaBr shifted the reversal potential in the positive direction and decreased both the outward and inward currents (Fig. 7). Finally, replacement of NaCl in the bath solution by NaI had little effect on the reversal potential or the amplitudes of the inward and the outward currents (Fig. 8).

The Table summarizes the shifts in reversal potential observed following anion substitution. We

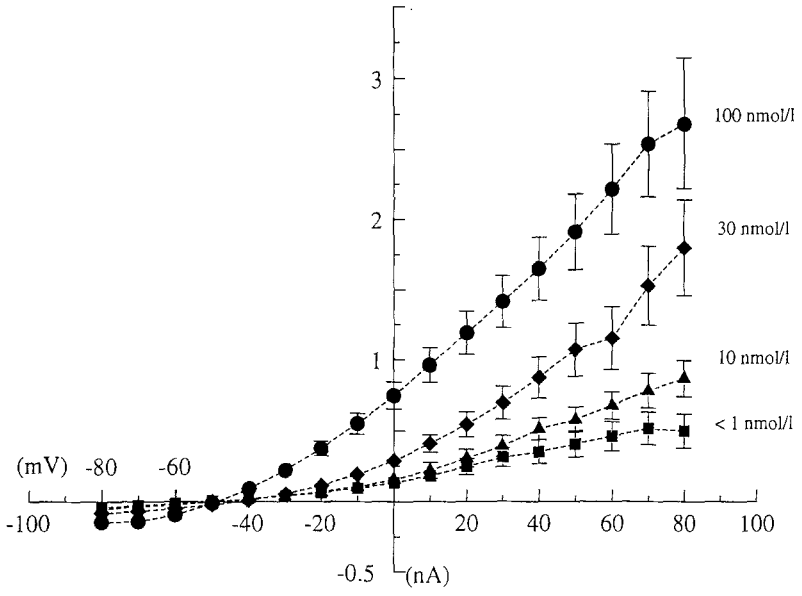

Fig. 3. Steady-state *I-V* relations of single sheep parotid cells with differing free Ca^{2+} concentrations in the pipette solution: <1 nmol/liter (filled squares, mean of nine experiments), 10 nmol/liter (filled triangles, mean of eight experiments), 30 nmol/liter (filled diamonds, mean of seven experiments) and 100 nmol/liter (filled circles, mean of nine experiments). Each bar represents the SEM.

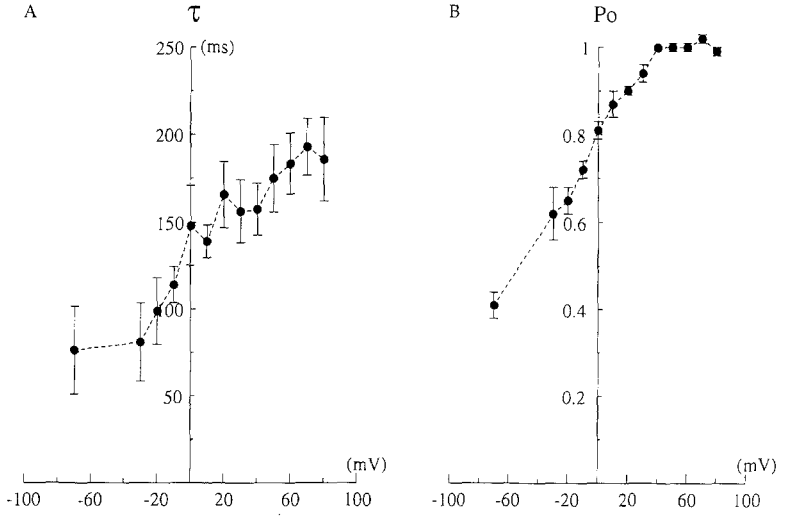

Fig. 4. Dependence of the relaxation time constant (*A*) and open probability relative to that at +40 mV (*B*) of the whole-cell Cl^- current measured by voltage pulses from a resting potential of −60 mV. The open probability was assumed to be equal to the amplitude of the current relaxation and was normalized to a value of 1 at +40 mV (*see* Materials and Methods). The NaCl pipette solution contained 10 nmol/liter free Ca^{2+} and the bath 5 mmol/liter Cs^+. Each point is the mean of 3–6 experiments.

used the magnitude of the shift in reversal potentials recorded with different anions to calculate the permeability of the test anion (X^-) relative to that of Cl^- (P_X/P_{Cl}). In this calculation, we have assumed that the currents measured under the conditions of the experiment were carried solely through Cl^- channels. The sequence of the relative permeabilities was: SCN^- (1.80) > I^- (1.09) > Cl^- (1) > NO_3^- (0.92) > Br^- (0.75). When the chord conductances between the reversal potential and +80 mV were calculated, the sequence of the relative conductances was: SCN^- (2.18) > I^- (1.07) > Cl^- (1) > Br^- (0.91) > NO_3^- (0.50).

Table. Effect of Cl^- replacement by different anions on current reversal potential

Anion	ΔE_{rev} (mV)	P_X/P_{Cl}	*n*
Br^-	−6.38 ± 1.33	0.75	4
I^-	2.04 ± 1.05	1.09	4
NO_3^-	−1.95 ± 1.98	0.92	4
SCN^-	13.82 ± 3.49	1.80	4

Results are means ± SEM of the number of experiments shown. P_X/P_{Cl} values were calculated assuming the anions to be the only permeant ions from the Goldman-Hodgkin-Katz equation. ΔE_{rev} is defined as the reversal potential in Cl^- minus the reversal potential in other anions.

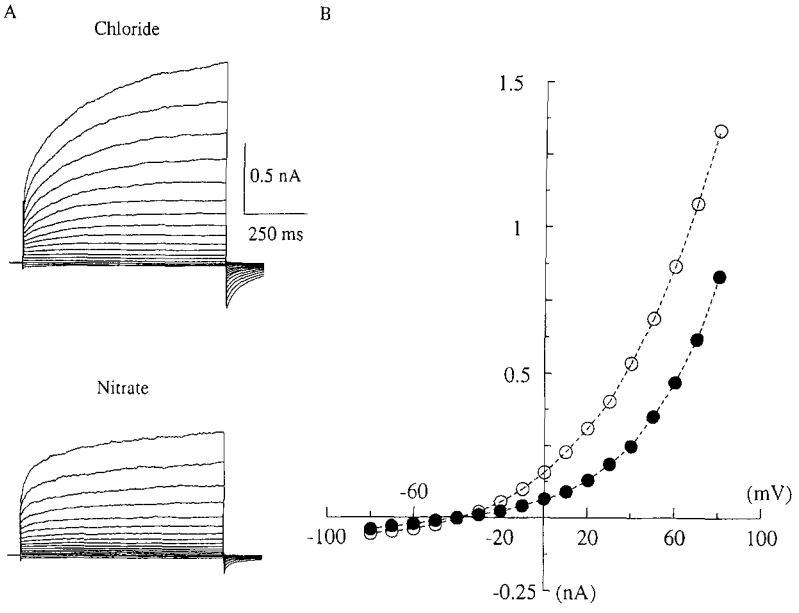

Fig. 5. (*A*) Whole-cell current responses of single sheep parotid secretory cells to test voltage pulses stepped from −60 mV to potentials ranging between −80 and +80 mV, prior to and following substitution of extracellular Cl^- with NO_3^-. (*B*) Steady-state *I-V* relations prior to and following substitution of extracellular Cl^- (open circles) with NO_3^- (filled circles). The data are derived from the experiment shown in *A*. The pipette solution contained 10 nmol/liter free Ca^{2+} and the bath 5 mmol/liter Cs^+.

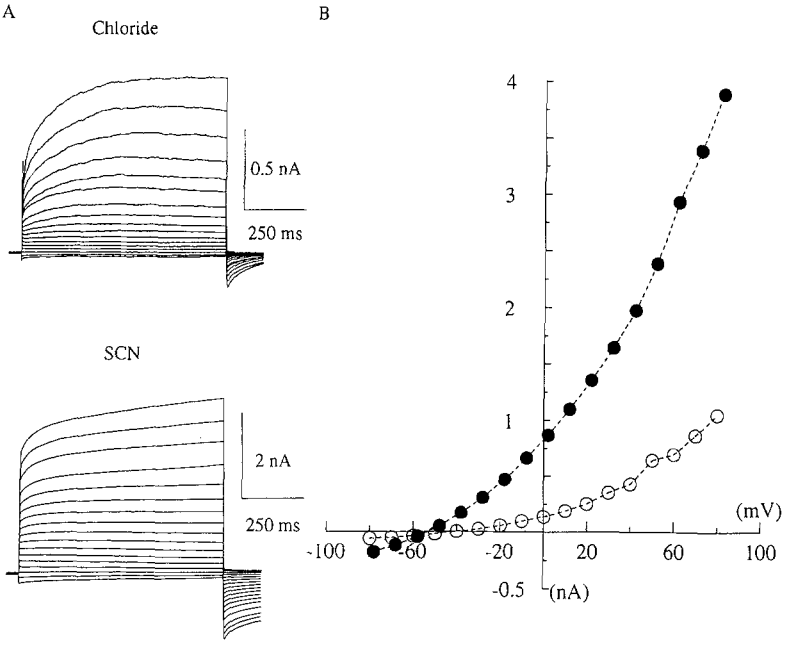

Fig. 6. (*A*) Whole-cell current responses of single sheep parotid secretory cells prior to and following substitution of extracellular Cl^- by isothiocyanate. (*B*) Steady-state *I-V* relations prior to and following substitution of extracellular Cl^- (open circles) with isothiocyanate (filled circles). The data are derived from the experiment shown in *A*. The experimental protocol is described in the legend to Fig. 5.

Effect of Cl^- Channel Blockers

To characterize the Cl^- current in sheep parotid cells pharmacologically, we first examined the effect of NPPB. Figure 9*A* and *B* show the representative tracings and current-voltage relations before and after addition of 10 μmol/liter NPPB. The blocker reduced the Cl^- current amplitude evoked by membrane depolarization (to +80 mV) to 45.9% ± 8.8 (n = 4) of the control value. The inhibitory effect of NPPB was more pronounced at a concentration of 30 μmol/liter, when the current at +80 mV was reduced to 27.3% ± 7.8 (n = 4) of the control value. Figure 9*C* shows the dose-response relation of the NPPB-induced change in whole-cell Cl^- current. The inhibitory effect of NPPB on the Cl^- current evoked by membrane depolarization from −60 to +80 mV was dose dependent over the range 10 to 300 μmol/liter, the effect being half-maximal at about 10 μmol/liter. In a single experiment with 0.3 mmol/liter NPPB, 94% inhibition was observed. This result suggests that the greater part of the current is carried through NPPB-blockable Cl^- channels.

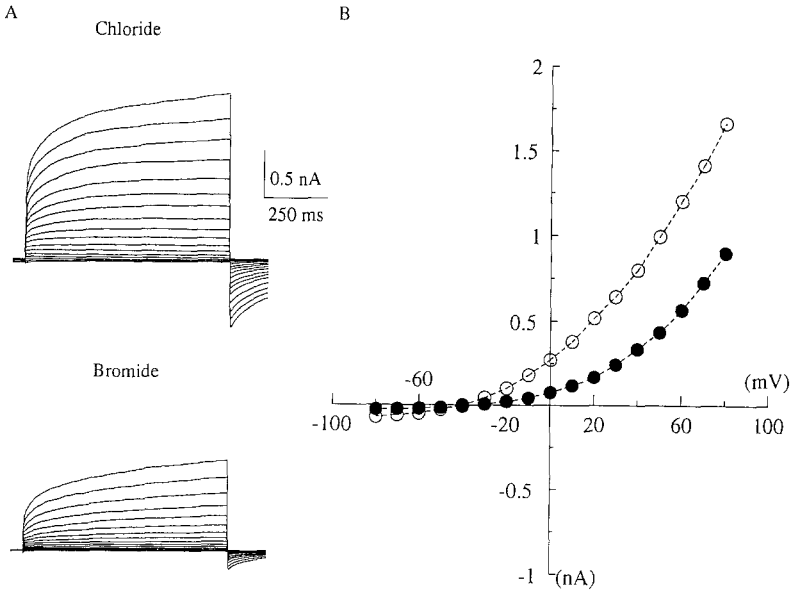

Fig. 7. (*A*) Whole-cell current responses of single sheep parotid secretory cells prior to and following substitution of extracellular Cl^- with Br^-. (*B*) Steady-state *I-V* relations prior to and following substitution of extracellular Cl^- (open circles) with Br^- (filled circles). The data are derived from the experiment shown in *A*. The experimental protocol is described in the legend to Fig. 5.

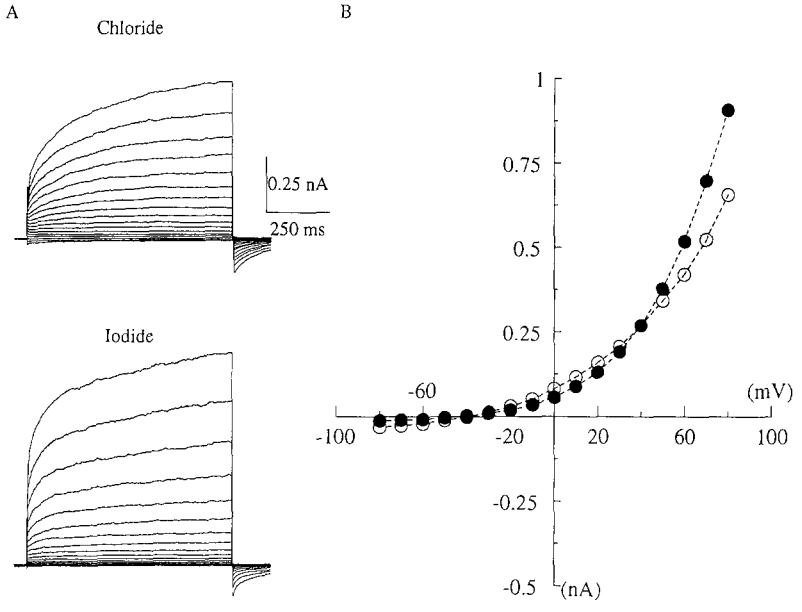

Fig. 8. (*A*) Whole-cell current responses of single sheep parotid secretory cells prior to and following substitution of extracellular Cl^- with I^-. (*B*) Steady-state *I-V* relations prior to and following substitution of extracellular Cl^- (open circles) with I^- (filled circles). The data are derived from the experiment shown in *A*. The experimental protocol is described in the legend to Fig. 5.

We also tested DIDS and furosemide on the Cl^- currents. Figure 10*A* and *B* shows representative tracings before and after the addition of DIDS (30 μmol/liter) and furosemide (100 μmol/liter), respectively. The addition of DIDS to the bathing solution caused a marked reduction in the whole-cell Cl^- currents. At 10 μmol/liter, DIDS reduced the current at +80 mV to 62.0% ± 3.4 (n = 4) of the control level. The inhibitory effect was more pronounced at a higher concentration of DIDS (30 μmol/liter), which reduced the current at +80 mV to 29.8% (n = 2) of the control value. Furosemide (100 μmol/liter) also inhibited the Cl^- current. At a concentration of 0.1 mmol/liter, furosemide reduced the current at +80 mV to 45.4% ± 6.6 (n = 3) of the control value and in a single experiment with a concentration of 300 μmol/liter, we observed 84% inhibition at +80 mV.

Discussion

Our studies show that the secretory cells of the sheep parotid gland contain a Ca^{2+}- and voltage-activated

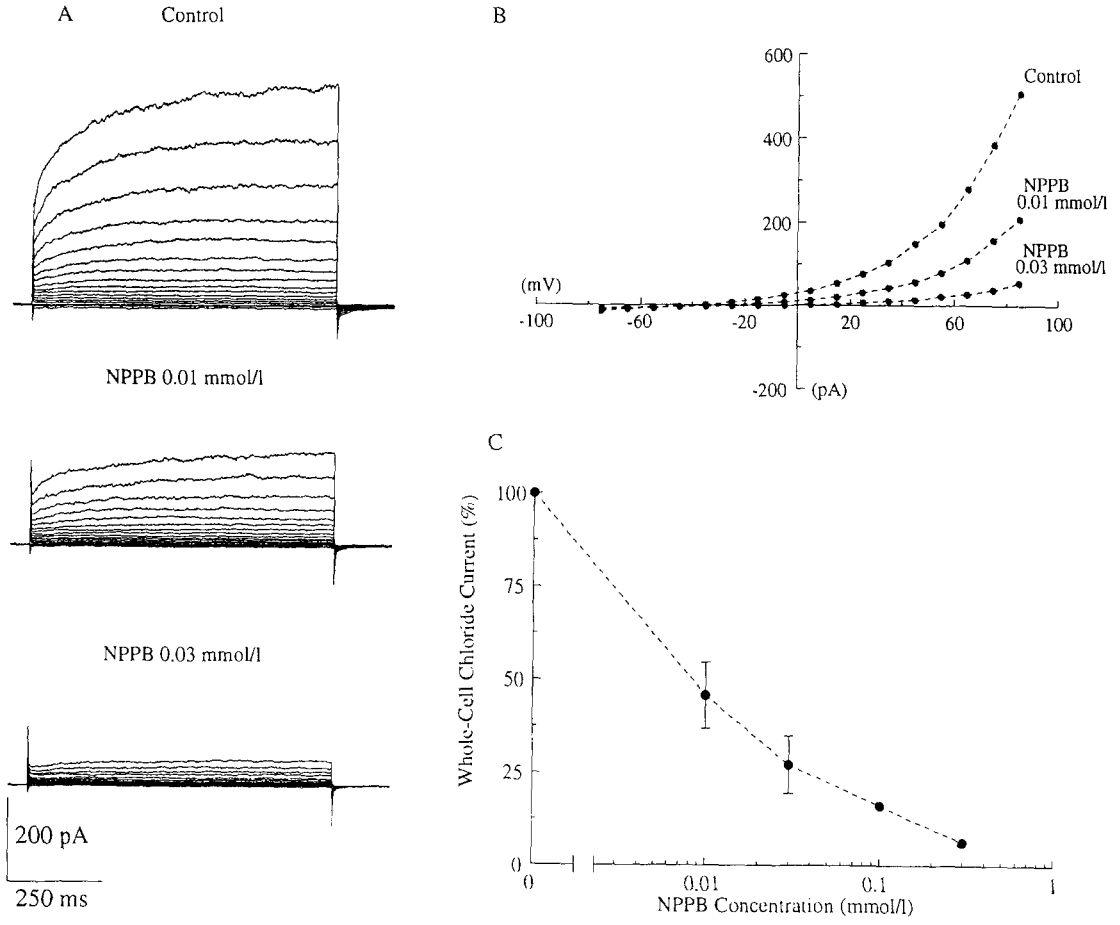

Fig. 9. (*A*) Whole-cell current responses of single sheep parotid secretory cells prior to and following two doses of NPPB given extracellularly. (*B*) Steady-state whole-cell *I-V* relations derived from the experiment shown in *A*. (*C*) Dose-response curve for the reduction of outward current at +80 mV produced by NPPB. The pipette solution contained 10 nmol/liter free Ca^{2+} and the bath 5 mmol/liter Cs^+.

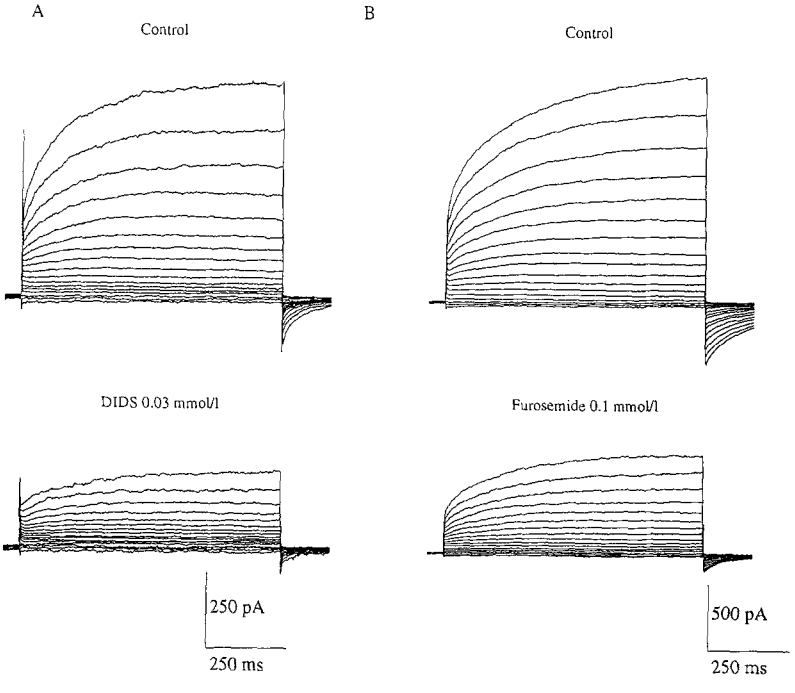

Fig. 10. (*A*) Whole-cell current responses of single sheep parotid secretory cells to test voltage pulses from −60 mV to test potentials ranging between −80 and +80 mV, prior to and following the addition of 30 μmol/liter DIDS to the bath solution. (*B*) Whole-cell current responses of single sheep parotid secretory cells to test voltage pulses from −60 mV to test potentials ranging between −80 and +80 mV, prior to and following the addition of 100 μmol/liter furosemide to the bath solution. The pipette solution contained 10 nmol/liter free Ca^{2+} and the bath 5 mmol/liter Cs^+.

Cl^- conductance. The view that the whole-cell currents described in this paper can be attributed to Cl^- channels is supported by the observations that: (i) glutamate substitution for Cl^- shifted the reversal potential of the whole-cell current from 0 to −55 mV and (ii) that known Cl^- channel blockers such as NPPB, DIDS and furosemide inhibit the current. It is not yet known which membrane domain contains these Cl^- channels, although the currently accepted model of secretion in epithelia predicts that the Cl^- channels should be in the apical membrane [5].

The properties of the Cl^- conductance distinguish it from the Cl^- conductances described in other epithelia. The Cl^- conductance associated with CFTR in several cell types is neither sensitive to intracellular Ca^{2+} [1] nor to membrane potential [26]. Furthermore, the ion selectivity of CFTR (Br^- (1.1) = Cl^- (1) > I^- (0.6)) [2], and its insensitivity to DIDS and NPPB [8] also distinguish it from the Cl^- conductance in sheep parotid secretory cells.

Like the Cl^- conductance in the sheep parotid, the small conductance Cl^- channel in pancreatic ducts is sensitive to NPPB, but can be distinguished from it by insensitivity of the channel in pancreatic ducts to DIDS and by its anion selectivity (NO_3^- (1.73) > Br^- (1.2) > I^- (1.0)) [12]. The intermediate conductance outwardly rectifying Cl^- channels that are found in many secretory epithelia [11, 13] are sensitive to both NPPB and DIDS [23] and in some reports at least are activated by depolarization [17] but are distinguishable from the Cl^- conductance in sheep parotid secretory cells on the basis of their insensitivity to cytosolic Ca^{2+} [17] and their ion selectivity (SCN^- (2.3) > I^- (1.75) > NO_3^- (1.4) > Br^- (1.2) > Cl^- (1)) [18].

It would be expected that the Cl^- conductance in sheep parotid cells would be closely related to the Ca^{2+}-activated Cl^- conductances in the secretory cells of salivary and lacrimal glands. The best characterized of these is in the secretory cells of the rat lacrimal gland [9, 19]. Like the Cl^- conductance in the sheep parotid, the Ca^{2+}-activated Cl^- conductance in rat lacrimal secretory cells is activated by depolarization and blocked by furosemide [10]. There is also indirect evidence to suggest that it is blocked by DIDS [22], but its sensitivity to NPPB has not been published. Its kinetics even has the same dependency on the anion composition of the bathing media [9] as we have observed in the sheep.

The Cl^- conductance in the rat lacrimal differs in several respects from that described in the present paper. Its ion selectivity: I^- (2.7) > NO_3 (2.4) > Br^- (1.6) > Cl^- (1) [9] is quite different and its sensitivity to Ca^{2+} is less—the Cl^- conductance in rat lacrimal cells only becomes active when the cytsolic free Ca^{2+} exceeds 500 nmol/liter [9], a level higher than that required to activate the Cl^- conductance in the sheep parotid. Although some of this difference in Ca^{2+} sensitivity may be attributable to EGTA failing to control adequately the cytosolic free Ca^{2+} in our experiments (*see* Results and ref. [9]), the magnitude of the discrepancy in Ca^{2+} sensitivity suggests that this is a genuine difference between the Cl^- channels in these two tissues. The Cl^- conductance in sheep parotid cells is also approximately 10 times more sensitive to furosemide than is that in rat lacrimal cells [10]. These differences are not unique to the rat lacrimal—the limited information available on Ca^{2+}-activated Cl^- conductances found in two other secretory epithelia, rat pancreatic acini [26] and human airway epithelia [21], indicates that these are similar to that found in the rat lacrimal gland. The Cl^- conductance in rat pancreatic acinar cells, like that in rat lacrimal cells, appears only to be activated at levels of intracellular Ca^{2+} of 1 μmol/liter and above [21]. The Ca^{2+}-activated Cl^- conductance in airway epithelial cells, like the Cl^- conductance in sheep parotid cells, is inhibitable by DIDS [26], but has a different anion selectivity (I^- (1.7) > Cl^- (1)) [26]. Thus, although the Cl^- channels in sheep parotid cells may be related to the Ca^{2+}-activated Cl^- channels found in secretory epithelia such as the rat lacrimal gland, they exhibit significant differences from them. These differences may contribute to the unusual properties of the secretion process in the sheep parotid.

It is difficult to ascribe a physiological role to the Cl^- currents observed in our study without further information on the membrane domain in which the Cl^- channels are localized. An apical location would be in accordance with the most widely accepted model of salivary secretion [5], that based on secondary active uptake of Cl^- across the basolateral membrane with passive efflux of Cl^- across the apical membrane into the lumen. A basolateral location, on the other hand, would be in accordance with the model proposed by Marty et al. for the lacrimal gland [19], in which Cl^- uptake across the basolateral membrane is via Cl^- channels and is driven by the depolarizing effects of basolateral, nonselective cation channels. Applying this model to the sheep parotid, however, has the difficulty that unlike many other salivary secretory cells [6], the secretory cells of the sheep parotid do not appear to have significant numbers of nonselective cation channels [16, 25]. Whichever membrane domain contains the Cl^- channels, it seems reasonable to postulate that the Ca^{2+}-activated Cl^- current plays an important role in supporting agonist-evoked fluid secretion as it does in many other exocrine glands [24] because increases in cytosolic Ca^{2+} concentration induced by muscarinic agonists have been reported in sheep parotid secretory cells [7]. It may also be of impor-

tance that the resting cytosolic Ca^{2+} in sheep parotid secretory cells is approximately 100 nmol/liter, a concentration at which the Cl^- conductance is substantially activated and hence available to support the spontaneous secretion that is characteristic of unstimulated sheep parotid cells.

Since Wright and coworkers [27] have demonstrated that spontaneous secretion by sheep parotid glands is not blocked by infusion of furosemide and DIDS into the arterial blood, one might expect that the Ca^{2+}-activated Cl^- channels reported in the present study would not have an important role to play in spontaneous secretion. It is worth noting, however, that in Wright's studies, the furosemide concentration in the saliva rose only to $2–3 \times 10^{-7}$ mol/liter even when the concentration in blood was as high as 10^{-4} mol/liter [27]. If we assume that the Cl^- channels are located in the apical membrane, it seems reasonable to speculate that the furosemide concentration in the saliva in Wright's studies was not sufficient to block a Cl^- conductance of the type we observed in the present study.

This project was supported by the Australian Research Council. We thank Professor J.A. Young for his critical reading of the manuscript, Professor R. Greger (Physiologisches Institut, Freiburg, FRG) for providing NPPB and Drs. N. Sangster and J. Rothwell and Mr. R. Murphy for giving us access to their sheep.

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