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ORIGINAL ARTICLE

J.P. Winpenny · C.J. Mathews · B. Verdon
C.J.C. Wardle · J.A. Chambers · A. Harris
B.E. Argent · M.A. Gray

Volume-sensitive chloride currents in primary cultures of human fetal vas deferens epithelial cells

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Abstract Using the patch-clamp technique, we have identified a large, outwardly rectifying, Cl^--selective whole-cell current in primary cultures of human vas deferens epithelial cells. Whole-cell currents were time- and voltage-dependent and displayed inactivation following depolarising pulses ≥ 60 mV. Currents were equally permeable to bromide (P_{Br}/P_{Cl} = 1.05 ± 0.04), iodide (P_I/P_{Cl} = 1.06 ± 0.07) and Cl^-, but significantly less permeable to gluconate (P_{Gluc}/P_{Cl} = 0.23 ± 0.03). Currents spontaneously increased with time after establishing a whole-cell recording, but could be inhibited by exposure to a hypertonic bath solution which reduced inward currents by 68 ± 4%. Subsequent exposure of the cells to a hypotonic bath solution led to a 418 ± 110% increase in inward current, indicating that these currents are regulated by osmolarity. 4,4′-Diisothiocyanatostilbene-2,2′-disulphonic acid (100 μM) produced a rapid and reversible voltage-dependent block (60 ± 5% and 10 ± 7% inhibition of current, measured at ± 60 mV, respectively). Dideoxyforskolin (50 μM) also reduced the volume-sensitive Cl^- current, but with a much slower time course, by 41 ± 13% and 32 ± 16% (measured at ± 60 mV, respectively). Tamoxifen (10 μM) had no effect on the whole-cell Cl^- current. These results suggest that vas deferens epithelial cells possess a volume-sensitive Cl^- conductance which has biophysical and pharmacological properties broadly similar to volume-sensitive Cl^- currents previously described in a variety of cell types.

Key words Vas deferens · Patch-clamp · Whole-cell · Volume-sensitive Cl^- current

J.P. Winpenny (✉) · C.J. Mathews · B. Verdon
B.E. Argent · M.A. Gray
Department of Physiological Sciences, University Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK

C.J.C. Wardle · J.A. Chambers · A. Harris
Paediatric Molecular Genetics, Institute of Molecular Medicine, University of Oxford, The John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK

C.J. Mathews
Department of Physiology, McGill University, McIntyre Medical Sciences Building, Room 1026, 3655 Drummond Street, Montreal, QC, H3G 1Y6, Canada

Introduction

The vas deferens forms the distal part of the excurrent duct system of the male reproductive tract and receives fluid and spermatozoa from the epididymis and transmits them distally to the urethra. Sperm maturation takes place within the excurrent duct system and one function of the epithelia that line the epididymis and vas deferens is to create the correct luminal environment for this process to occur. Although it is well established that the epididymis acts as an ion-transporting epithelium [4, 5, 18], little is known about the ion-transporting properties of the vas deferens epithelium and, in particular, the various ion channels that are involved. Recent work from this laboratory has identified a large-conductance maxi-K^+ channel on the apical surface [32] and several Cl^- conductances regulated by adenosine 3′,5′-cyclic monophosphate (cAMP), calcium and osmolarity [19, 20] in vas deferens epithelial cells. We have suggested that the maxi-K^+ channel may be involved in producing the high potassium concentration found in the luminal fluid of the vas deferens, by forming a pathway for secretion of potassium into the lumen. The high potassium concentration serves an important physiological function as it inhibits sperm motility and helps maintain sperm quiescent during storage [32].

In order to maintain the correct luminal environment for the maturation and storage of sperm, the vas deferens epithelial cells undergo active transepithelial transport of osmotically active solutes (e.g. electrolytes and various organic compounds) [13, 29]. The vas

deferens epithelial cells are also known to accumulate various organic solutes, e.g. carnitine and inositol [13, 29]. Any entry of osmotically active solutes into the epithelial cells across the basolateral membrane may lead to an increased osmotic pressure and cell swelling. It would, therefore, be critical for these cells to have some mechanism to counter these osmotic forces and hence limit any volume change that may occur as a result of osmolyte accumulation. In many cells a reduction in cell volume, a process termed regulatory volume decrease (RVD), is achieved by the activation of Cl^- and K^+ conductances and the loss of KCl from cells. Separate Cl^- conductances, which are activated by changes in cellular osmolarity, have been reported in a variety of epithelial cells, including rat and human epididymal [4, 5], rat pancreatic duct [39], rat parotid acinar [1], ciliary [42], human airway [3, 9, 21, 27, 28], intestinal [17, 33, 41] and frog proximal tubule [30, 31] epithelial cells. Furthermore, a link between these Cl^- conductances and RVD has been identified in human epididymal [5], human small intestinal [17] and frog proximal tubule [30, 31] cells. In these cells, RVD is prevented by the presence of blockers of the volume-sensitive Cl^-conductance. In addition, recent reports of studies using MDCK [2] and other epithelial cells [5, 14, 16] have shown that volume-sensitive anion channels have a significant permeability to amino acids. This suggests that these channels may also serve another functional role as a pathway for amino acid transport (for a review see [34]). Presumably a pathway for the movement of these organic osmolytes could also have a role in the RVD process.

In this study we have identified a whole-cell Cl^- conductance regulated by cell volume in primary cultures of human fetal vas deferens epithelial cells, whose biophysical characteristics are broadly similar to volume-sensitive Cl^- conductances previously reported in relation to other epithelia. To our knowledge this is the first report of such a volume-sensitive Cl^- conductance in these cells. Some of our observations have been published in preliminary form [20].

Materials and methods

Human vas deferens cell culture

Primary monolayers of vas deferens epithelial cells were grown in Primaria plastic flasks from explants of mid-trimester human fetal vasa deferentes, as previously described [11]. Four normal fetuses were obtained within 48 h of prostaglandin-induced termination. Cultures of cells consist of two cell types; a large angular cell that does not appear tightly packed even in confluent areas, and a relatively small "cobblestone" cell that always appears in tightly packed colonies. Both cell types have been identified as epithelial on the basis of morphological, biochemical and immunocytochemical evidence [11].

The cultures were passaged onto glass coverslips (passage numbers between 2 and 4) at Oxford, and 2–4 days later sent from Oxford to Newcastle upon Tyne. After arrival in Newcastle upon Tyne, they were incubated for 1–6 days at 37 °C in the standard growth medium [11] minus cholera toxin before electrophysiological studies were performed.

Electrophysiology

Coverslips were transferred to a tissue bath (volume 1.5 ml) mounted on a Nikon Diaphot inverted microscope and viewed using phase-contrast optics. Current recordings were made at 21–23°C from the upper surface of cells using the whole-cell configuration of the patch-clamp technique, as previously described [39, 40]. Pipettes were pulled from borosilicate glass (Clarke Electromedical, UK) and had resistances, after fire-polishing, of 3–6 MΩ measured using the solutions described in "Solutions and Chemicals". Seal resistances were > 10 GΩ.

Whole-cell currents were recorded with an EPC-7 patch-clamp amplifier (List Electronic, Darmstadt, Germany) using two basic voltage-clamp protocols.

1. During continuous recording, the membrane potential (V_m) was held at 0 mV and then alternately clamped to ± 60 mV for 1 s. There was a 1-s interval at the holding potential between each pulse.
2. To obtain current/voltage (I/V) relationships, V_m was held at 0 mV and then clamped over the range ± 100 mV in 20-mV steps. Each voltage step lasted 500 ms, and there was an 800-ms interval at the holding potential between steps. Data were filtered at 1 kHz and sampled at 2 kHz with a Cambridge Electronic Design 1401 interface (CED, Cambridge, UK) and stored on either a digital tape recorder or the computer hard disk.

I/V plots were constructed using either the average initial whole-cell current, measured over a 4-ms period starting 7 ms into the voltage pulse, or the average plateau whole-cell current measured over a 4-ms period starting 495 ms into the voltage pulse. The currents were not leak corrected. Series resistance (R_s) was typically three times the pipette resistance, and R_s compensation (40–70%) was routinely used. V_m measurements have been corrected for current flow (I) across the uncompensated fraction of R_s using the relationship: $V_m = V_p - IR_s$, where V_p is the pipette potential. Junction potential corrections were applied to V_m as previously described [39, 40]. Reversal potentials (E_{rev}) were obtained by interpolation from I/V plots after fitting 3rd or 4th order polynomials using least-squares regression analysis. The anion permeability ratios were derived from E_{rev} values using the Hodgkin-Katz modification of the Goldman equation as previously described [39, 40].

The input capacitance of cells was routinely measured using the analogue circuitry of the EPC-7 amplifier and compensated prior to the start of recording. Capacitance values were used to calculate current density, which is expressed as picoamperes per picofarad (pA/pF).

Solutions and chemicals

In order to look at Cl^--selective currents in isolation from potassium currents, tetraethylammonium (TEA) was used as the major cation in the pipette solution. The pipette solution contained (in mM): 135.0 TEA-Cl, 2.0 $MgCl_2$, 2.0 ethylene glycol-bis (β-aminoethyl ether) *N,N,N′,N′*,-tetraacetic acid (EGTA), 1.0 disodium ATP, and 10.0 *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulphonic acid (HEPES), pH to 7.20 with 1.0 M NaOH. The solution was filtered through a 0.2-μm membrane filter before use. The osmolarity of this solution was 290 mosmol/l and the calculated free Ca^{2+} concentration was < 0.1 nM.

The standard bath solution contained (in mM): 138.0 NaCl, 4.5 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 5.0 glucose, and 10.0 HEPES, pH 7.40 with 1 M NaOH. The osmolarity of this solution was 290 mosmol/l. In the anion-replacement experiments, 100 mM NaCl was replaced by either 100 mM sodium gluconate, 100 mM sodium iodide or 100 mM sodium bromide.

Hypertonic and hypotonic bath solutions were prepared either by the addition of 90 mM sucrose to the standard bath solution, resulting in a solution of osmolarity 390 mosmol/l, or by diluting the standard bath solution with distilled water (33.3% v/v), resulting in a solution of osmolarity 200 mosmol/l, respectively.

Stock solutions of 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS, 100 mM), dideoxyforskolin (50 mM), and tamoxifen (10 mM) were made up in dimethylsulphoxide (DMSO). When added to the standard bath solution the final concentrations were 100 μM DIDS, 50 μM dideoxyforskolin and 10 μM tamoxifen. The final DMSO concentration in the standard bath solution was 0.1% in each case. This was shown to have no effect on the volume-sensitive Cl^- current in vas deferens epithelial cells. All chemicals were purchased from Sigma and were of the highest purity available.

Statistics

Values are given as means ± SEM (*n* = number of observations). Data were analysed using the *t*-test and the Mann-Whitney *U*-test. A *P* value of < 0.05 was taken as being significant.

Results

Development of volume-sensitive current

In a series of experiments in which TEA was used as the predominant cation in the pipette-filling solution 96% (26/27) of cells tested developed a volume-sensitive Cl^- conductance after establishing a whole-cell recording.

Figure 1A shows the time course of development of the whole-cell current. The current trace starts 2 min into whole-cell recording, with small currents (13 and 6 pA/pF measured at E_{rev} ± 60 mV; Fig. 1A, B1) which gradually increase over several minutes reaching a peak value (47 and 20 pA/pF measured at E_{rev} ± 60 mV; Fig. 1A, B2) approximately 360 s into the recording. Figure 1A also shows data from the same cell 33 min after break-in and confirms that the conductance is sensitive to an osmotic gradient across the cell membrane, as a hypertonic bath solution (390 mosmol/l) slowly inhibited the outward and inward currents by 68% and 58%, respectively. Similar results were obtained in six other experiments. The mean reduction in current density for the seven experiments was 72.1 ± 4.2% and 68.3 ± 4.4% (measured at E_{rev} ± 60 mV, respectively). Currents could be reactivated by exposing the cells to a hypotonic bath solution (200 mosmol/l), upon which the outward and inward current density was increased by 837 ± 403% and 418 ± 110%, respectively. The maintenance of this conductance did not seem to be dependent on intracellular calcium since intracellular calcium was buffered to < 0.1 nM by 2 mM EGTA.

Biophysical characteristics of volume-sensitive current

Figure 1B2 illustrates typical peak current traces, obtained when V_m was held at 0 mV and pulsed to ± 100 mV in 20-mV steps. The average peak currents were 96.1 ± 10.3 pA/pF and −66.5 ± 8.1 pA/pF at E_{rev} ± 60 mV, respectively (*n* = 26 see Fig. 1D). Although the time to reach peak current did vary (range 2–7 min), the mean time to peak current amplitude for cells for which run-up data were available was 4.6 ± 0.6 min (*n* = 16). The currents showed characteristic time-dependent inactivation at depolarising step potentials ≥ 60 mV (Fig. 1B2). The volume-sensitive currents also exhibited an outwardly rectifying instantaneous *I*/*V* plot (Fig. 1C) with an E_{rev} of −3.2 ± 0.8 mV (*n* = 24) close to the calculated equilibrium potential for Cl^- of −1.7 mV under our conditions. Figure 1D summarises the initial current densities obtained at different times after obtaining the whole-cell configuration. For technical reasons, the earliest *I*/*V* data we obtained were 1 min after establishing the whole-cell recording configuration. As Fig. 1D shows, even at this early time point a substantial current was already present (39.5 ± 9 pA/pF and 28.9 ± 6 pA/pF at E_{rev} ± 60 mV; *n* = 5) and in four out of five cells this initial current already had volume-sensitive biophysical characteristics. We then investigated the initial current densities 2 min (54.6 ± 15 pA/pF and 34.2 ± 10 pA/pF at E_{rev} ± 60 mV; *n* = 9) after going whole-cell and 3 min (56.2 ± 14 pA/pF and 46.4 ± 10 pA/pF at E_{rev} ± 60 mV; *n* = 6) after going whole-cell. The initial current densities at both these time points were larger than the current densities at 1 min, confirming the "run-up" nature of the response. All the initial currents, whether at 1, 2 or 3 min after obtaining the whole-cell configuration, ran-up to peak current values, as illustrated in Fig. 1D. Taken together, these biophysical characteristics suggest that vas deferens epithelial cells possess a volume-sensitive Cl^- conductance.

Anion selectivity of volume-sensitive current

Further demonstration that these currents were Cl^--selective was provided by experiments in which 100 mM of the extracellular Cl^- was replaced by gluconate. Figure 2 shows the *I*/*V* plot from a representative experiment using a single vas deferens cell in which extracellular Cl^- was partially replaced by gluconate, iodide and bromide. As can be seen, gluconate caused a positive shift in the E_{rev} indicating that this anion is less permeable than Cl^-. This manoeuvre was performed using 15 cells and in each a positive shift in E_{rev} was seen (mean 18.9 ± 0.9 mV; *n* = 15), indicating a significant selectivity of the whole-cell conductance for Cl^- over gluconate. The anion permeabilities relative to Cl^- were calculated from the shifts in E_{rev} using the Goldman-Hodgkin-Katz equation. We have assumed that anion replacement does not alter any cation conductance in the vas deferens cells. The calculated value for P_{Gluc}/P_{Cl} was 0.23 ± 0.03 (*n* = 15).

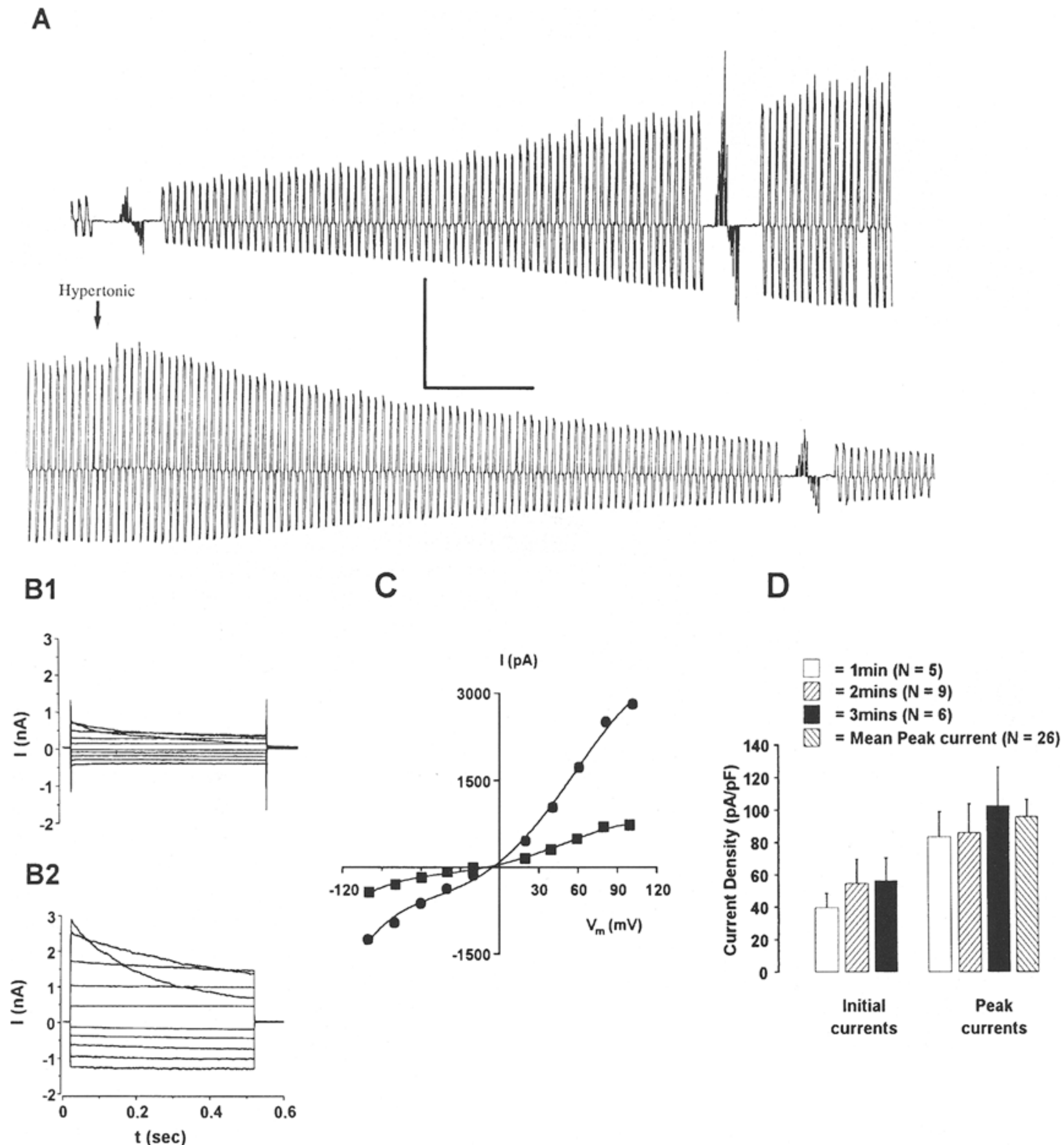


Fig. 1A–D Effect of time on whole-cell current after obtaining the whole-cell configuration. **A** Continuous recording starting 2 min after the establishment of the whole-cell configuration. Current developed steadily, in this case, reaching a peak amplitude after approximately 360 s. Following 33 min of the experiment the bath osmolarity was raised to a level hypertonic to the pipette solution upon which the current steadily decreased to control levels. Current traces were obtained using the ± 60 mV protocol and were interrupted by the ± 100 mV protocol to establish *I*/*V* relationships (see Materials and methods). The *scale bars* represent 1500 pA and 60 s respectively. **B** Currents elicited by the ± 100 mV protocol 2 min after establishment of the whole-cell configuration (**B1**) and after the full development of volume-sensitive currents (**B2**). **C** Instantaneous *I*/*V* plot for the currents shown in **B**. *I*/*V* data points were obtained by measuring currents over a 4-ms period 7 ms into the voltage pulse. (*Squares* Initial currents, *Circles*, peak volume-sensitive currents). **D** Summary of values obtained for initial currents measured at 1, 2 and 3 min after establishing the whole-cell configuration and the corresponding peak current values from the data at 1, 2 and 3 min after break-in. The mean peak current value from all experiments is also shown for comparison. Currents were measured at the reversal potential, E_{rev}, + 60 mV and have been normalised to cell input capacitance

Figure 2 also shows the *I*/*V* plots obtained upon replacement of 100 mM of the extracellular Cl^- with iodide and bromide. As can be seen there is little change in the E_{rev} values with these substitutions. When 100 mM bromide was present in the bath solution the mean shift in E_{rev} from control was -0.7 ± 0.7 mV ($n = 5$). Similarly, with 100 mM iodide in the extracellular solution, the mean shift in E_{rev} from control was -0.9 ± 1.2 mV ($n = 4$). The calculated values for P_{Br}/P_{Cl} and P_I/P_{Cl} were 1.05 ± 0.04 and 1.06 ± 0.07, respectively. These results are not significantly different from the permeability of this

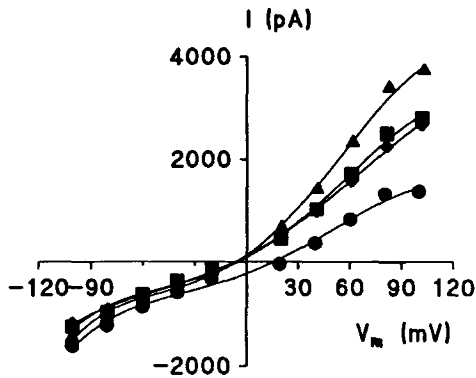


Fig. 2 Anion selectivity of volume-sensitive currents. Representative, instantaneous I/V plots recorded 60 s after the substitution of 100 mM of normal bath Cl^- (*squares*) for 100 mM iodide (*triangles*) or 100 mM bromide (*diamonds*) or 100 mM gluconate (*circles*)

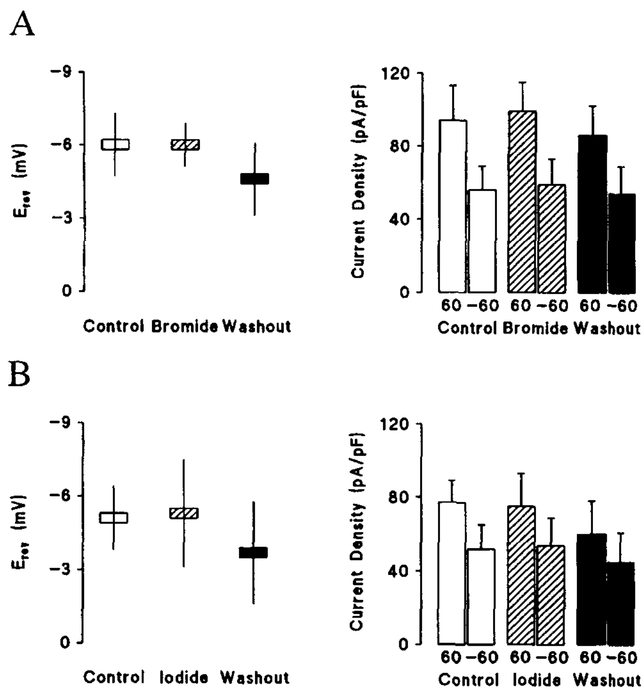


Fig. 3A, B Mean anion E_{rev} and current density data. **A** The mean E_{rev} data for bromide and its associated current density values for control, in the presence of bromide and washout conditions (n = 5). **B** The mean E_{rev} for iodide and its associated current density values for control, in the presence of iodide and washout conditions (n = 4)

conductance to Cl^-, indicating that the conductance is not selective amongst these halides. Figure 3 shows the mean data for the E_{rev} values and their associated current densities before, during and after addition of bromide and iodide. There is no significant differences between these parameters, indicating that both bromide and iodide do not affect E_{rev} or conductance.

Time and voltage dependence of volume-sensitive current

The volume-sensitive Cl^- conductance of the vas deferens epithelial cells was time-dependent, with current inactivation during depolarising pulses and slight current activation during hyperpolarising pulses (see Fig. 1B2). The extent of inactivation or activation depended on the previous voltage history of the cell.

To further characterise the inactivation process, a conditioning prepulse to -80 mV was applied for 2 s in order to fully activate all the channels prior to applying depolarising voltage pulses to +120 mV, in 20-mV steps (Fig. 4A). Inactivation of the outward Cl^- currents was observed at depolarising potentials of ≥ 60 mV. Figure 4C shows the I/V plot for the inactivating Cl^- conductance. Instantaneous current, measured 7 ms after the start of the voltage pulse (open symbols), represents current flow through the activated conductance, whilst plateau current, measured 495 ms into the voltage pulse (closed symbols), provides evidence of the time-dependent inactivation process (n = 4).

Similarly, to further characterise the activation process of the volume-sensitive current, a conditioning prepulse to +80 mV for 2 s was applied in order to inactivate all the channels prior to applying voltage pulses to -120 mV in 20-mV steps (Fig. 4D). Current activation was observed during hyperpolarising voltage steps of ≥ –40 mV. An I/V plot for the activation of the whole-cell Cl^- conductance is shown in Fig. 4F. The time courses of current inactivation and activation were found to be fitted by single exponential functions (Fig. 4B and E, respectively). The corresponding rate coefficients for current inactivation and activation are plotted in Fig. 5, as a function of voltage, together with their linear least-squares fits. As can be seen, the rate coefficients for inactivation and activation were found to be faster for stronger depolarising and hyperpolarising pulses respectively. The slope of these plots shows that the activation process is approximately 3 times as sensitive to voltage as that of the inactivation process (-0.31 and $0.10\ s^{-1}.\ mV^{-1}$, respectively).

Pharmacological inhibition of volume-sensitive current

Figures 6 and 7 compare the effects of 100 μM DIDS, 50 μM dideoxyforskolin, and 10 μM tamoxifen on peak volume-sensitive currents. Addition of 100 μM DIDS to the perfusate was found to rapidly reduce the volume-sensitive Cl^- current (Fig. 6A). The DIDS block was voltage-dependent, outward current being inhibited to a much greater extent than inward current, resulting in an inwardly rectifying I/V plot. The average decrease was 60.4 ± 5.2% for outward currents

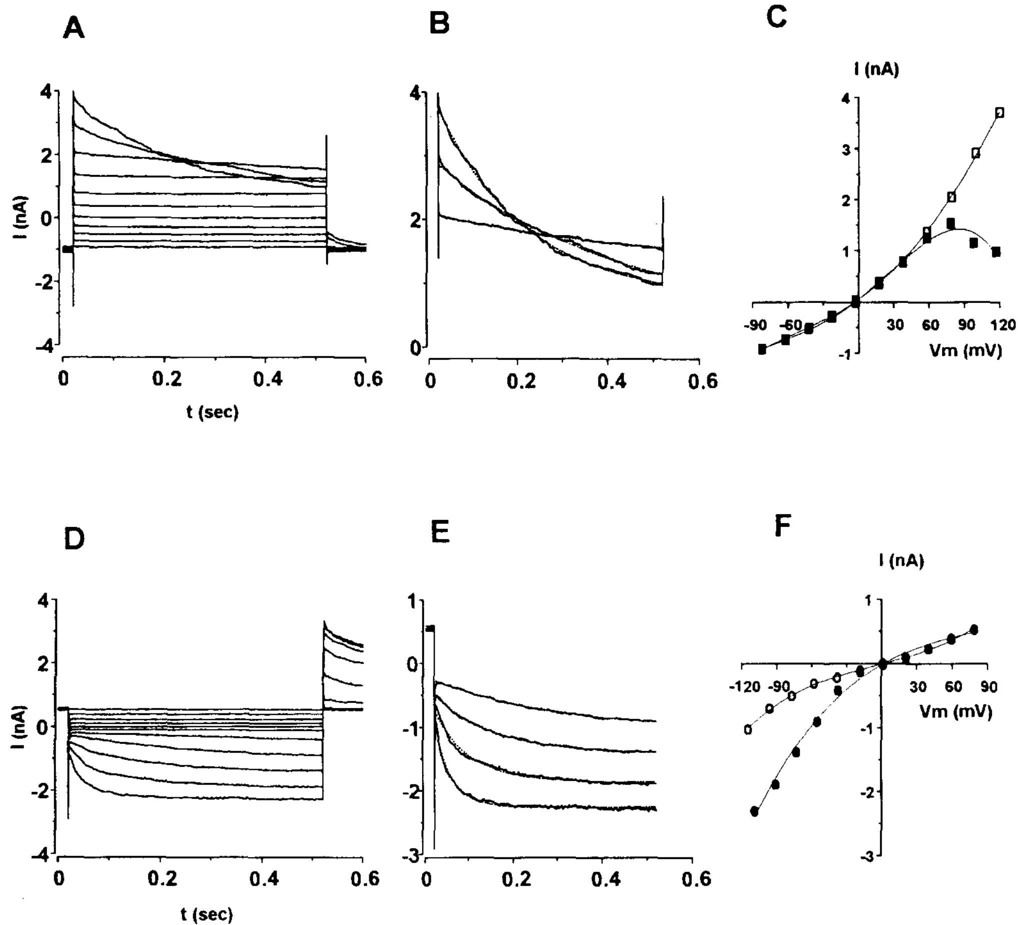


Fig. 4A–F Time and voltage dependence of the volume-sensitive currents. **A–C** Time- and voltage-dependent inactivation of currents. **A** The current traces obtained in response to holding the membrane potential at −80 mV for 2 s and then pulsing from −80 to +120 mV for 500 ms, in 20-mV steps. Currents were measured at 7 and 495 ms into the pulse. **B** The same current traces obtained in **A** from voltage pulses to +80, +100 and +120 mV (*solid lines*) and the corresponding single exponential fits to these current traces (*dotted lines*). The parameters of the fit were τ (ms) = 457, 433, and 200 for +80, +100 and +120 mV respectively. **C** *I*/*V* plots of currents measured in A, *open symbols* indicate initial current, and *closed symbols* represent plateau current. **D–F** Time- and voltage-dependent activation of currents. **D** The current traces obtained in response to holding the membrane potential at +80 mV for 2 s and then pulsing from +80 to −120 mV for 500 ms, in 20-mV steps. Currents were measured at 7 and 495 ms into the voltage pulse. **E** The same current traces obtained in **D** from voltage pulses to −60, −80, −100 and −120 mV (*solid lines*) and the corresponding single exponential fits to these current traces (*dotted lines*). The parameters of the fit were τ (ms) = 276, 135, 84, 39 ms for −60, −80, −100, and −120 mV, respectively. **F** *I*/*V* plots for the currents shown in **D**, *open symbols* indicate initial current, and *closed symbols* represent plateau current

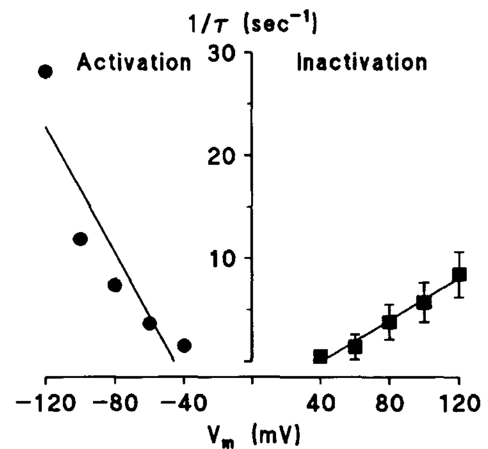


Fig. 5 Rate coefficients ($1/\tau$) of activation (*circles*) and inactivation (*squares*) of volume-sensitive Cl^- currents as a function of voltage. Rate coefficients were derived from single exponential fits to records in Fig. 4A, D as illustrated in Fig. 4B, E. *Solid lines* represent linear least-squares fits to the data

measured at 60 mV and 9.5 ± 7.2% for inward currents measured at −60 mV (see Fig. 7).

The effect of dideoxyforskolin, an inhibitor of *P*-glycoprotein [24], on the volume-sensitive conductance was also investigated. Valverde and coworkers [37] have reported that the volume-activated Cl^- conductance expressed following transfection of fibroblasts with the full-length human MDR1 cDNA was reduced by both forskolin and dideoxyforskolin. Dideoxyforskolin was used in this study since, unlike forskolin, it lacks the ability to activate adenylyl cyclase [25], thus avoiding the possibility that any effect of the compound results

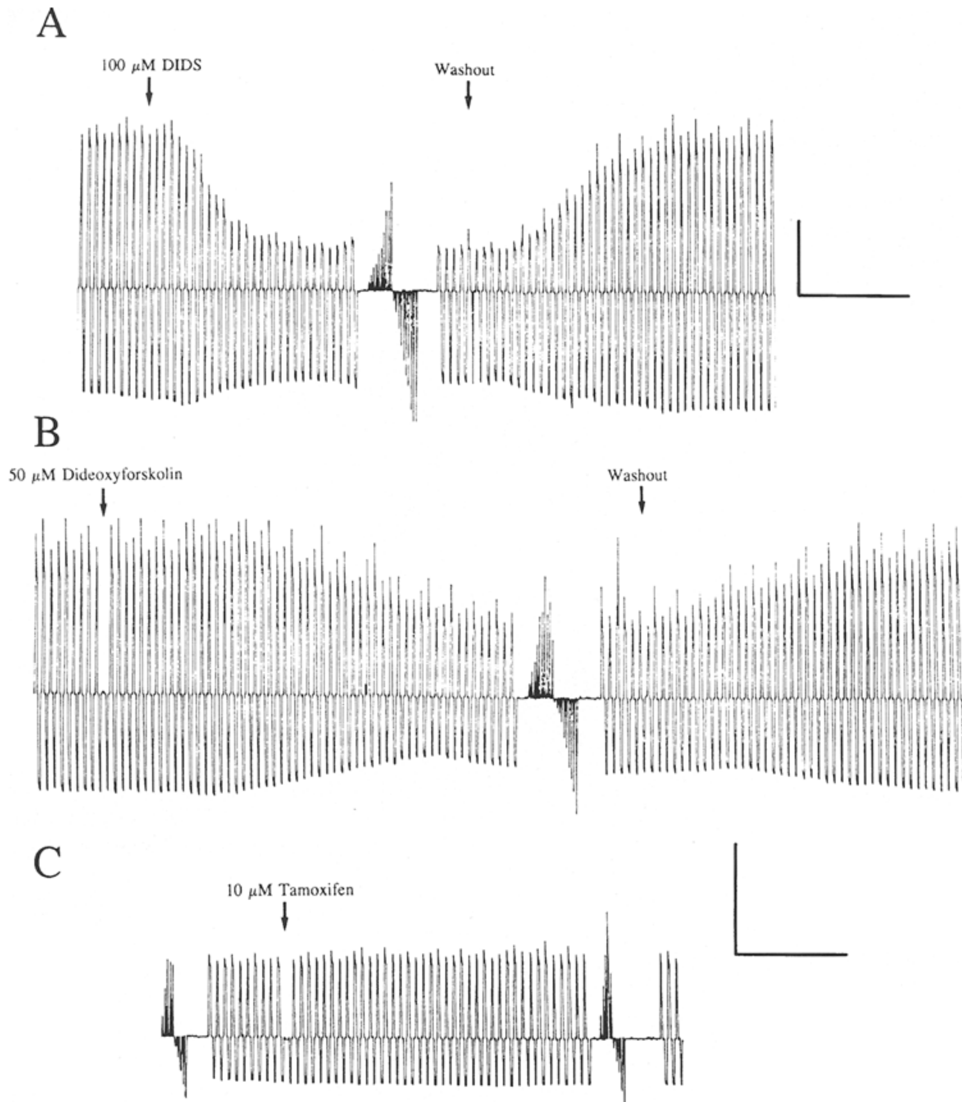
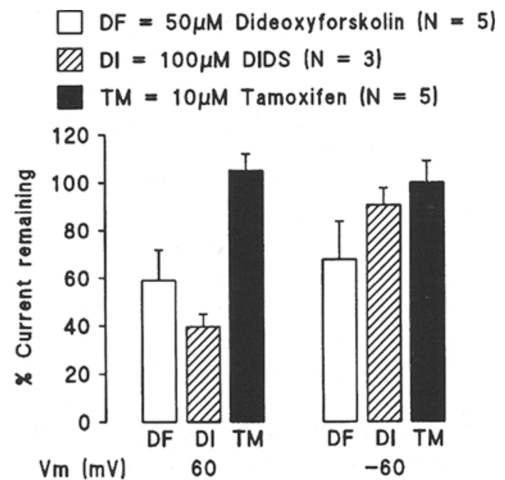


Fig. 6A–C Inhibition of volume-sensitive currents. Representative whole-cell current traces in response to the ± 60 mV protocol (see Materials and methods) **A** 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (*DIDS*, 100 μM) was added where indicated, causing a rapid decrease in outward whole-cell current, which was reversed by washout. The *scale bars* represent 4000 pA and 60 s respectively. **B** Dideoxyforskolin (50 μM) was added where indicated, causing a reduction in inward and outward whole-cell currents, which was reversed by washout. **C** Tamoxifen (10 μM) was added where indicated. The *scale bars* for **B** and **C** represent 3000 pA and 60 s respectively

from an increase in intracellular cAMP. Dideoxyforskolin (50 μM) caused a reversible reduction in both outward and inward volume-sensitive currents, of 41.0 ± 12.7 and 32.3 ± 15.9% respectively ($n = 5$; Figs. 6B, 7). Block by dideoxyforskolin was not voltage-dependent (there being no statistical difference between outward and inward current at ± 60 mV) and was much slower than the effect of DIDS. In addition, recovery from the drug was also much slower.

Fig. 7 Summary of the inhibition data measured at E_{rev} ± 60 mV. Data are represented as the percentage of control current remaining



The anti-oestrogen tamoxifen has been reported to selectively inhibit a volume-sensitive Cl^- conductance in the T84 colonic epithelial cell line [38] and rat pancreatic duct cells [39]. This drug also represents the most selective inhibitor of volume-sensitive Cl^- current so far described. Figures 6C and 7 shows that tamoxifen, to our surprise, had no effect on the vas deferens Cl^- conductance, even when used at double the concentration (10 μM; $n = 5/5$) that completely abolished the volume-sensitive Cl^- conductance of T84 [38] and rat pancreatic duct cells [39].

Discussion

Biophysical characteristics of the volume-sensitive Cl^- current

In this study, we have demonstrated the presence of a volume-sensitive whole-cell Cl^- conductance in primary cultures of human vas deferens epithelial cells. The major biophysical characteristics of this conductance are an outwardly rectifying I/V relationship and time-dependent inactivation at depolarising potentials and time-dependent activation at hyperpolarising potentials. These characteristics are typical of volume-sensitive Cl^- currents previously reported in relation to several other epithelial cells (e.g. epididymis [4, 5], pancreatic ducts [39], ciliary epithelium [42], airway [3, 9, 21, 27, 28], and gut [17, 33, 41]).

Volume-sensitive Cl^- currents developed spontaneously, with isosmotic solutions in bath and pipette, after going whole-cell and gradually increased with time. The simplest explanation for this is that the cells swell due to the intracellular environment becoming hypertonic, maybe as a result of restricted diffusion of intracellular macromolecules or incomplete mixing of the cellular contents with the pipette solution. This is supported by experiments showing that the volume-sensitive Cl^- current was reduced on exposure to a hypertonic bath solution. Similar observations have been reported in relation to a number of preparations[9, 21, 41]. The volume-sensitive Cl^- current seemed to be independent of intracellular calcium as this was buffered to < 0.1 nM. Similar results have been described in relation to other epithelial cells [1, 4, 5, 17, 21, 33, 42] and adds to the growing body of evidence that suggests that volume-sensitive Cl^- currents involved in RVD in epithelial cells are Ca^{2+}-independent, although exceptions do exist [31, 39]. Furthermore, it suggests that this Cl^- conductance is separate from the calcium-activated Cl^- conductance in these cells that we have previously described [19].

The activation and inactivation kinetics of this volume-sensitive Cl^- conductance were both fitted by single exponential functions. This is in agreement with the volume-sensitive Cl^- conductance in T84 [41] and human nasal epithelial [9] cells, but is in contrast to a recent report from rat C6 glioma cells [15]. The activation and inactivation kinetics of the vas deferens volume-sensitive Cl^- conductance were also considerably faster than reported for the Cl^- conductance in T84 cells [41], but were similar to that reported for nasal epithelial cells [9]. The voltage dependence of the activation and inactivation kinetics, however, was qualitatively similar in all three cell preparations and the slope of the voltage dependence shows that the rate constant for the activation process is approximately 3 times as sensitive to voltage as that of the inactivation process. This is similar to the values reported in connection with T84 and nasal epithelial cells [9, 41].

Anion selectivity of volume-sensitive Cl^- current

Anion replacement experiments with gluconate confirmed that the volume-sensitive current in vas deferens epithelial cells was Cl^--selective. The gluconate permeability ($P_{Gluc} = 0.23$) of vas deferens is similar to values found by others for volume-sensitive Cl^- channels (range 0.11 to 0.25) [6, 27, 37]. Furthermore, the fact that gluconate has a significant permeability indicates that the diameter of the vas deferens channel pore is greater than 0.6 nm. In contrast to gluconate, the volume-sensitive Cl^- conductance was not selective for the halides over chloride ($P_{Br} = 1.05$ and $P_I = 1.06$), which is similar to the swelling-induced Cl^- channel of the rat epididymis ($P_{Br} = 1.07$ and $P_I = 1.16$) [4]. At first sight these permeabilities to iodide and bromide would seem to be different from permeabilities of other epithelial cells previously reported [3, 6, 17, 27, 33, 38, 39]. However, on closer inspection, it is noticeable that there is a large variation in P_{Br} and P_I values obtained from different tissues (e.g. $P_{Br} = 1.05$ and $P_I = 1.19$ [27]; $P_{Br} = 1.56$ and $P_I = 2.60$ [3]; $P_{Br} = 1.41$ and $P_I = 1.82$ [39]). Even in the same T84 cell line the variation is large (e.g. $P_{Br} = 1.04$ and $P_I = 1.19$ [27]; $P_{Br} = 1.60$ and $P_I = 2.60$ [41]). Recent results from several preparations [2, 5, 27] show that the volume-sensitive Cl^- conductances in these cells are permeable to the amino acids, aspartate ($P_{Asp} = 0.12$–0.19) and glutamate ($P_{Glut} = 0.18$–0.20). These permeability values are very similar to the gluconate permeability of the vas conductance and, although not measured in our preparation, it is possible that these amino acids could permeate the volume-sensitive Cl^- channel in vas deferens cells.

Pharmacological inhibitors of the volume-sensitive Cl^- current

The volume-sensitive Cl^- current in vas deferens epithelial cells was inhibited by the disulphonic stilbene, DIDS. Outward currents were inhibited to a much greater extent than inward currents, indicating

that the block was voltage-dependent. Similar results in a number of preparations have been reported [4–6, 17, 33, 39]. Since DIDS is negatively charged the voltage dependence suggests that DIDS is binding to a positively charged site on the channel. The reversible nature of the block indicates that DIDS does not covalently modify the channel.

Dideoxyforskolin also reversibly inhibited both outward and inward currents. However, the time course of block and recovery from the drug was much slower compared to DIDS. A possible explanation is that dideoxyforskolin may block the channel from the intracellular side, whereas DIDS probably blocks from the extracellular side of the channel. Valverde and collaborators [37] have reported that when full-length human MDR1 cDNA was permanently transfected into NIH3T3 fibroblasts, the cells displayed volume-activated whole-cell Cl^- currents and suggested that *P*-glycoprotein was acting as a volume-activated Cl^- channel. This idea was supported by data that showed that these currents were blocked by agents that had previously been reported to inhibit the *P*-glycoprotein-mediated drug transport, including dideoxyforskolin, forskolin, verapamil, nifedipine, quinine and quinidine [7]. In contrast, McEwan and colleagues [22] have reported that, in T84 cells, vinblastine secretion via *P*-glycoprotein, and volume-activated Cl^--secretion showed little correlation. Furthermore, no correlation has been found between MDR1 expression and volume-sensitive Cl^-current magnitude, in drug-sensitive and drug-resistant epithelial cell lines [28]. More recently, Morin et al. [23] have shown that MDR1 expressed in *Xenopus* oocytes does not produce a volume-sensitive conductance. This conflicting evidence suggests that *P*-glycoprotein may not be a channel itself but may be involved in the regulation of endogenous volume-sensitive Cl^- channels and, indeed, this has been more recently proposed by Higgins and colleagues [10, 12] to explain their results. MDR1 RNA is not expressed in cultures of vas deferens epithelial cells at significant levels (A. Harris, unpublished observation), which suggests that *P*-glycoprotein is unlikely to underlie or regulate the large volume-activated Cl^-conductance found in vas deferens cells. This conclusion is supported by a recent report by Tominaga et al [36] who have provided clear evidence for neither a direct nor a regulatory relationship between *P*-glycoprotein and a volume-activated Cl^- conductance in a human small intestinal epithelial cell line (Intestinal 407).

We also tested the effect of the anti-oestrogen, tamoxifen, which has been reported to block volume-activated Cl^-currents in T84 [38] and rat pancreatic duct [39] cells. In contrast to these reports, 10 μM tamoxifen had no effect on the volume-sensitive Cl^- current of vas deferens epithelial cells. Valverde et al. [38] have reported that tamoxifen blocks the P-glycoprotein transporter and the presumed *P*-glycoprotein associated Cl^- channel. The absence of an effect of tamoxifen on the volume-sensitive Cl^- current in vas deferens cells is consistent with the absence of *P*-glycoprotein in these cells. Furthermore, Nilius et al. [26] have reported that tamoxifen and dideoxyforskolin had variable effects on volume-sensitive Cl^-currents in a number of nonepithelial cell lines and Tominaga et al. [36] have shown varying effects of Tamoxifen and dideoxyforskolin, depending on whether they were added before or after swelling-induced activation of the Cl^- current. Taken together it would seem questionable that tamoxifen, could be used to discriminate volume-sensitive Cl^- currents from other Cl^- currents, as proposed by Valverde et al. [38].

Comparison to other volume-sensitive Cl^- channels

Jentsch and colleagues have reported the cloning and expression of a novel Cl^- channel that was gated by voltage and was volume-sensitive [8, 35]. The channel showed about 50% sequence homology with both the *Torpedo* electroplax Cl^- channel (ClC-0) and the rat skeletal muscle Cl^- channel (ClC-1), and has, therefore, been named ClC-2. Currents recorded from *Xenopus* oocytes expressing ClC-2 were activated by hypotonic solutions in the absence of calcium, following a delay similar to that reported for the vas deferens. The currents displayed time-dependent inactivation and activation at depolarising and hyperpolarising potentials, respectively, broadly similar to that described here for vas deferens epithelial cells. However, time-dependent activation of the current was only observed at potentials more negative than −90 mV; even a voltage pulse to −180 mV produced no saturation of the activation process during a 30-s pulse. In contrast, the current described in the vas deferens cells activated at potentials more negative than −60 mV and was fully activated at a potential of −100 mV within a 500-ms pulse. In addition the *I*/*V* plot of ClC-2 was inwardly rectifying in contrast to the outwardly rectifying *I*/*V* plot of the vas deferens conductance. Furthermore the ClC-2 conductance was not blocked by 1 mM DIDS [8], whilst DIDS inhibited the volume-sensitive Cl^- current in the vas deferens cells. These data would tend to suggest that the volume-sensitive Cl^- conductance of vas deferens epithelial cells is a separate, and distinct Cl^- conductance from ClC-2.

Physiological role of the volume-sensitive Cl^- current in vas deferens cells

The volume-sensitive Cl^- conductance in primary cultures of human vas deferens epithelial cells may be one of the ways by which these cells regulate their cell volume. Together with a volume-sensitive K^+ conductance, this Cl^- conductance could contribute to an RVD following osmotic swelling. Furthermore, it is possible

that this Cl^- conductance could provide a secretory route for organic solutes (e.g. amino acids) into the vas deferens lumen. Banderali and Roy [2] and others [5, 14, 16] have described volume-sensitive Cl^- channels that are permeable to amino acids. Movement of organic osmolytes would also aid the ability of the cells to volume regulate.

In conclusion, our work provides evidence for the presence of a volume-sensitive Cl^- conductance in primary cultures of human vas deferens epithelial cells with characteristics broadly similar to those reported previously for volume-sensitive Cl^- conductances in other epithelial cells.

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