J. Membrane Biol. 124, 275–284 (1991)

The Journal of
Membrane Biology

Chloride Channels on Epithelial Cells Cultured from Human Fetal Epididymis

C.E. Pollard*, A. Harris†,**, L. Coleman†, and B.E. Argent

Department of Physiological Sciences, University Medical School, Newcastle upon Tyne NE2 4HH, and †Paediatric Research Unit, Division of Medical and Molecular Genetics, United Medical and Dental Schools, Guy's Hospital, London, SE1 9RT, United Kingdom

Summary. Using single-channel recording techniques, we have detected two types of outwardly rectifying chloride channel on epithelial cells cultured from human fetal epididymis. A small-conductance channel (2.8–5.0 pS) was spontaneously active in 29% of cell-attached patches but rapidly disappeared on patch excision. This channel often occurred in clusters and exhibited slow kinetics with open and closed times of the order of tens or hundreds of msec; an open-state probability that was essentially independent of voltage; and a very low permeability to bicarbonate relative to chloride. Exposing epididymal cells to either forskolin (3 μM) or adrenaline (1 μM) activated this channel (up to 350-fold), suggesting that it may be involved in cyclic AMP-mediated anion secretion by the male reproductive tract. The large-conductance channel (14 to 29 pS) was never detected in cell-attached patches but could be activated by depolarization (40 mV) in 3% of excised, inside-out patches. Once activated, opening of this 'large' channel was voltage independent, and it had a relatively high permeability to both gluconate ($P_{gluconate}/P_{chloride}$ = 0.24) and bicarbonate ($P_{bicarbonate}/P_{chloride}$ = 0.4). The proportion of excised patches that contained this channel was increased 2.5-fold by prior stimulation of the epididymal cells; however, because the channel was never observed in cell-attached patches its physiological role must remain uncertain.

Key Words epididymis · epithelial transport · patch clamp · chloride channels · cyclic AMP-regulated chloride channel

Introduction

The epididymis forms the proximal part of the excretory duct system in the male reproductive tract and is a complex organ which secretes and absorbs ions, water and organic solutes [9, 21, 32, 33, 37]. By way of its transport properties, the epididymal epithelium normally provides the correct luminal environment for sperm maturation.

Recently, it has been shown that primary cultures of epithelial cells isolated from the cauda (distal) epididymis of the rat possess an electrogenic secretory mechanism for chloride and bicarbonate ions [9, 32, 33, 36]. This process is stimulated by a variety of agents, all of which increase intracellular cyclic AMP [36], and may facilitate ejaculation by maintaining the fluidity of the epididymal contents [32, 34]. Furthermore, it has also been shown that bicarbonate ions are important for the initiation of sperm motility at ejaculation [27].

On the basis of ion replacement [9, 32], transport inhibitor [32, 33] and intracellular pH studies [35], it has been suggested that epididymal anion secretion occurs by a mechanism similar to that proposed for other epithelia [35]. As such, there is an anion accumulation step at the basolateral side of the cell (mediated by Na/K/2Cl cotransporters, Na^+/H^+ exchangers and Cl^-/HCO_3^- exchangers), while anion exit at the apical membrane is mediated by ion channels [35]. However, to date, this transport model has not been confirmed by cellular electrophysiological studies.

The aim of this study was to characterize the chloride channels on human epididymal cells and to identify which channel(s) might be involved in cyclic AMP-mediated anion secretion. Our results indicate that both large- and small-conductance, outwardly rectifying, chloride channels are present in this epithelium. Only the small-conductance channel was detected in cell-attached patches, and its activity could be markedly increased by forskolin and adrenaline. This suggests that the 'small' channel is regulated by cyclic AMP and thus might be involved in electrogenic anion secretion by the

* *Present address:* Fisons plc., Pharmaceutical Division, Bakewell Road, Loughborough, Leicestershire, LE11 ORH, U.K.

** *Present address:* Institute of Molecular Medicine, The John Radcliffe Hospital, Headington, Oxford, OX39DU, U.K.

human male reproductive tract. Some of our results have been published in preliminary form [28].

Materials and Methods

Human Epididymal Cell Cultures

Primary monolayers of epididymal cells were grown on collagen-coated glass coverslips from explants of second trimester human fetal epididymes as previously described [19]. Three normal fetuses, and one fetus with Wellveger's syndrome, were obtained within 48 hr of prostaglandin-induced terminations (two fetuses) or spontaneous abortions (two fetuses). Once established, these cultures contain two populations of epithelial cells; relatively large, angular cells that do not appear tightly packed even in confluent monolayers, and smaller round cells that occur in tightly packed colonies. Both cell types have been identified as epithelial on the basis of morphological, biochemical and immunocytochemical evidence [19].

The cultures were established at Guy's Hospital, London, and then sent by express postal service to Newcastle upon Tyne. After arrival in Newcastle they were incubated for 1 to 9 days in the standard growth medium [19] minus cholera toxin before electrophysiological studies were performed. A total of 20 cultures (between two and seven from each fetus), maintained for between 6 and 16 weeks, were used in this study.

Electrophysiology

Single-channel current recordings were made at 21–23°C using the patch-clamp technique [18]. Most patches (89%) were obtained from the upper surface of flat cells in confluent or nonconfluent areas of the monolayers. However, in order to increase the sealing rate, we occasionally rounded the cells by brief incubation with 1.5% wt/vol trypsin (Gibco). From the electrophysiological point of view there were no obvious differences between untreated and trypsinized cells, between the large and small cells in the cultures, and between cultures of different ages. Therefore, all the electrophysiological data have been pooled.

Full details of the electrophysiological techniques used in this study are described elsewhere [14–16]. The tissue bath (volume 1.5 ml) was grounded, and potential difference across excised, inside-out patches was referenced to the extracellular face of the membrane. In the cell-attached configuration, the potential difference across the patch is equal to the cell membrane potential (V_m) minus the pipette clamp potential (V_p). Since the membrane potential is unknown, we give V_p values in this recording configuration. Outward current, the flow of positive charge from the inside to the outside of the membrane, is indicated as an upward deflection on all the records. Junction potentials were measured using a flowing 3M-KCl electrode [14] and the appropriate corrections applied to our data. The current reversal potential (E_{rev}) was obtained by fitting a second or third order polynomial to the rectifying I/V plots using least squares regression analysis. We give chord conductances between E_{rev} and ±50 mV. Anion permeability ratios for channels in excised patches were calculated from the shift in E_{rev} values following ion substitution using the Hodgkin-Katz modification of the Goldman equation as follows [16]:

$$\frac{P_X}{P_{Cl}} = \frac{[Cl^-]_o \times 10^{E_{rev}/58.7} - [Cl^-]_i}{[X]_i - [X]_o \times 10^{E_{rev}/58.7}} \quad (1)$$

where P is permeability; [Cl] and [X] are the molar concentrations of chloride and the replacement anion, respectively, i and o denote the inside and outside of the membrane, respectively, and E_{rev} is the reversal potential (mV).

Channel activity is expressed in two ways; either as single-channel open state probability (P_o) or as the product of channel number (N) and P_o (NP_o). To determine P_o, current records were digitized at 10 kHz using a CED 1401 interface (Cambridge Electronic Design, UK) and analyzed using a two-threshold transition algorithm which employed a 50% threshold crossing parameter to detect events. P_o was calculated as the fraction of total time that channels were open using a minimum of 120 sec of data. When multi-channel patches were used for P_o determinations, we assumed that the total number of channels present was equal to the maximum number of simultaneous current transitions. To obtain NP_o we first divided the current record into 30-sec segments and then calculated the area under the current record for each segment. These area values are proportional to NP_oI, where N is the number of active channels, P_o is the single-channel open-state probability, and I is the single-channel current. To calculate NP_o each NP_oI value was divided by the area under the notational current trace obtained if one channel was open for 30 sec.

Solutions and Chemicals

During seal formation, and when recording in the cell-attached mode, the bath contained an extracellular type Na^+-rich solution (in mM): 138 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 glucose, and 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.4. For some experiments either the Na^+ in this solution was totally replaced with K^+, or the Cl^- was partially replaced with either gluconate or HCO_3^-. The composition of these solutions was as follows. K^+-rich (mM): 142.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 glucose, and 10 HEPES, pH 7.4. Gluconate-replacement (mM): 38 NaCl, 100 Na-gluconate, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 glucose, and 10 HEPES, pH 7.4. HCO_3^- replacement (mM): 38 NaCl, 100 $NaHCO_3$, 4.5 KCl, 1 $MgCl_2$, 5 glucose, and 10 HEPES, pH 7.9. Bath solution changes were accomplished by gravity feed (5 ml/min) from a bank of reservoirs. When these solutions were used in the pipette, glucose was omitted and they were filtered through a 0.2 μM membrane filter.

In some experiments the cells were continuously stimulated by exposing them to one or a combination of stimulants throughout the experimental period, which usually lasted for 0.5–6 hr. In other experiments, cells were briefly exposed to stimulants while a cell-attached recording was in progress. The stimulants employed and their final bath concentrations were: forskolin (3 μM), adrenaline (1 μM), dibutyryl cyclic AMP (0.1 mM) and isobutylmethylxanthine (0.1 mM) (all from Sigma). All other chemicals were purchased from commercial sources and were of the highest purity available.

Statistics

Significance of difference between means was determined using Student's t test. The level of significance was set at $P \leq 0.05$. All values are expressed as mean ± SE (number of observations).

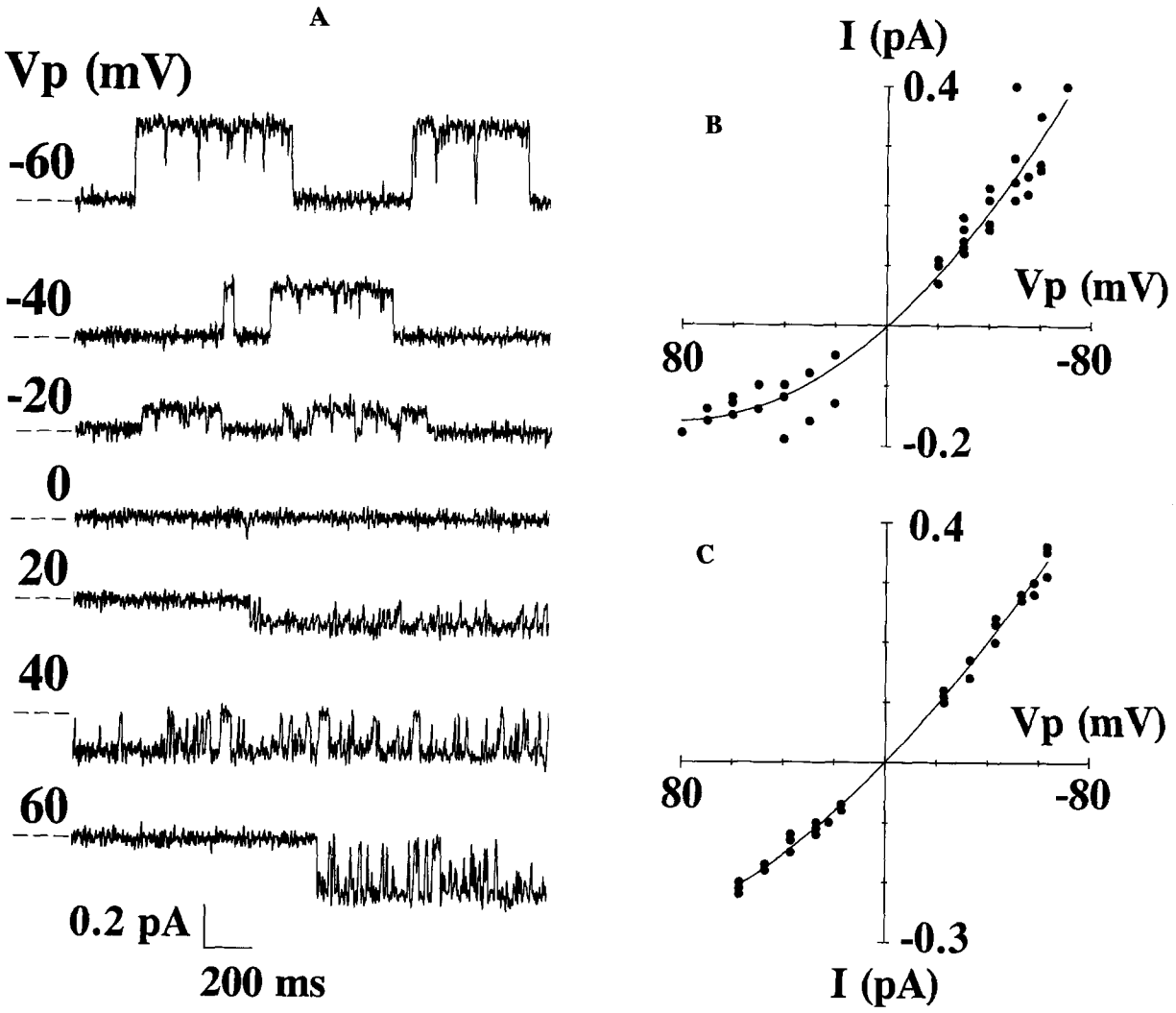

Fig. 1. The small-conductance channel in cell-attached patches. (*A*) Typical current traces recorded at the pipette potentials (V_p) indicated. Dashed lines show current level when all channels are closed. Solutions: bath, Na^+-rich; pipette, Na^+-rich. (*B*) Single-channel *I/V* plot with Na^+-rich solutions in pipette and bath. Data are from five patches on cells in five different monolayers. (*C*) Single-channel *I/V* plot. Solutions: bath, Na^+-rich; pipette, K^+-rich. Data are from three patches on cells in three different monolayers. Current records have been low-pass filtered at 100 Hz. Lines were fitted by second-order polynomial least squares regression analysis.

Results

The Small-Conductance Channel

Cell-attached patches on the upper surface of cells in both confluent and nonconfluent areas of monolayers were either quiescent or contained up to 11 small-conductance channels. Figure 1*A* shows cell-attached currents recorded from a patch that contained one active channel. When this patch was depolarized the channel exhibited slow kinetics with open and closed times of the order of tens to hundreds of msec; however, rapid closing events appeared when the patch was hyperpolarized and inward currents were flowing through the channel. With a Na^+-rich solution containing 148.5 mM Cl^- in the pipette, the unitary currents reversed at $V_p = -0.5$ mV (i.e., close to the resting membrane potential of the cell), and the conductance was 5.0 pS for outward current and 2.8 pS for inward current (ratio of outward to inward conductances = 1.8). Thus this channel is an outward rectifier in cell-attached patches. Replacing the Na^+-rich solution in the recording pipette with a K^+-rich solution had no marked effect on the *I/V* plot (Fig. 1*C*); under these conditions the reversal potential was 0.2 mV and the conductances for outward and inward currents 5.2 and 3.8 pS, respectively. This indicates that the small-conductance channel is either completely

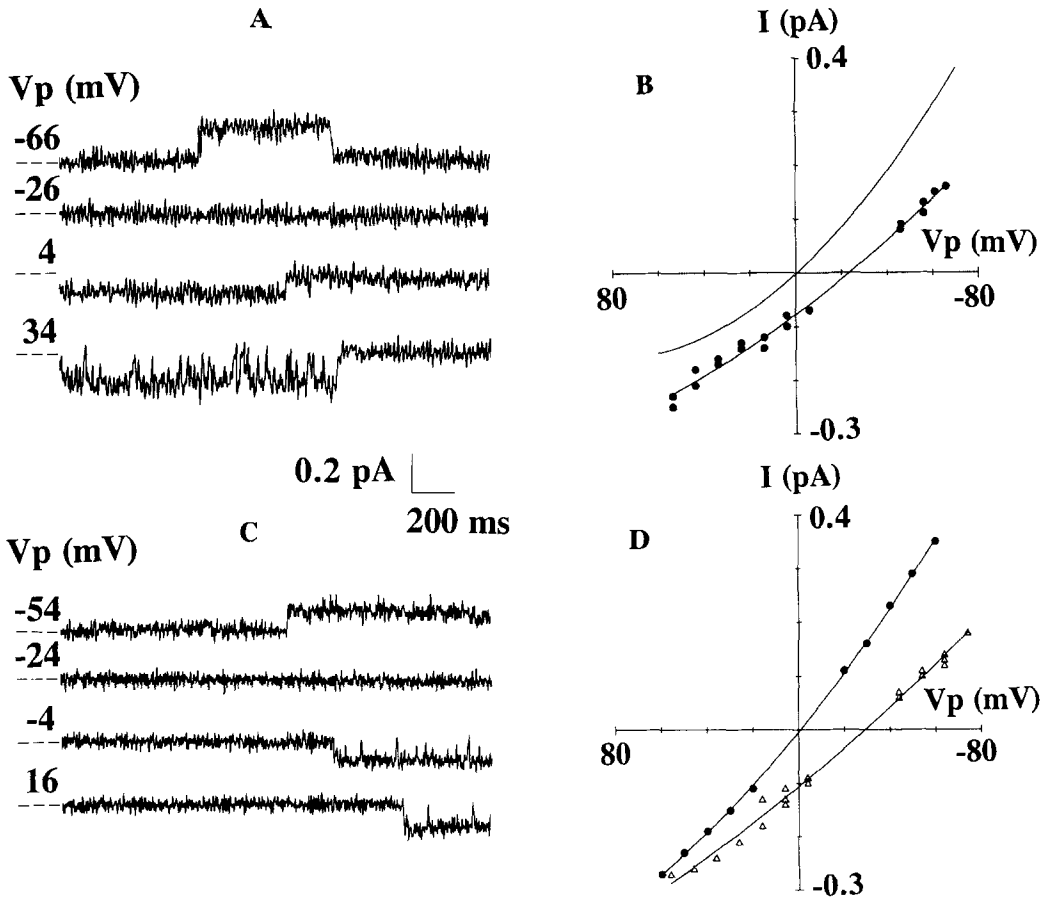

Fig. 2. Anion selectivity of the small-conductance channel. (*A*) Typical single channel currents recorded after substituting 100 mM Cl^- in the recording pipette with gluconate. The bath solution was Na^+ rich. (*B*) *I/V* plots. (●), Bath, Na^+ rich; pipette, gluconate replacement. Data are from two patches on two different cells. For clarity the control *I/V* plot (some data as in Fig. 1*B*) is shown without the individual data points. (*C*) Single-channel currents recorded after replacing 100 mM Cl^- in the pipette solution with HCO_3^-. The bath solution was Na^+ rich. (*D*) *I/V* plots. Solutions: (●), Bath, Na^+ rich; pipette, Na^+ rich (pH 7.9). Data are from one patch. (△), Bath Na^+ rich; pipette, bicarbonate replacement (pH 7.9). Data are from three patches on cells in two different monolayers. Current records have been low-pass filtered at 100 Hz. Lines were fitted by second-order polynomial least squares regression analysis.

nonselective or selects for chloride over sodium and potassium.

We tested for anion selectivity of the 'small' channel in cell-attached patches by replacing 100 mM Cl^- in the pipette solution with other anions. If the channel selects for chloride over the replacement anion then this manoeuvre will cause a negative (rightward) shift in the single-channel current reversal potential. A shift of −29 mV will occur if the replacement anion is impermeant provided: (i) that the change in pipette anion composition does not affect the intracellular concentration of permeant ions, and (ii) that the electrochemical gradient for chloride is identical for all cells used in the analysis [16]. We adopted this approach to determine anion selectivity because it proved impossible to retain activity of the small-conductance channel in excised patches. Figure 2*A* and *B* shows that replacing 100 mM Cl^- in the pipette solution with gluconate caused the reversal potential to shift by −25 mV (mean of two experiments, individual values were −24 and −25 mV), confirming that the 'small' channel is anion selective.

Figure 2*C* and *D* shows data from similar experiments in which we examined the bicarbonate permeability of the channel. Because the HCO_3^--replacement pipette solution has a pH of 7.9, a Na^+-rich pipette solution with the same pH was used to obtain the control *I/V* plot (filled circles in Fig. 2*D*). Replacing 100 mM of the chloride in the Na^+-rich pipette solution with bicarbonate (pH 7.9) caused the reversal potential to shift by −29 mV (mean of three experiments, individual values were −27, −28 and −33 mV), indicating that the 'small' channel has a very low bicarbonate permeability relative to chloride (Fig. 2*D*).

The current traces illustrated in Figs. 1 and 2 suggest that opening of the small-conductance chan-

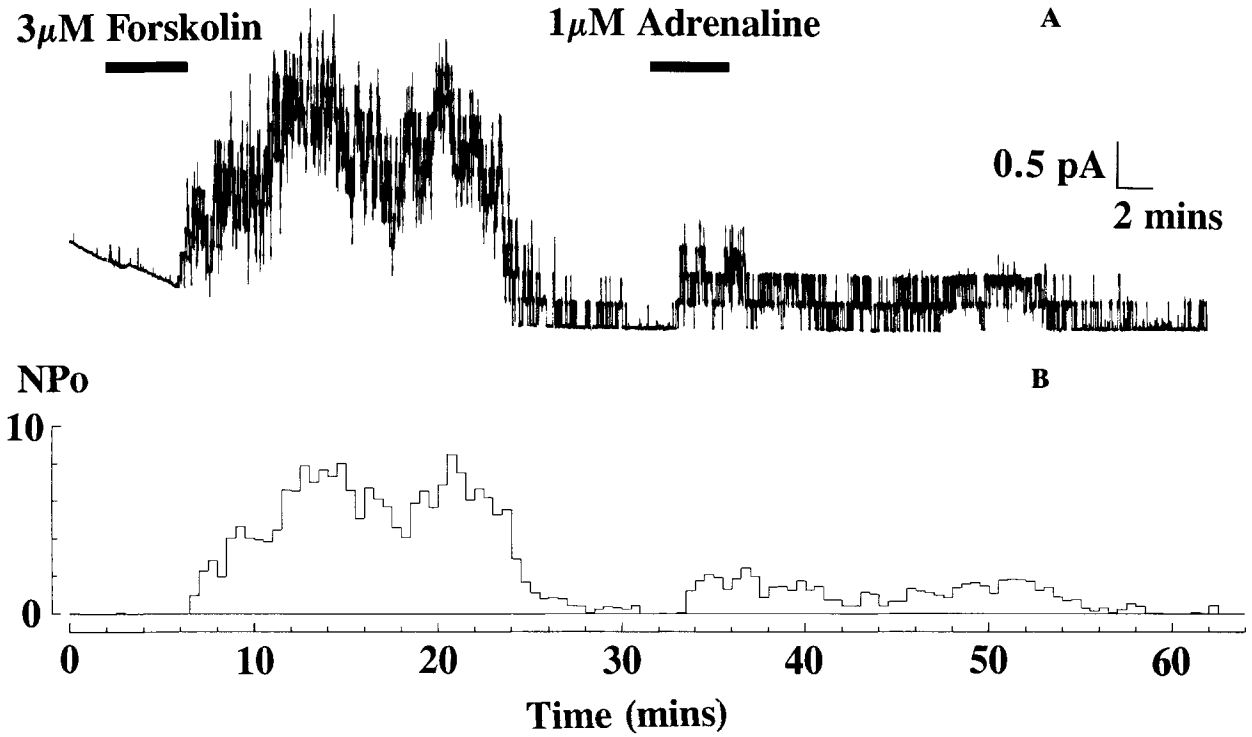

Fig. 3. Simulation of small-conductance channel activity by forskolin and adrenaline. (*A*) Single-channel currents recorded at V_p = −55 mV from a cell-attached patch. Low pass filtered at ~50 Hz by the frequency response of the chart recorder. Solutions: bath and pipette, Na^+ rich. For the duration of the horizontal bars a Na^+-rich solution containing either 3 μM forskolin or 1 μM adrenalin was perfused through the bath. The falling current baseline at the start of the experiment was caused by a gradual increase in seal resistance. (*B*) Channel activity. The initial baseline current drift and the large number of channels in this patch precluded single-channel P_o analysis using our computer software, so we have expressed channel activity as NP_o (*see* Materials and Methods). The baseline current over the period 5 to 24 min was estimated by interpolation using the current level just before channel activation and the level at the next fully closed state.

nel is not markedly voltage dependent. In one experiment we tested for this more carefully by switching the pipette potential between −60 mV (depolarizing the patch) and 60 mV (hyperpolarizing the patch) at 30-sec intervals. The total recording time was 120 sec at each potential, and the calculated P_o values were 0.07 at −60 mV and 0.03 at 60 mV. This indicates that channel activity can be increased by membrane depolarization; however, the fact that a 120-mV change in membrane potential was required to doubled P_o indicates that voltage is unlikely to have a physiological role in regulating the channel.

Figure 3 shows that exposing epididymal cells to forskolin (3 μM) and adrenaline (1 μM) increased channel activity (expressed as the product NP_o), and that these effects were reversed after washout of the stimulants from the tissue bath. In this particular experiment, forskolin increased channel activity by a maximum of 355-fold and adrenaline by 123-fold. During stimulation with forskolin there were up to eleven small-conductance channels simultaneously open in the patch (Fig. 3). In all probability, the ~5 min delay between addition of forskolin and stimulation of channel activity partly reflects the time required for bath fluid exchange, and partly the time taken for forskolin to cross the cell membrane and activate the catalytic subunit of adenylate cyclase. The activation delay with adrenaline, which will act on plasma membrane receptors, was only ~1.5 min. Reversible activation of the channel was also observed in two out of two additional experiments with forskolin and adrenaline performed on different monolayers. In both the forskolin trials channel activity was stimulated 102-fold, whereas with adrenaline the increases were 23-fold and three-fold. These results suggest that regulation of the channel on intact cells may be mediated by intracellular cyclic AMP.

The Large-Conductance Channel

Activity of the 'small' channel disappeared after patches were excised into the inside-out recording configuration. However, in these excised patches, and in others that had been quiescent during cell-

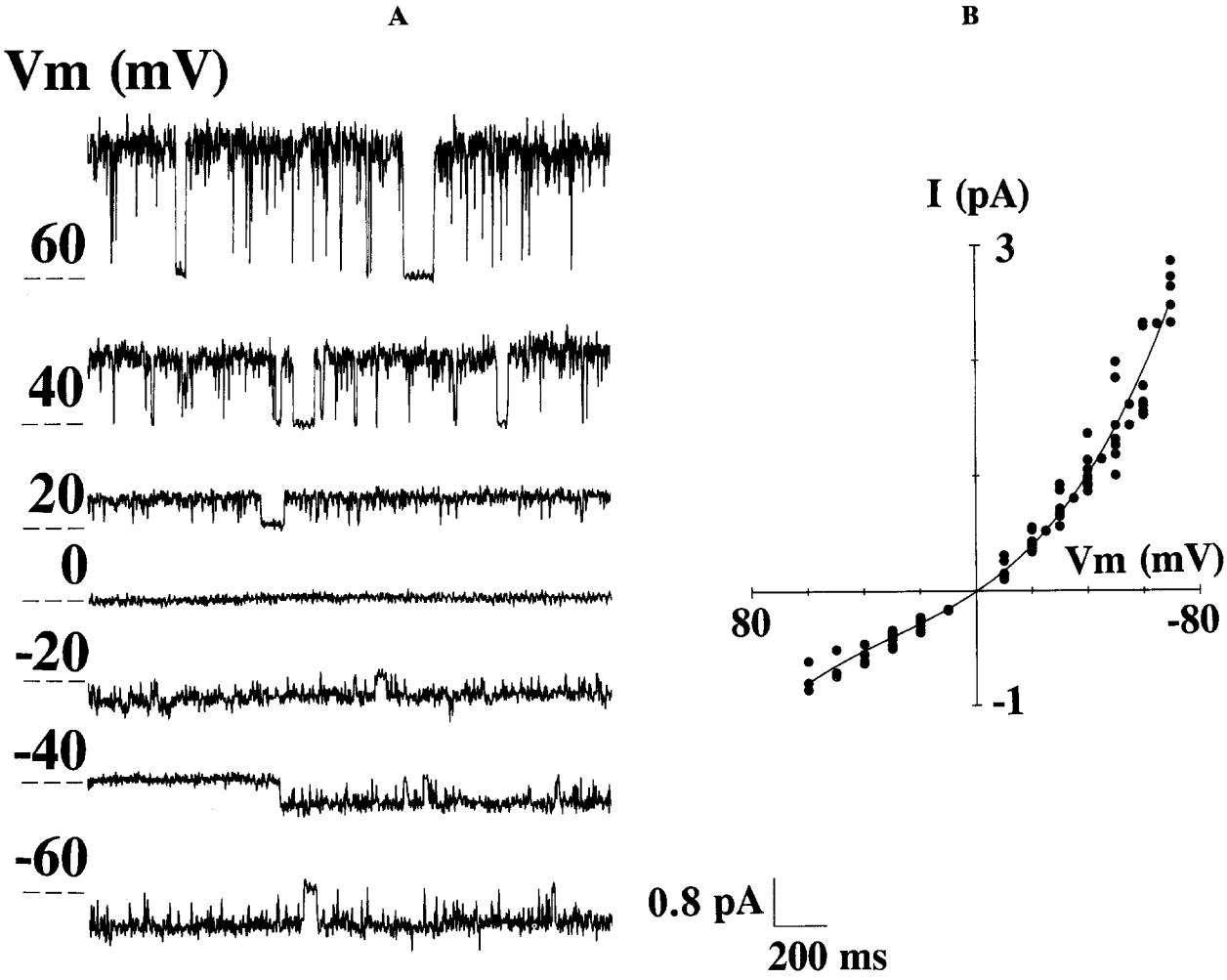

Fig. 4. The large-conductance channel in excised, inside-out patches. (*A*) Typical current traces recorded at the membrane potentials (V_m) indicated. Solutions: Bath and pipette, Na^+ rich. Current records have been low-pass filtered at 500 Hz. (*B*) Single-channel *I/V* plot. Solutions: Bath and pipette, Na^+ rich. Data are from 10 patches excised from different cells.

attached recording, we could often initiate activity of a larger conductance channel by depolarizing the patch (40 mV for about 20 sec). Because there was no obvious correlation between activation of a 'large' channel and the presence of 'small' channels prior to patch excision it seems unlikely that the former could arise by an association of 'small' channels. We never observed activity of the large-conductance channel in cell-attached patches.

Figure 4*A* shows single-channel currents flowing through the 'large' channel in an excised patch bathed in symmetrical Na^+-rich solutions. One channel was present in this patch and it remained open for several sec during which time there was a considerable degree of open-channel noise (Fig. 4*A*). With a Na^+-rich solution containing 150 mM Cl^- on both sides of the patch, the unitary currents reversed at 0 mV and the *I/V* plot displayed outward rectification with conductances of 29 pS for outward and 13 pS for inward currents (ratio of outward to inward conductance = 2.2) (Fig. 4*B*).

Details of the ion selectivity of the large-conductance channel are shown in Fig. 5. Switching from a Na^+-rich to a K^+-rich bath solution had no effect on the *I/V* plot in excised patches, indicating that the channel does not select for sodium or potassium (Fig. 5*B*). On the other hand, imposing a threefold chloride concentration gradient across an excised patch, by replacing bath chloride with gluconate, shifted the reversal potential by −20 mV (Fig. 5*A* and *B*). In one other experiment of this type, the shift in reversal potential was −17 mV. From these results $P_{gluconate}/P_{chloride}$ for the 'large' channel can be calculated as 0.24.

Figure 5*D* shows that increasing the pH of the Na^+-rich pipette solution from 7.4 to 7.9 had no

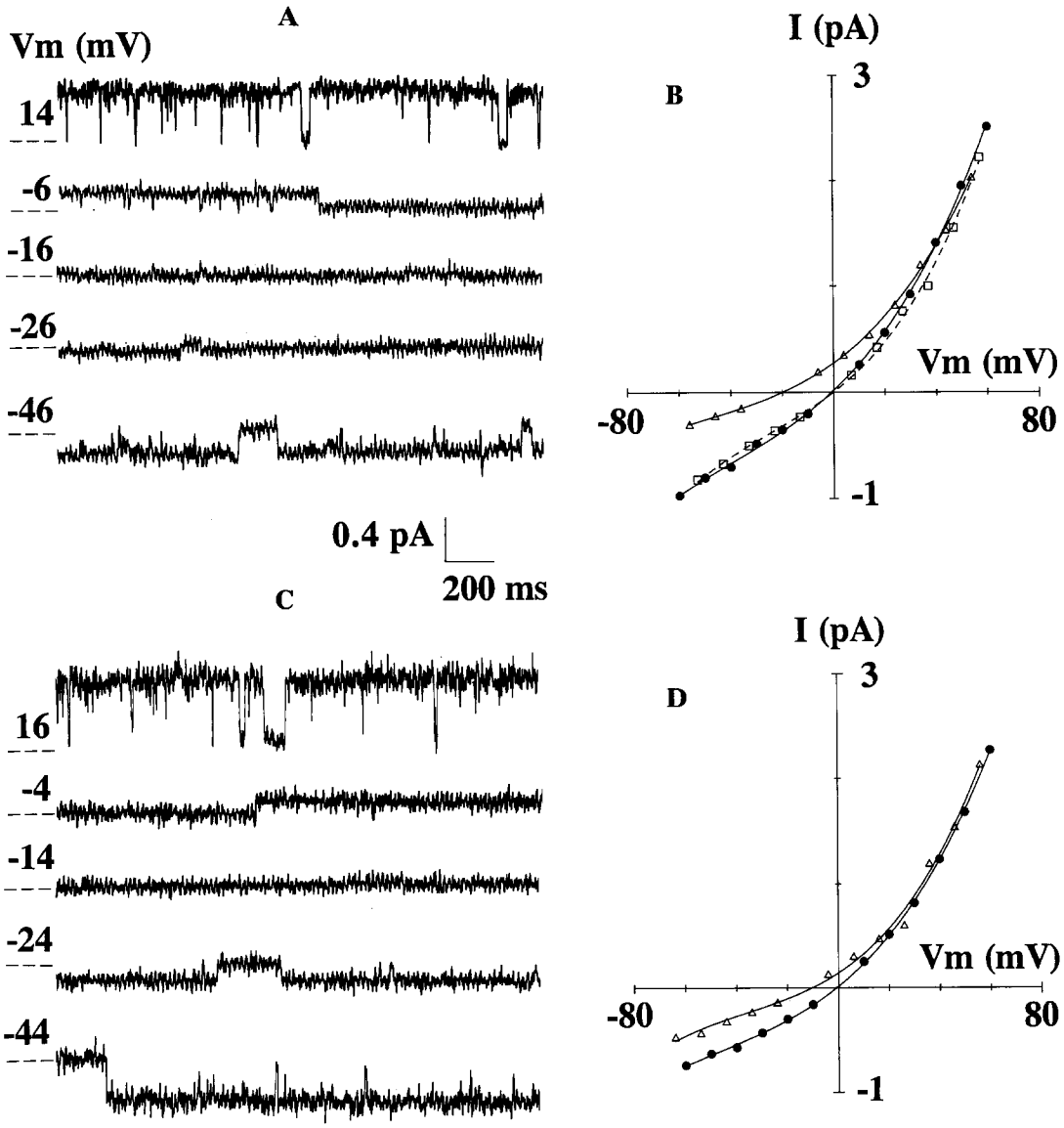

Fig. 5. Anion selectivity of the large-conductance channel in excised, inside-out patches. (*A*) Single-channel currents recorded at the membrane potentials (V_m) indicated. Solutions: Bath, gluconate replacement; pipette, Na^+ rich. (*B*) Single-channel *I/V* plots. (●) Bath and pipette, Na^+ rich. (□—data fit indicated by dashed line) Bath, K^+ rich; pipette, Na^+ rich. (△) Bath, gluconate replacement; pipette, Na^+ rich. (*C*) Single-channel currents. Solutions: bath, bicarbonate replacement; pipette, Na^+ rich. (*D*) Single-channel *I/V* plots. (●) Bath, Na^+ rich (pH 7.9); pipette, Na^+ rich. (△) Bath, bicarbonate replacement (pH 7.9); pipette, Na^+ rich. Current records have been low-pass filtered at 300 Hz. Lines were fitted by third-order polynomial least squares regression analysis. All these data were obtained from one excised patch.

effect on the *I/V* plot for the large-conductance channel. However, when two thirds of the bath chloride was replaced with bicarbonate (pH 7.9) the reversal potential was shifted by −10 mV. In another experiment of this type the shift in reversal potential was −16 mV (Fig. 5*C* and *D*). Taken together, these results give a calculated $P_{\text{bicarbonate}}/P_{\text{chloride}}$ of 0.4.

Inspection of the current traces in Fig. 4*A* suggests that like the 'small' channel, activity of the larger conductance channel is not voltage dependent. We checked this more carefully by holding an excised patch at −25 mV for 50 sec and then switching the potential to 25, −50 and 50 mV for the same period of time. This cycle was then repeated six times, giving a total recording time of 300 msec at each voltage. The calculated single-channel P_o values were 0.63, 0.59, 0.64 and 0.72 at −25, 25, −50 and 50 mV, respectively. These results confirm that once activated, opening of this 'large' channel is not affected by membrane voltage.

Table. The proportion of patches on unstimulated and stimulated epididymal cells that contained active chloride channels[a]

Cl^- channel	C-A patches		I-O patches	
	Unstimulated	Stimulated	Unstimulated	Stimulated
Small conductance	54/189 (29%)	42/219 (19%)	—	—
Large conductance	0/189 (0%)	0/219 (0%)	7/248 (3%)	31/370 (8%)

[a] Stimulated cells were exposed to a mixture of dibutyryl cyclic AMP (0.1 mM), isobutylmethylxanthine (0.1 mM) and forskolin (1 μM) for at least 30 min prior to gigaseal formation. Note that for the 'large' channel the number of excised, inside-out (I-O) patches is greater than the number of cell-attached (C-A) patches. This results from the fact that gigaohm seals were not always established in the C-A configuration but developed when the patch was excised.

Occurrence of the 'Small' and 'Large' Channels

The frequency with which we observed the two types of outwardly rectifying chloride channel is detailed in the Table. Note that 'small' channel activity was detected in 29% of cell-attached patches on unstimulated cells, and that channel activity disappeared when these patches were excised. On the other hand, the 'large' channel was never observed in cell-attached patches and could be activated in only 3% of excised patches derived from unstimulated cells. Exposing the cells to dibutyryl cAMP (0.1 mM), isobutylmethylxanthine (0.1 mM) and forskolin (1 μM) for at least 30 min prior to giga-seal formation reduced by one-third the proportion of cell-attached patches containing the small-conductance channel (Table). However, the same procedure more than doubled (to 8%) the proportion of excised patches that contained the larger channel (Table).

Discussion

Our major finding is that two types of outwardly rectifying chloride channel are present on epithelial cells cultured from human fetal epididymis. The 'large' channel had conductances of 13 and 29 pS for inward and outward currents, respectively, while for the 'small' channel these values were 2.8 and 5 pS, respectively. We did not detect the 20 pS nonrectifying channel that is present on the apical membrane of rat cauda epididymis [8].

'Large' Channel

This chloride channel was never observed in cell-attached patches but was activated by depolarization (40 mV) in 3% of patches excised from unstimulated cells. Its characteristics can be summarized as follows: (i) Outward rectification with the ratio of outward-to-inward conductance = 29/13 pS = 2.2. This rectification must be an inherent property of the channel since it occurs when equal chloride concentrations are present on both sides of the patch. (ii) Rarely more than two channels per patch. (iii) Open state probability independent of voltage once the channel is activated. (iv) Significant permeability to gluconate; $P_{gluconate}/P_{chloride} = 0.24$. (v) Relatively high permeability to bicarbonate; $P_{bicarbonate}/P_{chloride} = 0.4$. A similar, outwardly rectifying, chloride channel has previously been identified on cultured rat epididymal cells [2], in a number of other anion secreting epithelia (enterocytes [10, 12], T_{84} and HT_{29} cell lines [17, 20, 31], pancreatic duct cells [15, 30]), and in airways epithelium where its regulation by protein kinase A and C-kinase has been reported to be defective in cystic fibrosis (CF) [22, 24, 25, 29].

As we never detected activity of this large-conductance chloride channel in cell-attached patches on epididymal cells, its physiological role must remain uncertain. We came to the same conclusion regarding the function of this channel in the pancreas [15]. However, the proportion of excised patches that contained the channel was more than doubled (from 3 to 8%) by prior exposure of the epididymal cells to a cocktail of stimulants which increases intracellular cyclic AMP. This may indicate that at least the number of large-conductance channels can be modulated by this cyclic nucleotide. Outwardly rectifying whole-cell chloride conductances have been detected in osmotically swollen epithelial cells [38] and in dividing lymphocytes [3], perhaps indicating a role for this channel in cell volume regulation. Outward rectification is also a feature of the Ca^{2+}-activated whole cell chloride currents in T_{84} and airways epithelial cells [6].

'Small' Channel

This was the chloride channel we detected most frequently; it occurred in 29% of all cell-attached patches on unstimulated cells. Its characteristics can be summarized as follows: (i) Outward rectification with the ratio of outward to inward conductances = 5/2.8 pS = 1.8. (ii) Clustering with up to 11 channels per patch. (iii) An open-state probability which is essentially independent of voltage over the physiological range of membrane potentials. (iv) A very low bicarbonate permeability relative to chloride. (vii) Rapid rundown following patch excision.

Previously, small-conductance chloride channels have been identified in lacrimal acinar cells (1–2 pS) [26], in shark rectal gland (11 pS) [13], in the choroid plexus (2–7 pS) [5], and on thyroid epithelial cells (5.5 pS) [4]. However, the 'small' chloride channel in the epididymis more closely resembles a cyclic AMP-regulated channel that we originally identified on rat and human pancreatic duct cells [14–16]. In these cells the channel facilitates bicarbonate secretion via a Cl^-/HCO_3^- exchanger by allowing chloride to recycle across the apical membrane. Recently, a chloride channel with broadly similar properties has also been identified on the human colonic cell line T_{84} [31].

Activity of the 'small' channel in the epididymis could be markedly stimulated when cells were exposed to either forskolin or adrenaline during cell-attached recording. In the presence of forskolin up to 11 channels were observed to open simultaneously in cell-attached patches, and increases in channel activity (expressed as NP_o) of up to 350-fold were detected. These results show that the small-conductance channel in the epididymis can be upregulated by cyclic AMP and, therefore, that it might play a role in electrogenic anion secretion [9, 32, 33]. Stimulation of 'small' channel activity by cyclic AMP during cell-attached recording was also observed in rat and human pancreatic duct cells [14, 15] but did not occur in T_{84} cells [31]. Nevertheless, prior exposure of T_{84} cells to stimulants did increase the number of cell-attached patches containing active channels by greater than sixfold [31]. While we did not observe this effect in the human epididymis, or in a previous study on human pancreatic duct cells [15], the proportion of patches on rat pancreatic duct cells that contained small-conductance channels did increase by 2.3-fold after exposure to stimulants which elevate intracellular cyclic AMP [14].

In our previous studies on pancreatic duct cells we did occasionally observe small-conductance channels in excised patches, and under these conditions the *I/V* plot was always linear provided there was no chloride concentration gradient across the patch (*see* Fig. 2*B* in ref. 14; Fig. 4*B* in ref. 15; and Fig. 2*C* in ref. 16). This suggests that outward rectification of the 'small' channel in cell-attached patches is due to a lower chloride concentration in the cytoplasm of the epididymal cells compared to the pipette solution. These small-conductance channels may, therefore, underlie the linear, cyclic AMP-activated, whole-cell chloride currents recently detected (using the patch-clamp technique) in some chloride secreting epithelia [6]. Moreover, a linear whole cell chloride conductance [1, 11, 23], together with small conductance chloride channels of a type very similar to those we described in this report [23], also appear in epithelial and nonepithelial cells that have been transfected with the normal CF gene. This suggests that the 'small' channel is at least regulated by the CF gene product, and may even be the gene product itself [1, 23]. Recently, we have shown that cultured epididymis and vas deferens both express the CF gene [7]. Therefore, a defect in 'small' channel regulation might well underlie the degenerative changes in these structures that render more than 97% of CF males sterile.

We thank Mr. N. Hampton for help with data analysis and Dr. M.A. Gray for helpful discussions. This work was funded by the Cystic Fibrosis Research Trust (UK), the Cystic Fibrosis Foundation (USA), and a small grants research award from the University of Newcastle upon Tyne. Ethical committee approval was obtained for this work.

References

1. Anderson, M.P., Rich, D.P., Gregory, R.J., Smith, A.E., Welsh, M.J. 1981. Generation of cAMP-activated chloride currents by expression of CFTR. *Science* **251**:679–682
2. Ashford, M.L.J. 1986. Single chloride channels in cultured rat epididymal cells. *J. Physiol.* **371**:142P
3. Bubien, J.K., Kirk, K.L., Rado, T.A., Frizzell, R.A. 1990. Cell cycle dependence of chloride permeability in normal and cystic fibrosis lymphocytes. *Science* **248**:1416–1419
4. Champigny, G., Verrier, B., Gérard, C., Mauchamp, J., Lazdunski, M. 1990. Small conductance chloride channels in the apical membrane of thyroid cells. *FEBS Lett.* **259**:263–268
5. Christensen, O., Simon, M., Randlev, T. 1989. Anion channels in a leaky epithelium. A patch clamp study of choroid plexus. *Pfluegers Arch.* **415**:37–46
6. Cliff, W.H., Frizzell, R.A. 1990. Separate Cl^- conductances activated by cAMP and Ca^{2+} in Cl^--secreting epithelial cells. *Proc. Natl. Acad. Sci. USA* **87**:4956–4960
7. Coleman, L., Harris, A. 1991. Immortalization of male genital duct epithelium: An assay system for the cystic fibrosis gene. *J. Cell Sci.* **98**:85–89
8. Cook, D.I., Huang, S.J., Wilson, S.M., Wong, P.Y.D., Young, J.A. 1990. Ion channels in the apical plasma membrane of the rat cauda epididymis. *J. Physiol.* **423**:57P
9. Cuthbert, A.W., Wong, P.Y.D. 1986. Anion secretion in cultured rat epididymal epithelium. *J. Physiol.* **378**:335–345
10. Diener, M., Rummel, W., Mestres, P., Lindemann, B. 1989. Single chloride channels in colon mucosa and isolated colonic enterocytes of the rat. *J. Membrane Biol.* **108**:21–30

11. Drumm, M.L., Pope, H.A., Cliff, W.H., Rommens, J.M., Marvin, S.A., Tsui, L.-S., Collins, F.S., Frizzell, R.A., Wilson, J.M. 1990. Correction of the cystic fibrosis defect in vitro by retrovirus-mediated gene transfer. *Cell* **62:**1227–1233
12. Giraldez, F., Murray, K.J., Sepúlveda, F.V., Sheppard, D.N. 1989. Characterization of a phosphorylation-activated Cl^--selective channel in isolated *Necturus* enterocytes. *J. Physiol.* **416:**517–537
13. Gögelein, H., Schlatter, E., Greger, R. 1987. The 'small' conductance chloride channel in the luminal membrane of the rectal gland of the dogfish. *Pfluegers Arch.* **409:**122–125
14. Gray, M.A., Greenwell, J.R., Argent, B.E. 1988. Secretin-regulated chloride channel on the apical plasma membrane of pancreatic duct cells. *J. Membrane Biol.* **105:**131–142
15. Gray, M.A., Harris, A., Coleman, L., Greenwell, J.R., Argent, B.E. 1989. Two types of chloride channel on duct cells cultured from human fetal pancreas. *Am. J. Physiol.* **257:**C240–C251
16. Gray, M.A., Pollard, C.E., Harris, A., Coleman, L., Greenwell, J.R., Argent, B.E. 1990. Anion selectivity and block of the small conductance chloride channel on pancreatic duct cells. *Am. J. Physiol.* **259:**752–761
17. Halm, D.R., Rechkemmer, G.R., Schoumacher, R.A., Frizzell, R.A. 1988. Apical membrane chloride channels in a colonic cell line activated by secretory agonists. *Am. J. Physiol.* **254:**C505–C511
18. Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100
19. Harris, A., Coleman, L. 1989. Ductal epithelial cells cultured from human fetal epididymis and vas deferens: Relevance to sterility in cystic fibrosis. *J. Cell Sci.* **92:**687–690
20. Hayslett, J.P., Gögelein, H., Kunzelmann, K., Greger, R. 1987. Characteristics of apical chloride channels in human colonic cells (HT_{29}). *Pfluegers Arch.* **410:**487–494
21. Hinton, B.T., Turner, T.T. 1988. Is the epididymis a kidney analogue?. *News Physiol. Sci.* **3:**28–31
22. Hwang, T.-C., Lu, L., Zeitlin, P.L., Gruenert, D.C., Huganir, R., Guggino, W.B. 1989. Cl^- channels in CF: Lack of activation by protein kinase C and cAMP-dependent protein kinase. **244:**1351–1353
23. Kartner, N., Hanrahan, J.W., Jensen, T.J., Naismith, A.L., Sun, S., Ackerley, C.A., Reyes, E.F., Tsui, L.-C., Rommens, J.M., Bear, C.E., Riordan, J.R. 1991. Expression of the cystic fibrosis gene in non-epithelial invertebrate cells produces a regulated anion conductance. *Cell* **64:**681–691
24. Li, M., McCann, D., Liedtke, C.M., Nairn, A.C., Greengard, P., Welsh, M.J. 1988. Cyclic AMP-dependent protein kinase opens chloride channels in normal but not cystic fibrosis airway epithelium. *Nature* **331:**358–360
25. Li, M., McCann, J.D., Anderson, M.P., Clancy, J.P., Liedtke, C.M., Nairn, A.C., Greengard, P., Welsh, M.J. 1989. Regulation of chloride channels by protein kinase C in normal and cystic fibrosis airway epithelia. *Science* **244:**1353–1356
26. Marty, A., Tan, Y.P., Trautmann, A. 1984. Three types of calcium-dependent channel in rat lacrimal glands. *J. Physiol.* **357:**293–325
27. Okamura, N., Tajima, Y., Sugita, Y. 1988. Decrease in bicarbonate transport activities during epididymal maturation of porcine sperm. *Biochem. Biophys. Res. Commun.* **157:**1280–1287
28. Pollard, C.E., Harris, A., Coleman, L., Argent, B.E. 1990. Chloride channels on human epididymal cells *in vitro*. *J. Physiol.* **426:**72P
29. Schoumacher, R.A., Shoemaker, R.L., Halm, D.R., Tallant, E.A., Wallace, R.W., Frizzell, R.A. 1987. Phosphorylation fails to activate chloride channels from cystic fibrosis airway cells. *Nature* **330:**752–754
30. Tabcharani, J.A., Jensen, T.J., Riordan, J.R., Hanrahan, J.W. 1989. Bicarbonate permeability of the outwardly rectifying anion channel. *J. Membrane Biol.* **112:**109–122
31. Tabcharani, J.A., Low, W., Elie, D., Hanrahan, J.W. 1990. Low-conductance chloride channel activated by cAMP in the epithelial cell line T_{84}. *FEBS Lett.* **270:**157–164
32. Wong, P.Y.D. 1988. Mechanism of adrenergic stimulation of anion secretion in cultured rat epididymal epithelium. *Am. J. Physiol.* **254:**F121–F133
33. Wong, P.Y.D. 1988. Inhibition by chloride channel blockers of anion secretion in cultured epididymal epithelium and intact epididymis of rats. *Br. J. Pharmacol.* **94:**155–163
34. Wong, P.Y.D., Chan, T.P.T. 1988. Adrenergic control of electrogenic anion secretion in primary cultures of rat epididymal cells. *In*: Gastrointestinal and Hepatic Secretions: Mechanism and Control. J.S. Davison and E.A. Shaffer, editors. pp. 216–219. University of Calgary Press, Calgary
35. Wong, P.Y.D., Huang, S.J. 1989. Intracellular pH measurement in primary monolayer cultures of rat epididymal cells. *Pfluegers Arch.* **413:**414–421
36. Wong, P.Y.D., Huang, S.J. 1990. Secretory agonists stimulate a rise in intracellular cyclic AMP but not Ca^{2+} and inositol phosphates in cultured rat epididymal epithelium. *Exp. Physiol.* **75:**321–337
37. Wong, P.Y.D., Yeung, C.H. 1978. Absorptive and secretory functions of the perfused rat cauda epididymidis. *J. Physiol.* **275:**13–26
38. Worrell, R.T., Butt, A.G., Cliff, W.H., Frizzell, R.A. 1989. A volume-sensitive chloride conductance in human colonic cell line T84. *Am. J. Physiol.* **256:**C1111–C1119

Received 25 February 1991; revised 24 May 1991