Biochimica et Biophysica Acta, 1104 (1992) 160–164

BBAMEM 75523

Halide permeation through 10 pS and 20 pS anion channels in human airway epithelial cells

Malgorzata A. Wilk-Blaszczak, Andrew S. French and S.F. Paul Man

Departments of Physiology and Medicine, University of Alberta, Edmonton, Alberta (Canada)

(Received 11 June 1991)

Key words: Chloride channel; Airway epithelium; Mole fraction; Cystic fibrosis; Ion permeation

Halide permeability sequences were obtained from reversal potential measurements of single-channel currents through 10 pS and 20 pS anion channels in human airway epithelial cells. The sequences obtained were $Cl^- > I^- > Br^- \geq F^-$ for the 10 pS channel and $Cl^- > I^- \geq Br^- \geq F^-$ for the 20 pS channel. However, the permeability differences were not large, the greatest being 0.66 for the ratio of fluoride to chloride permeability in the 20 pS channel. Single-channel currents were also measured with solutions of constant halide concentration but varying ratios of chloride to fluoride ions. An anomalous mole fraction effect was observed for the 20 pS channel but not for the 10 pS channel, suggesting that the former is a multi-ion channel. Comparison of the halide permeability sequences of these two channels with those of whole-cell currents in other epithelial cells does not support their involvement in any of the known whole-cell epithelial currents.

Introduction

The apical membranes of airway epithelial cells contain anion channels whose function is crucial for controlling the chloride and sodium fluxes through the epithelium. These ionic fluxes affect the viscoelastic properties of the mucus and hence the effectiveness of mucociliary clearance. Chloride permeability is reduced in cystic fibrosis, causing defective mucociliary clearance [1–3].

Single-channel experiments have identified at least four types of anion channels in human airway epithelia [4]. The most widely studied are the outwardly rectifying channels, which have a conductance of ≈ 45 pS in physiological chloride concentrations at zero membrane potential [5–8]. However, anion channels with linear conductances of ≈ 20 pS and ≈ 10 pS have also been described in human and canine airway [5,8,9] with the 20 pS channels possibly containing two groups [10], and large anion channels of ≈ 350 pS conductance have been observed in a variety of epithelia, including human airway [4,8]. Recently, fluctuation analysis and single-channel experiments have suggested that the human airway epithelium also contains a significant number of anion channels with conductances below 5 pS [11,12].

The relative contributions of these different channels to normal and pathological airway functions are not yet known. One method for estimating their contributions to whole-cell or total epithelial fluxes is to discover their relative permeabilities to different anions and then compare the permeability sequences found with each type of measurement. The halide permeability sequence of the rectifying channel has been estimated in human airway cells [7] and in the T84 colonic cell line [13]. Halide permeability sequences have also been used as one method of distinguishing different chloride currents in T84 cells [14].

The permeability of the 20 pS channel in the human airway has been measured for several anions but not for halides other than chloride [10]. These measurements were used to construct a model of permeation in the 20 pS channel in which a single anion occupied a relatively weak binding site in the channel.

We have now measured the relative halide permeabilities of 10 pS and 20 pS anion channels in human nasal airway epithelial cells. In addition, we used solutions containing mixtures of Cl^- and F^- ions in order to test the hypothesis that permeation through these channels occurs by single ion binding.

Correspondence: A.S. French, Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

Methods

Nasal epithelial tissues, obtained from turbinate surgery on 15 human subjects, were treated with 0.2% protease and 0.1% DNase in calcium-free medium (DME-F12) at 4°C for 16 to 24 h. The enzymes were neutralized by adding fetal bovine serum (FBS) to the mixture (final concentration 10%) for 30 min, and then the cells were detached from the epithelial strips by gentle mechanical agitation. Free cells were filtered through a Nitex nylon mesh (60 μm), centrifuged at 200 $\times g$ for 10 min, pelleted and then resuspended in 10% FBS in DME-F12. Trypan blue exclusion was used to test viability, which was usually greater than 90%. The cells were washed once more and then plated at low density on collagen coated dishes and incubated in 5% CO_2 at 37°C. The culture medium contained DME-F12 supplemented with insulin (2 μg/ml), transferrin (7.5 μg/ml), hydrocortisone (18 ng/ml), cholera toxin (10 ng/ml), T_3 (2 ng/ml), epidermal growth factor (13 ng/ml), endothelial cell growth factor (7.5 μg/ml), and antibiotics (gentamycin 50 μg/ml, streptomycin 100 μg/ml, and penicillin-G 60 μg/ml). Experiments were performed on single cultured cells. The temperature was kept at 36 ± 1°C during all experiments by means of a thermostatically controlled stage. Experiments were performed with cells between 1 and 4 days after plating.

Single-channel currents were recorded from excised patches of apical membranes in the inside-out configuration using a List EPC-7 amplifier [15]. Pipets were fabricated from thick walled microfilament borosilicate glass using a horizontal puller (Sutter Instruments P-87) in three steps, coated with Sylgard (Dow Corning), and fire polished with a microforge. Pipet resistances were 16–25 MΩ when filled with 145 mM choline chloride solution and gave seal resistances of about 30 GΩ. In order to minimize junction potentials, a NaCl-agar-filled bridge was used to connect the reference electrode to the bathing solution. Pipet offset potentials were measured and corrected before forming a seal. Changes in junction potential at the reference electrode due to changing halide solutions in the bath were calculated from the Henderson equation [16] and subtracted from the measured membrane potential to obtain the potential across the patch. All potentials are reported relative to zero in the extracellular solution and positive currents are outwards throughout.

Channel current signals from the amplifier were fed into a digital VCR recorder adaptor (Medical Systems, PCM-1) and stored on video tape. A digital computer sampled the data at 10 kHz with a 12-bit analog-to-digital convertor. Filtering was carried out after sampling by a Gaussian digital filter.

The procedures used for data analysis were based largely on those described by Colquhoun and Sigworth [17]. The half-amplitude criterion was used as a threshold to distinguish between open and closed states. Event durations were corrected for filter rise-time by a polynomial approximation [17]. Distributions of open and closed times were created and the probability of the channel being in the open state was calculated. Only openings longer than the filter dead-time were used to compute the mean channel current amplitude.

The pipet solution used in all experiments contained (mM): 145 choline chloride, 0.2 $CaCl_2$, 0.1 $MgCl_2$, 10 Hepes (pH 7.35). Choline is not measurably permeable through either of the cation channels that have been observed in human airway epithelial cells [18]. The initial bath solution for all experiments contained (mM): 145 NaCl, 0.2 $CaCl_2$, 0.1 $MgCl_2$, 10 Hepes (pH 7.35). For halide exchange experiments the NaCl was replaced with NaBr, NaI or NaF. Changes in the bath solution were made by moving the pipet tip into a small chamber separated from the main dish. A flow system with very small dead space allowed rapid solution exchanges.

Relative ion permeabilities were calculated from reversal potentials, V_r:

$$V_r = \frac{RT}{F} \ln \frac{[Cl^-]_o + (P_A/P_{Cl})[A^-]_o}{[Cl^-]_i + (P_A/P_{Cl})[A^-]_i} \quad (1)$$

[19] where subscripts 'o' and 'i' denote the outside and inside solutions, respectively, P_A is the permeability of an anion, A^-, and R, T, and F are the gas constant, temperature, and Faraday constant, respectively. Reversal potentials were determined from current-voltage relationships plotted for the different anions.

Results

From a total of 236 successful patches formed on human nasal airway cells, 43 anion channels were observed with conductances in the range 8.3–12.5 pS, and 27 in the range 15.2–26.0 pS, all with linear current-voltage relationships. A value of 12 pS was used previously to separate these two groups of channels [8] and the upper value of 12.5 pS used here for the 10 pS channels gave a clear separation between the two groups. The halide permeation properties of the two groups were also well separated by this division. These two groups will be referred to as 10 pS and 20 pS channels throughout. In addition, more than 20 other patches contained anion permeable channels with linear conductances which were probably between 8 and 30 pS, but were not accurately characterized because the seal failed. Patches containing rectifying channels in the range 45–60 pS and large channels (≈ 350 pS) were observed but not processed further. Seven other patches contained more than one anion channel and were not analyzed. Most patches also contained very

small channels (≈ 5 pS) which will be described in a separate publication. The results described here were obtained from thirteen 10 pS channels and eighteen 20 pS channels which were stable throughout the complete set of solution changes required for each experiment.

Fig. 1 shows typical single-channel recordings of 10 pS and 20 pS channels with each of the different halide solutions in the bath. Additionally, recordings in symmetrical chloride solutions are shown with membrane potentials of +50 mV and −50 mV to illustrate the linear conductance. In the case of the 10 pS channel there were no distinct changes in current amplitude on shifting to different halide solutions, while the 20 pS channel was clearly less permeant to fluoride ions.

Fig. 2 shows current-voltage relationships for a 10 pS channel with each of the different halide solutions in the bath. At positive membrane potentials the current would be caused by chloride ion movement from the pipet in each case, while at strongly negative membrane potentials the effects of different halide permeabilities were seen. In this, and other current-voltage plots, the data from symmetrical chloride experiments were fitted by a linear regression line, but the solid lines through the remaining data were drawn by eye.

Current-voltage relationships of this type were drawn for individual 10 pS channels and the reversal potentials were used to calculate the relative permeabilities from Eqn. 1. The relative permeabilities were: Cl^- 1.0 ($n = 43$), I^- 0.97 ± 0.05 ($n = 4$), Br^- 0.86 ± 0.09 ($n =$

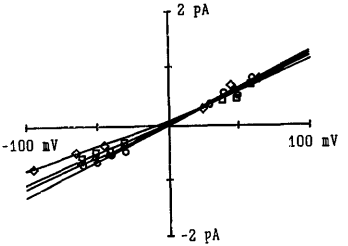

Fig. 2. Current-voltage relationships for a 10 pS channel with different halides in the bathing solution. Halide concentrations were: 145 mM Cl^- (circles), 145 mM Br^- (squares), 145 mM I^- (triangles), and 145 mM F^- (diamonds). The pipet solution contained 145 mM Cl^-.

3), F^- 0.80 ± 0.14 ($n = 6$). Single-tailed Student's t-tests applied to these data indicated that each pair of mean values in the sequence were significantly different ($P < 0.05$) except for the difference $Br^- > F^-$.

Fig. 3 shows current-voltage relationships for a 20 pS channel with different halide solutions in the bath. In this case the resulting permeability ratios were: Cl^- 1.0 ($n = 27$), I^- 0.79 ± 0.12 ($n = 6$), Br^- 0.76 ± 0.13 ($n = 4$), F^- 0.66 ± 0.05 ($n = 5$). For this sequence, t-

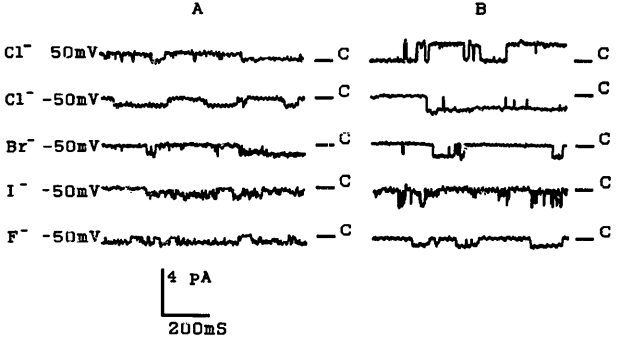

Fig. 1. Original recordings of 10 pS (A) and 20 pS (B) anion channels from excised inside-out patches of human nasal epithelial cells in different halide solutions. Membrane potentials and ionic species (145 mM) are indicated at the left. The pipet solution always contained 145 mM Cl^-. The data were all filtered at 500 Hz, and the closed condition is indicated by the letter 'C' in each case.

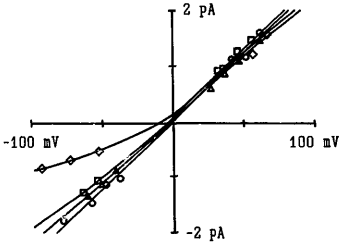

Fig. 3. Current-voltage relationships for a 20 pS channel with different halides in the bathing solution. Halide concentrations were: 145 mM Cl^- (circles), 145 mM Br^- (squares), 145 mM I^- (triangles), and 145 mM F^- (diamonds). The pipet solution contained 145 mM Cl^-.

tests indicated that only the difference $Cl^- > I^-$ was significant. The difference $I^- > F^-$ was also significant, but $I^- > Br^-$ and $Br^- > F^-$ were not.

Permeation through the 20 pS anion channel has previously been modelled by single ion binding in the channel [10]. In order to test the single ion model for both the 10 pS and 20 pS channels, we looked for anomalous mole fraction behavior in solutions containing varying proportions of chloride and fluoride ions in a total of 145 mM halide. These two ions were used because they had the largest permeability differences in both cases. Fig. 4 shows typical recordings from a 20 pS anion channel with five different mixtures of chloride and fluoride in the bathing solution.

The relative values of single-channel currents at -50 mV are shown in Fig. 5 for the five different Cl^-/F^- mixtures. Data are plotted for both 10 pS and 20 pS channels, and the solid lines show the predicted linear relationships if movements of the two ions through the

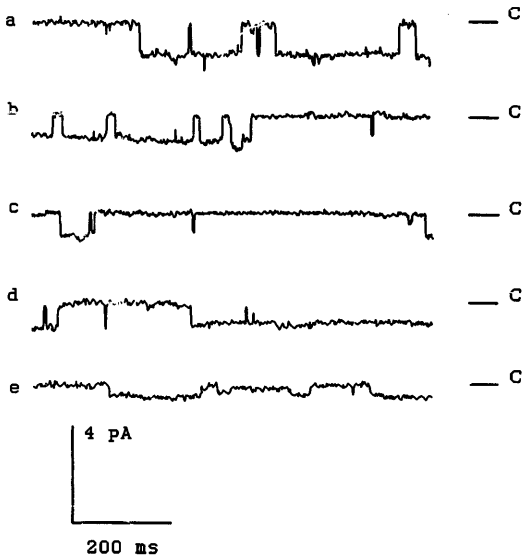

Fig. 4. Recordings obtained from a 20 pS anion channel with varying Cl^- and F^- concentrations in the bathing solution. The mixtures were: 145 mM Cl^- (a), 109 mM Cl^-/36 mM F^- (b), 72.5 mM Cl^-/72.5 mM F^- (c), 36 mM Cl^-/109 mM F^- (d), 145 mM F^- (e). The pipet solution contained 145 mM Cl^- solution and the membrane potential was −50 mV. The data were all filtered at 500 Hz, and the closed condition is indicated in each case.

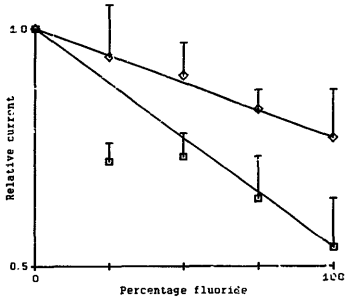

Fig. 5. The effect of varying the relative concentration of F^- to Cl^- on current flowing through 10 pS (diamonds) and 20 pS (squares) anion channels. Means and standard deviations are shown. For the 10 pS channels Five experiments were used. For the 20 pS channels the numbers of experiments were: 8 (0% F^-), 4 (25% F^-), 6 (50% F^-), 5 (75% F^-) and 7 (100% F^-). The membrane potential was −50 mV for all experiments. The solid lines join the mean values for 0 and 100% F^-.

channels were completely independent of each other. For the 10 pS channels, there was no evidence of any deviation from a linear mixture of the two ion currents, but for the 20 pS channels there was a clear deviation from linearity.

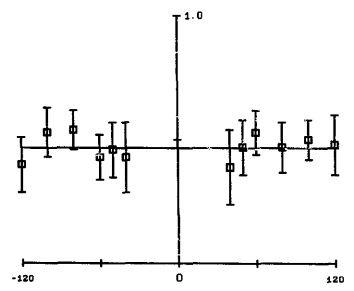

Fig. 6. The probability of 10 pS anion channels being open as a function of membrane potential. Mean ± standard deviations are shown for six different channels in symmetric 145 mM chloride solutions. The solid horizontal line shows the mean value of 0.47 for all of the recordings.

The open probability of the 20 pS anion channel was described previously and was not dependent on membrane potential [10]. Fig. 6 shows comparable data for the 10 pS channel. The mean open probability of 0.47 ± 0.18 was considerably higher than the value of 0.27 obtained for the 20 pS channel, but again there was no detectable dependence on membrane potential.

Discussion

Including both the completely and incompletely characterized anion channels gave a total density of 38% channels per patch for linear anion channels below 26 pS. This can be compared to 26.2% reported previously for similar sized electrodes on human airway epithelial cells [8]. However, the earlier study reported that 20 pS channels made up ≈ 80% of the linear channels, while the present work found that 10 pS channels were more common, accounting for ≈ 60% of the total. The differences between the two reports can probably be accounted for by assuming that the earlier study missed a significant number of 10 pS channels, as originally suggested [8].

The halide permeability sequences obtained here can be summarized as: $Cl^- > I^- > Br^- \geq F^-$ for the 10 pS channel and $Cl^- > I^- \geq Br^- \geq F^-$ for the 20 pS channel. In both cases the differences in permeability were not strong, even between chloride and fluoride. These orders of permeability do not exactly follow the Stokes radii of the ions [20] or their dehydration energies [19]. Instead, the sequences resemble a mid-range Eisenmann sequence for anions [21], indicating that permeation through both pores is controlled by a combination of dehydration and weak interaction with an internal binding site. Previous measurements on the 20 pS channel suggested values of 5 Å for the pore diameter and 3.0 kT for the energy barrier inside the channel [10], which are in good agreement with these findings. Fluoride has also been found to be the least permeable halide ion through a range of other anion channels, including epithelial tissues [22].

The anomalous mole fraction effect (AMFE) has been used before as a test of ion channel permeation mechanisms for several different channels and using a variety of measurement techniques [19,23]. If more than one ion can occupy a channel pore at one time, or if there are other interactions between a permeant ion and the channel while a different ion is traversing the channel, the channel may behave differently when exposed to mixtures of two different ions than would be predicted from a linear combination of the behaviors found when the two ions are permeating separately.

In the present experiments, mixtures of F^- and Cl^- produced currents through the 10 pS channel which would be expected from a linear combination of the two ions acting independently. However, currents

through the 20 pS channel were only ≈ 80% of the linear prediction with 25% F^-. This kind of AMFE has been seen before in inwardly rectifying potassium channels with mixtures of K^+ and Tl^+ [24] and in L-type calcium channels with mixtures of Ca^{2+} and Ba^{2+} [23]. In each case the apparent conductance was reduced below the linear prediction with an asymmetric mixture of ions. Therefore, the 20 pS anion channel probably contains a multi-ion pore. The situation is not so clear for the 10 pS channel. Although no AMFE was seen, it is possible for the effect to be obscured by high driving forces of concentration or membrane potential [23]. This would be difficult to test with single-channel measurements of the 10 pS channel because of the small currents that would need to be measured.

The anion permeability sequence for the rectifying anion channel of epithelia has been examined in several studies. Reported sequences are: $I^- > Br^- > Cl^- > F^-$ [22], $I^- > Cl^- = Br^- > F^-$ [25], $Cl^- = Br^- = I^-$ [7] for airway epithelia, and $I^- > Br^- > Cl^- > F^-$ [13] for T84 cells. While not completely consistent, these findings suggest that there are significant permeation differences between the rectifying anion channel and the two linear anion channels examined here.

Halide permeation sequences have been used in attempts to distinguish the contributions of identified single channels to whole-cell currents in T84 cells. Calcium-dependent currents and volume-sensitive currents in these cells were both associated with rectifying channels [14,26], although these currents can be distinguished on other grounds [14]. A cAMP-dependent anion current could not be explained by the rectifying channel and was suggested to be caused by 20 pS or smaller conductance linear anion channels [14].

The permeability sequences for the volume-sensitive, calcium-activated, and cAMP-activated currents have been reported to be: $I^- > Br^- > Cl^-$, $I^- > Br^- > Cl^-$, and $Br^- > Cl^- > I^-$, respectively [14,26]. Although 20 pS or smaller anion channels were suggested to cause the cAMP-dependent current of T84 cells [14], none of these sequences match those reported here for the 10 pS and 20 pS channels, making it unlikely that either is responsible for the current. An alternative explanation is that even smaller channels are responsible, because anion channels of < 5 pS conductance have recently been found in airway epithelia by both single-channel and fluctuation analysis experiments [11,12].

In comparing different experimental permeability sequences, it must be noted that a range of temperatures of about 22°C-37°C have been used in different measurements. The present data were obtained at 37°C, as were some other recent measurements [7,14]. The wide range of data used to construct Eisenmann sequences for anions [21] probably included a similar range of temperatures, while the models used to explain those anion sequences were constructed at 25°C. The effects of temperature on permeability sequences have not been thoroughly investigated. Current models of multiple ion permeation do not suggest strong effects of temperature on relative ion permeability [19], but the possibility of temperature effects cannot be excluded.

A linear, voltage-insensitive, whole-cell anion current was produced by expression of the cystic fibrosis transmembrane conductance regulator (CFTR) in airway epithelial cells [27]. In insect cells, CFTR produced single anion channels with a mean conductance of 8.4 pS [28], which is within the range of conductances found here for the 10 pS channel. However, expression of normal CFTR in several different cell lines produced whole-cell currents with the permeability sequence: $Br^- > Cl^- > I^- > F^-$, and the relative permeability ratios were much larger than observed here [29]. Thus, the possible contributions of these voltage-insensitive 10 pS and 20 pS channels to currents in normal and cystic fibrosis airway epithelial cells remain uncertain. Resolution of these questions will require more information about halide selectivity in whole-cell currents of airway cells.

Acknowledgements

We thank Marek Duszyk for advice and encouragement throughout this work, Paul Labrecque for assistance in cell preparation, and the Department of Otolaryngology of the University of Alberta Hospital for providing human tissues. This work was supported by the Medical Research Council of Canada and the Canadian Cystic Fibrosis Foundation.

References

1 Boucher, R.C., Cotton, C.U., Gatzy, J.T., Knowles, M.R. and Yankaskas, J.R. (1988) J. Physiol. (Lond.) 405, 77–103.
2 McPherson, M.A. and Dormer, R.L. (1991) Mol. Aspects Med. 12, 1–81.
3 Welsh, M.J. (1987) Physiol. Rev. 67, 1143–1184.
4 Vaughan, P.C. and French, A.S. (1989) Prog. Biophys. Mol. Biol. 54, 59–79.
5 Frizzell, R.A., Rechkemmer, G. and Shoemaker, R.L. (1986) Science 233, 558–560.
6 Welsh, M.J. (1986) Science 232, 1648–1650.
7 Kunzelmann, K., Pavenstädt, H. and Greger, R. (1989) Pflügers Arch. 414, 291–303.
8 Duszyk, M., French, A.S. and Man, S.F.P. (1989) Can. J. Physiol. Pharmacol. 67, 1362–1365.
9 Shoemaker, R.L., Frizzell, R.A., Dwyer, T.M. and Farley, J.M. (1986) Biochim. Biophys. Acta 858, 235–242
10 Duszyk, M., French, A.S. and Man, S.F.P. (1990) Biophys. J. 57, 223–230.
11 Duszyk, M., French, A.S. and Man S.F.P. (1991) Biophys. J. 59, 645a.
12 Grygorczyk, R. (1991) Biophys. J. 59, 646a.

13 Halm, D.R., Rechkemmer, G.R., Schoumacher, R.A. and Frizzell, R.A. (1988) Am. J. Physiol. 254, C505-C511.
14 Cliff, W.H and Frizzell, R.A. (1990) Proc. Natl. Acad. Sci. USA 87, 4956-4960.
15 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85-100.
16 McInnes, D.A. (1939) The Principles of Electrochemistry, Rheinhold Publishing Corp., Washington, DC.
17 Colquhoun, D. and Sigworth, F.J. (1983) Single-channel recording (Sakmann, B. and Neher, E., eds.), pp. 191-263, Plenum Press, New York.
18 Duszyk, M., French, A.S. and Man, S.F.P. (1991) Biomed. Res. 12, 17-23.
19 Hille, B. (1984) Ion Channels of Excitable Membranes, Sinauer Associates, Sunderland, MA.
20 Robinson, R.A. and Stokes, R.H. (1959) Electrolyte Solutions, Butterworths, London.
21 Wright, E.M. and Diamond, J.M. (1977) Physiol. Rev. 57, 109-156.
22 Frizzell, R.A. (1987) Trends Neurosci. 10, 190-195.
23 Friel, D.D. and Tsien, R.W. (1989) Proc. Nat. Acad. Sci. USA 86, 5207-5211.
24 Hagiwara, S., Miyazaki, S., Krasne, S. and Ciani, S. (1977) J. Gen. Physiol. 70, 269-281.
25 Welsh, M.J., Li, M., McCann, J.D., Clancy, J.P. and Anderson, M.P. (1989) Ann. N.Y. Acad. Sci. 574, 44-51.
26 Worrell, R.T., Butt, A.G., Cliff, W.H. and Frizzell, R.A. (1989) Am. J. Physiol. 256, C1111-C1119.
27 Rich, D.P., Anderson, M.P., Gregory, R.J., Cheng, S.H., Paul, S., Jefferson, D.M., McCann, J.D., Klinger, K.W., Smith, A.E. and Welsh, M.J. (1990) Nature 347, 358-363.
28 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. and Riordan, J.R. (1991) Cell 64, 681-691.
29 Anderson, M.P., Gregory, R.J., Thompson, S., Souza, D.W., Paul, S., Mulligan, R.C., Smith, A.E. and Welsh, M.J. (1991) Science 253, 202-205.