The cystic fibrosis transmembrane conductance regulator chloride channel

Iodide block and permeation

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INTRODUCTION

Cystic fibrosis (CF) is a common autosomal-recessive disease characterized by abnormally low chloride conductance in epithelial cell membranes (1). The CF gene sequence predicts a protein having two sets of six transmembrane spanning regions, two nucleotide (ATP) binding folds, and a highly charged regulatory domain with concensus phosphorylation sites for protein kinases A and C (2, 3). An ohmic, low-conductance chloride channel is generated when this protein (named CFTR for cystic fibrosis transmembrane conductance regulator) is expressed in insect cells, and the channels and CFTR protein appear with the same time course (4). The same Cl channel is also produced when the gene is expressed in CFTR-transfected CHO cells (5) and in *Xenopus* oocytes injected with cRNA (6). Moreover, a macroscopic Cl conductance having properties consistent with this channel is generated in HeLa cells transiently expressing CFTR, and the apparent selectivity of this conductance can be altered by mutating residues within the first and sixth transmembrane spanning regions (7). Taken together, the single channel and mutagenesis data strongly suggest CFTR is the cAMP-activated Cl channel which has been characterized previously in human pancreatic duct (8), thyroid (12), and the colonic cell line T84 (9).

The channel generated in CFTR-expressing cells can be activated in excised patches by exposure to protein kinases (5), making it possible to study single channel selectivity in detail. Iodide selectivity has recently been used for identifying this channel macroscopically (7, 10, 11), but previous studies of iodide permeability have led to conflicting results. In this paper we use CHO cells stably transfected with the CFTR gene to study iodide effects at the single channel level under biionic conditions. We report that iodide blocks the channel at normal (150 mM) ionic strength and that permeability to iodide is higher than to chloride when measured from the cytoplasmic side.

RESULTS AND DISCUSSION

Iodide block

Fig. 1 shows channel activity recorded from an inside-out patch at ±50 mV when the pipette solution contains 150 mM NaCl and the bath solution contains 150 mM NaCl (Fig. 1 *b*) or 75 mM NaCl + 75 mM NaI (Fig. 1 *c*). Single channel events were easily resolved and had similar amplitudes at both potentials when bathed symmetrically by 150 mM Cl (Fig. 1 *b*). However, when the bath (i.e., cytoplasmic) solution was exchanged for the mixture containing 75 mM Cl and 75 mM I (Fig. 1 *c*), single channel currents were depressed at +50 mV and disappeared completely at −50 mV. As shown in Fig. 2 *a*, varying iodide concentration in the bath solution had little effect on the reversal potential; however, the slope conductance declined monotonically from 6.82 ± 0.33 pS to 3.91 ± 0.23 as the mole fraction of iodide was increased (Fig. 2 *b*). Iodide block is clearly voltage dependent; however, the slope conductance and open probability were not noticeably affected by membrane potential between +20 and +80 mV.

Iodide and nitrate permeation from the inside

Fig. 3 *a* compares current-voltage (I/V) curves when the bath solution contains Cl, NO_3, or I. Permeability ratios were calculated as: $P_X/P_{Cl} = Cl_i \exp [(E_X - E_{Cl})F/RT]/X_i$; where Cl_i and X_i are the activities of chloride and test anion X in the bath, respectively, and $E_X - E_{Cl}$ is the change in zero current potential when Cl on one side of the membrane is replaced by X. Iodide and nitrate permeabilities were similar (Fig. 3 *a*); however, only iodide caused block. The reversal potential shifted from 0 mV in symmetrical 150 mM NaCl to +9.6 ± 3.3 mV with 150 mM iodide in the bath and to +10.5 ± 2.1 mV with 150 mM nitrate in the bath. Using activity coefficients for I and Cl of 0.769 and 0.732, respectively, these shifts indicate $P_I/P_{Cl} = 1.66 \pm 0.2$ ($\bar{x} \pm$ SE; $n = 6$) and $P_{NO3}/P_{Cl} = 1.66 \pm 0.02$ ($\bar{x} \pm$ SE; $n = 5$). Although permeability ratios indicate the channel prefers iodide and nitrate to chloride, slope conductance was decreased when either anion replaced chloride.

This estimate for P_I/P_{Cl} is much higher than has been reported previously for whole cell conductance in T_{84} cells (0.4; reference 11) and CFTR-transfected HeLa cells (0.6; 7), but its accuracy is questionable because it

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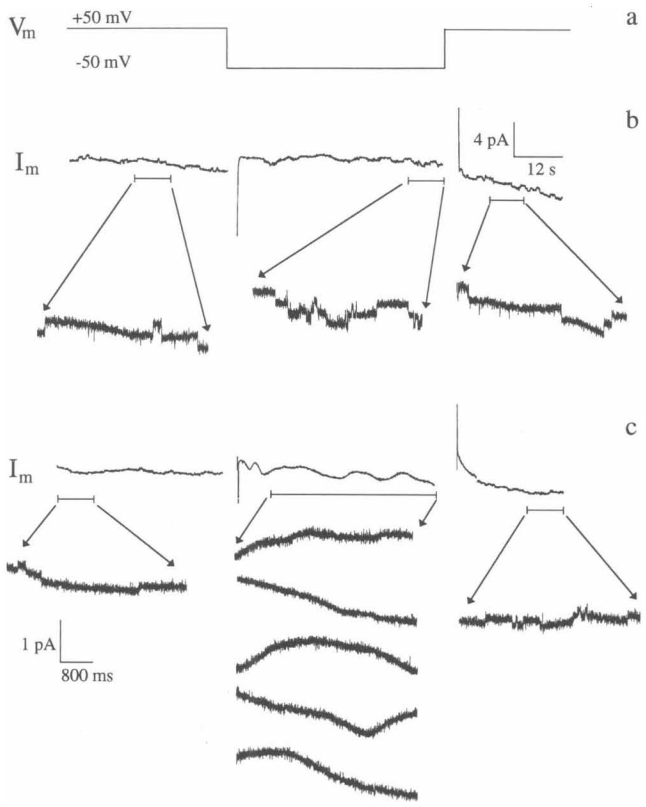

FIGURE 1 Blockade of the low-conductance chloride channel by cytoplasmic iodide. (*a*) Voltage protocol used to demonstrate iodide block. (*b*) Low-conductance Cl channel activity in symmetric 150 mM NaCl with 0.5 mM ATP + 180.5 nM protein kinase A catalytic subunit. (*c*) Single channel activity recorded when the bath contains 75 mM sodium iodide and 75 mM sodium chloride. Transitions were not observed at −50 mV.

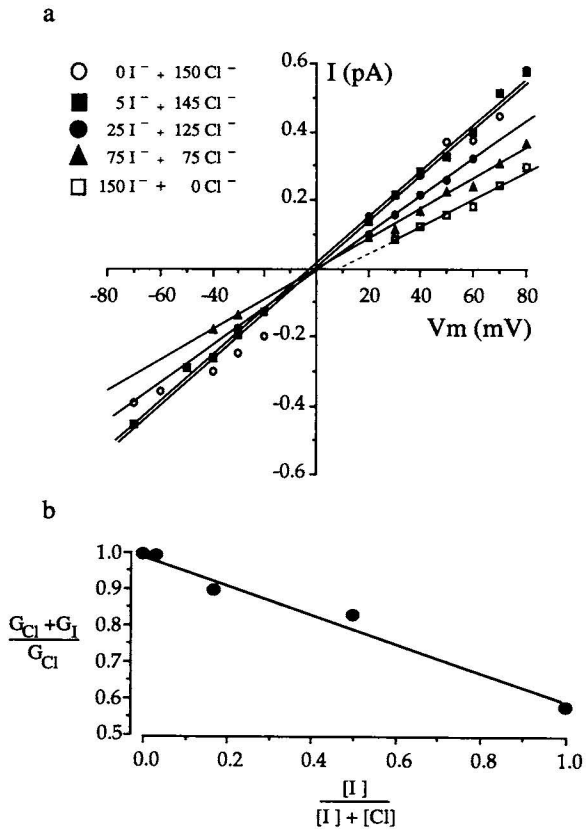

FIGURE 2 Effect of different intracellular iodide concentrations on the conductance of the low-conductance chloride channel. (*a*) Current-voltage relationship with different Cl and I concentrations. Current amplitude decreases as iodide concentration in the bath increases. (*b*) Single channel conductance declines monotonically as the mole fraction of iodide in the bath solution is increased.

was derived from a reversal potential that had to be extrapolated due to the iodide block. However, during preliminary experiments we noticed that iodide block did not occur under biionic conditions when the salt concentration was elevated to 400 mM. Fig. 3 *b* shows an example of currents recorded with 400 mM iodide solution in the bath and 400 mM chloride in the pipette. The reversal potential, which could be deter-

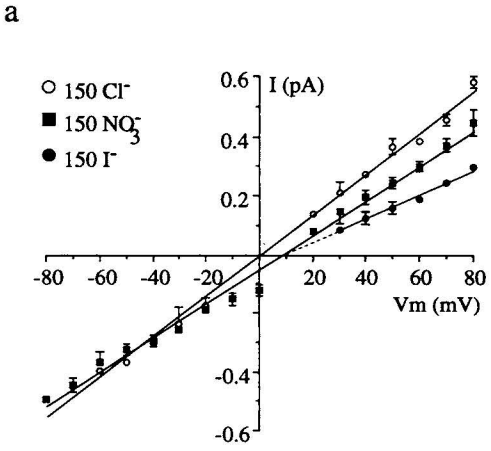

FIGURE 3 Selectivity of the low-conductance chloride channel to iodide and nitrate. (*a*) Current-voltage (I/V) relationship measured under biionic conditions (150 mM solutions). The reversal potentials for iodide and nitrate appear similar although block makes it necessary to extrapolate the value for iodide. (*b*) Iodide block is avoided by using 400 mM NaCl in the pipette and 400 mM NaI in the bath. The reversal potential can be observed clearly under these conditions and indicates a selectivity ratio of 1.90. (*c*) The selectivity ratio of the outward rectifier, which is also present in these patches and is generally considered to have high iodide permeability compared to the cAMP-stimulated Cl channel, is only 1.50 under these conditions.

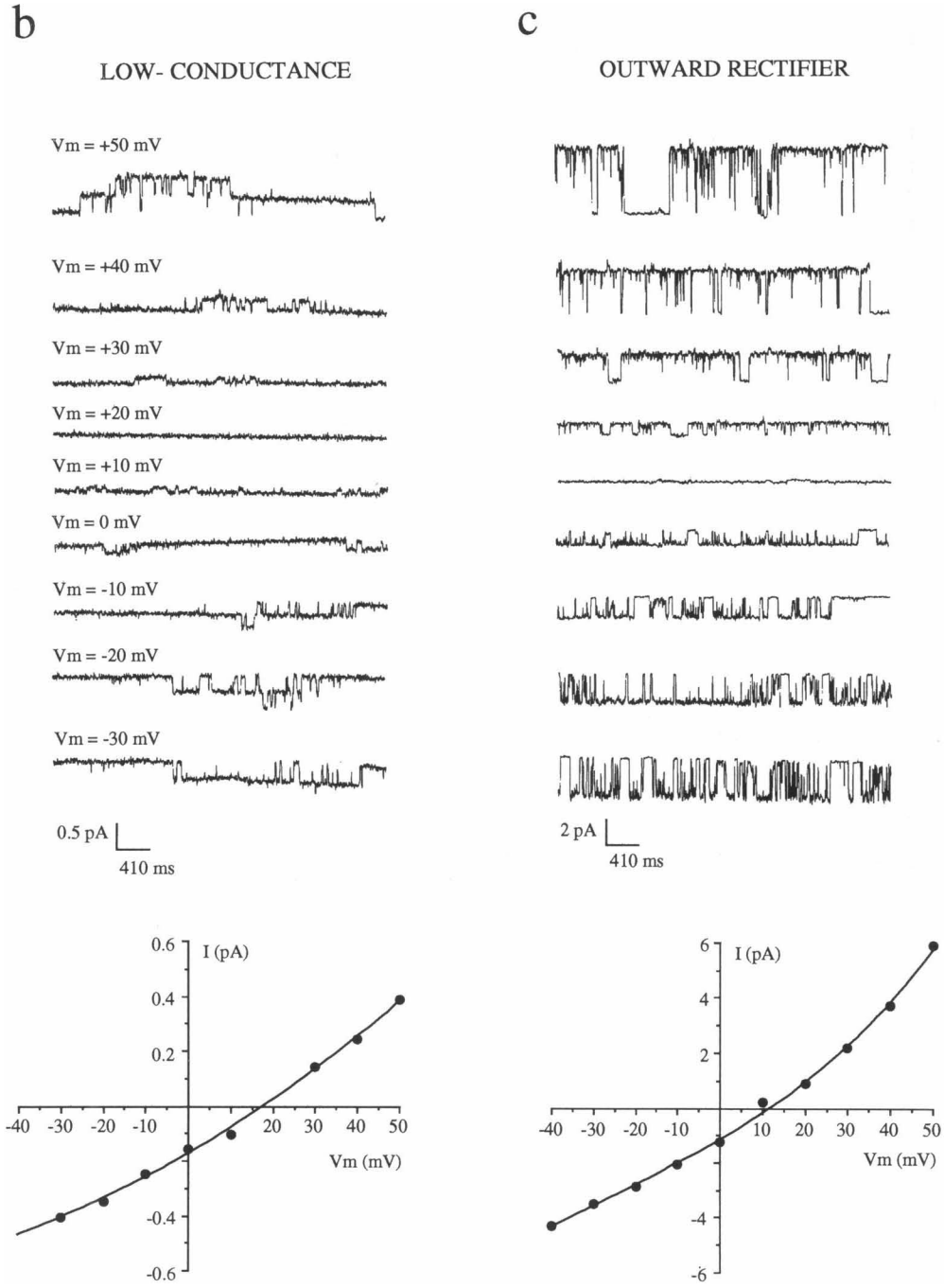

FIGURE 3 (continued)

mined unequivocally under these conditions, was 17.5 ± 0.9 mV ($x \pm$ SE, $n = 4$), indicating a P_I/P_{Cl} ratio of 1.90 ± 0.06. This ratio is significantly higher than that determined for outwardly rectifying channels, which were also observed under these conditions ($P_I/P_{Cl} = 1.50 \pm 0.01$; Fig. 3 *c*).

Iodide selectivity has become a widely used criterion for distinguishing the cAMP-activated Cl conductance mediated by CFTR from other anion channels such as the outward rectifier, which have high iodide permeability. Why then is the iodide:chloride permeability ratio for the CFTR chloride channel so high when measured from the inside (1.47), compared to previous ratios determined from the outside (0.4–0.6)? At this point we

can only speculate; however, it is intriguing that our estimate is almost identical to the one obtained from the outside after lysines at position 95 or 335 in CFTR had been mutated to negatively charged residues (7). A straight-forward interpretation would be that iodide is slowed by binding at this charged site, and that bound iodide contributes to the channel's selectivity filter for permeating anions, including iodide itself. Neutralizing the site by mutagenesis or by chloride binding (as in the present paper, with 150 mM extracellular [Cl]) would then increase both iodide conductance and the equilibrium iodide:chloride permeability ratio. Whether residues K95 and K335 in CFTR are also the site where iodide blocks the channel remains to be determined.

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