To investigate the possibility that inward rectification of CFTR Cl^- current is caused by a component of the recording solutions, we examined the effect on CFTR of a number of factors in the bath and pipette solutions. These included the nature of the biological buffer and the monovalent cation used in our recording solutions.

Previous work demonstrated that some biological buffers block Cl^- channels including CFTR (Hanrahan and Tabcharani, 1990; Ishihara and Welsh, 1997; Tabcharani et al., 1997). Ishihara and Welsh (1997) demonstrated that TRICINE is without effect on CFTR in contrast to other buffers including MOPS and TES. Therefore, we examined the effect of TRICINE-based solutions on the gating kinetics of CFTR using the $C_1 \leftrightarrow C_2 \leftrightarrow O$ kinetic scheme and by substituting TES (10 mM) by TRICINE (10 mM) in the bath and pipette solutions. Fig. S1 summarizes the effects of TES and TRICINE on the rate constants at −50 and +50 mV. In both TES- and TRICINE-based solutions α_1 and β_2 were decreased significantly and β_1 was unchanged (Fig. S1). In addition, α_2 was increased in the presence of both biological buffers. However, the increase in α_2 was only statistically significant in the presence of TES (Fig. S1). As explained in the section "Voltage changes the gating kinetics of CFTR" of RESULTS, these data suggest that voltage produces reciprocal changes in α_1 and the rate constants within the bursting state (i.e., α_2 and β_2) that tend to counteract each other. The data also indicate that changing the biological buffer is without effect on the gating kinetics of CFTR.

By using the large monovalent cation NMDG that fails to permeate cation channels, we record CFTR Cl^- currents uncontaminated by cation currents. However, it is feasible that NMDG might alter the biophysical properties of CFTR. Therefore, we examined the effect of NaCl-based solutions on the inward rectification of CFTR Cl^- currents by substituting NMDGCl (147 mM) by NaCl (147 mM) in the bath and pipette solutions. Fig. S2 A demonstrates that the I-V relationship of CFTR Cl^- currents recorded in the presence of symmetrical 147 mM NaCl solutions exhibits inward rectification. Fig. S2 B demonstrates that the magnitude of inward rectification observed in the presence of NaCl-based solutions did not differ from that in the presence of NMDGCl-based solutions (NaCl: I at +100 mV = 72 ± 6% of that at −100 mV (n = 4); NMDGCl: I at +100 mV = 74 ± 2% of that at −100 mV (n = 10); $P > 0.5$).

To investigate further the effect of monovalent cations, we studied the gating kinetics of CFTR using the $C_1 \leftrightarrow C_2 \leftrightarrow O$ kinetic scheme. Fig. S3 summarizes the effects of 147 mM NMDGCl and 147 mM NaCl solutions on

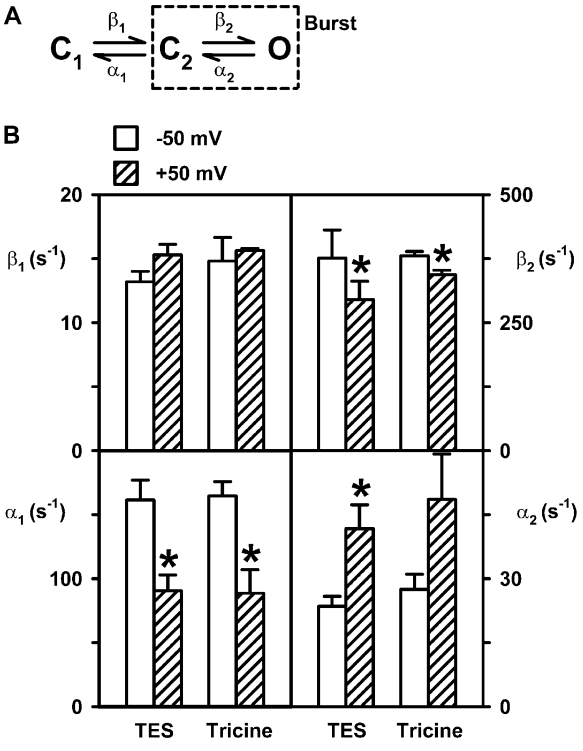

FIGURE S1. Effect of the biological buffer on single-channel kinetics. (A) The $C_1 \leftrightarrow C_2 \leftrightarrow O$ kinetic scheme that describes CFTR channel gating (Winter et al., 1994). States C_1, C_2, and O represent two closed states and one open state, respectively, while β_1, β_2, α_1, and α_2 represent the rate constants describing transitions between the open and closed states. States enclosed within the dashed box represent the bursting state. (B) Rate constants at the indicated voltages determined by the maximum likelihood fit to the model shown in A when the recording solutions contained either TES (10 mM) or TRICINE (10 mM). Data are means ± SEM (TES, n = 8; TRICINE, n = 3) at each voltage. The asterisks indicate values that are significantly different from the −50 mV data ($P < 0.05$). Measurements were made using excised inside-out membrane patches from C127 cells expressing wild-type human CFTR. Membrane patches contained only a single active CFTR Cl^- channel and were bathed in symmetrical 147 mM Cl^- solutions. All intracellular solutions contained PKA (75 nM) and ATP (1 mM).

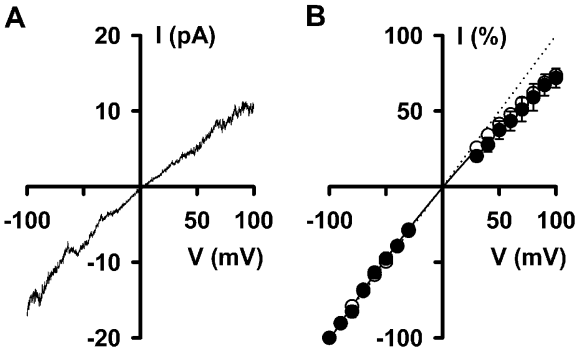

FIGURE S2. I-V relationships of CFTR Cl^- currents. (A) I-V relationship of CFTR Cl^- currents recorded in the presence of symmetrical 147 mM NaCl solutions. (B) I-V relationships of CFTR Cl^- currents recorded in the presence of either 147 mM NaCl solutions (filled circles) or 147 mM NMDGCl solutions (open circles) expressed as a percentage of the current value at −100 mV. Data are means ± SEM (NaCl, $n = 4$ and NMDGCl, $n = 10$) at each voltage. Error bars are smaller than symbol size except where shown. The continuous lines are the fits of second order regressions to the data. The dotted line shows the predicted ohmic I-V relationship. Other details as in Fig. S1.

the rate constants at −50 and +50 mV. In both NMDGCl- and NaCl-based solutions α_1 and β_2 were decreased significantly, α_2 was increased significantly, and β_1 was unchanged (Fig. S3). Like the effects of TES and TRICINE on channel gating, these data suggest that voltage produces reciprocal changes in α_1 and the rate constants within the bursting state (i.e., α_2 and β_2) that tend to offset each other. The data also indicate that changing the monovalent cation is without effect on the gating kinetics of CFTR.

Based on the above data, we suggest that neither TES nor NMDG are responsible for the inward rectification of the CFTR Cl^- channel. Comparison of the different solutions employed in studies where inward rectification of the CFTR Cl^- channel has been observed (Zhao et al., 1996; Linsdell and Hanrahan, 1999; Lansdell et al., 2000; Cai and Sheppard, 2002; Linsdell and Gong, 2002; this study) suggests that CsEGTA and Mg^{2+} are unlikely to be responsible for the inward rectification of CFTR. These data contrast with the results of studies of K_{ir} channels, which indicate that EGTA and Mg^{2+} cause a voltage-dependent block of current flow through these K^+ channels (Vandenberg, 1987; Guo and Lu, 2002). Similarly, anion conduction studies indicate that inward rectification of the CFTR Cl^- channel is observed when other anions are substituted for Cl^- in both the intra- and extracellular solutions (Linsdell, 2001). This suggests that inward rectification of current flow through the CFTR pore is a common feature of anion flow through the CFTR Cl^- channel.

Finally, studies of the mechanism of inward rectification of K_{ir} channels (Lopatin et al., 1994) raise the possibility that an unidentified agent might be responsible for the inward rectification of CFTR Cl^- currents. Several lines of

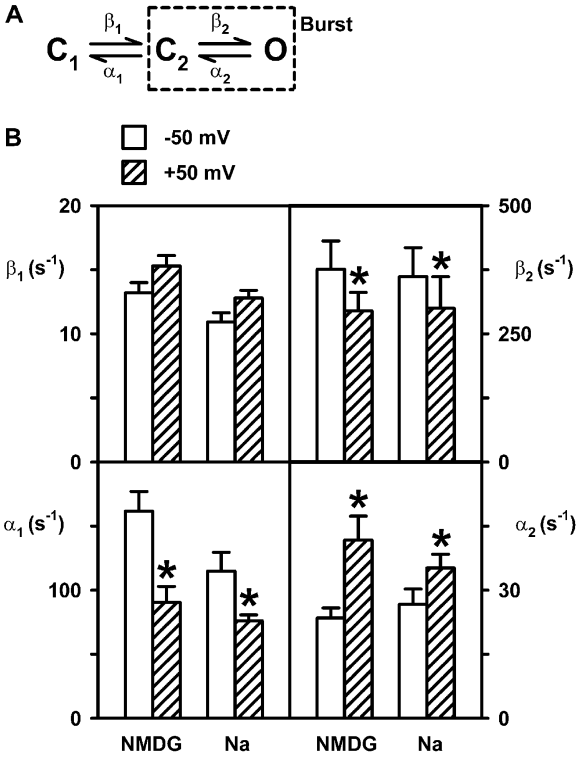

FIGURE S3. Effect of monovalent cations on single-channel kinetics. (A) A linear three-state model of CFTR channel gating (see Fig. 1A and Winter et al., 1994). (B) Rate constants at the indicated voltages determined by the maximum likelihood fit to the model shown in A when the recording solutions contained either NMDGCl (147 mM) or NaCl (147 mM). Data are means ± SEM (NMDG, $n = 8$ and NaCl, $n = 3$) at each voltage. The asterisks indicate values that are significantly different from the −50 mV data ($P < 0.05$). Other details as in Fig. 1.

evidence suggest that this possibility is remote. First, excised membrane patches were moved ~500 μm away from the surface of cells during experiments. Second, the intracellular surface of the membrane patch was extensively washed during experiments by the perfusion of different solutions. Third, inward rectification was observed when CFTR Cl^- channels were reconstituted into artificial lipid bilayers (Zhao et al., 1996). Fourth, if rectification is caused by an unidentified factor, it is common to both native epithelial cells (Quinton and Reddy, 2000) and recombinant cells (Linsdell and Hanrahan, 1999; Lansdell et al., 2000; Cai and Sheppard, 2002; Linsdell and Gong, 2002). Together, the data suggest that inward rectification is unlikely to be caused by channel block.

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