A Mutation in CFTR Modifies the Effects of the Adenylate Kinase Inhibitor Ap_5A on Channel Gating

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ABSTRACT Mutations in the gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR) cause cystic fibrosis. The CFTR anion channel is controlled by ATP binding and enzymatic activity at the two nucleotide-binding domains. CFTR exhibits two types of enzymatic activity: 1), ATPase activity in the presence of ATP and 2), adenylate kinase activity in the presence of ATP plus physiologic concentrations of AMP or ADP. Previous work showed that P^1,P^5-di(adenosine-5′)pentaphosphate (Ap_5A), a specific adenylate kinases inhibitor, inhibited wild-type CFTR. In this study, we report that Ap_5A increased activity of CFTR with an L1254A mutation. This mutation increased the EC50 for ATP by >10-fold and reduced channel activity by prolonging the closed state. Ap_5A did not elicit current on its own nor did it alter ATP EC50 or maximal current. However, it changed the relationship between ATP concentration and current. At submaximal ATP concentrations, Ap_5A stimulated current by stabilizing the channel open state. Whereas previous work indicated that adenylate kinase activity regulated channel opening, our data suggest that Ap_5A binding may also influence channel closing. These results also suggest that a better understanding of the adenylate kinase activity of CFTR may be of value in developing new therapeutic strategies for cystic fibrosis.

INTRODUCTION

The cystic fibrosis transmembrane conductance regulator (CFTR) forms an anion channel expressed primarily in epithelia (1–4). Like other ATP-binding cassette (ABC) transporters, CFTR contains two nucleotide-binding domains (NBDs) and two membrane-spanning domains (MSDs). In addition, it contains a regulatory (R) domain. Among ABC transporters, CFTR is unique because it forms a channel through which anions flow passively, driven in either direction by the electrochemical gradient across the membrane.

Activity of the CFTR anion channel requires kinase-mediated phosphorylation of the R domain (1,2,5). The binding of ATP to the NBDs and their enzymatic activity then open and close the channel. Structural evidence from other ABC proteins indicates that the two NBDs form a complex in a rotationally symmetric head-to-tail dimer (6–8). The NBD dimers form two ATP-binding sites; each ATP is sandwiched between the Walker A motif (the P-loop) of one NBD and the signature (LSGGQ) motif of the other NBD. In CFTR, ATP-binding Site 1 includes the Walker A motif of NBD1 and the signature motif of NBD2. The Walker A motif of NBD2 and the signature motif of NBD1 contribute to ATP-binding Site 2.

Both ATP-binding sites control channel opening and closing (9–12). The interaction of ATP with Site 1 is required for normal gating. When mutations prevent ATP from binding to Site 2, ATP-binding Site 1 can gate the channel, albeit with reduced activity (11). Normal gating also requires the interaction of ATP with ATP-binding Site 2, and the majority of the enzymatic activity of CFTR occurs at this site (9,13–15). Previous work showed that CFTR NBDs possess the capacity for two different kinds of enzymatic activity. In the presence of ATP alone, ATPase activity ($Mg^{2+}\cdot ATP \rightarrow Mg^{2+} + P_i + ADP$) is required for channel gating (1,2,5). This observation is consistent with the ATPase activity reported for several other ABC transporters (16). However, when ATP is added in the presence of physiologic concentrations of AMP, adenylate kinase activity ($Mg^{2+}\cdot ATP +$ AMP D $Mg^{2+}\cdot ADP + ADP$) regulates channel activity (17–20). The two enzymatic activities (ATPase and adenylate kinase) appear to share an ATP-binding site — ATP-binding Site 2. Adenylate kinase activity has been described in isolated CFTR NBDs by several groups (17,18,21), and mixing NBD1 and NBD2 caused a synergistic enhancement of adenylate kinase activity (20). However, another study (21) reported no synergistic or additive effects on adenylate kinase activity when combining NBD1 and NBD2. In addition, that study failed to detect adenylate kinase activity after solubilization of recombinant CFTR from membranes, although the adenylate kinase inhibitor P^1,P^5-di(adenosine-5′)pentaphosphate (Ap_5A) inhibited Cl^- transport when CFTR was reconstituted into membranes (21). The reason for these discrepancies remains unclear. Interestingly, another recent report (22) indicates that a different ABC protein, Rad50, also functions as ATPase and as an adenylate kinase. Although structural and functional studies have ascertained where ATP binds in the NBDs of ABC transporters, the distinct AMP-binding site in CFTR has not been identified.

The goal of this study was to better understand how adenylate kinase activity controls CFTR. As a probe of adenylate kinase, we used Ap_5A, which is a specific inhibitor of adenylate kinases and known to bind simultaneously to the ATP-binding

Submitted June 25, 2008, and accepted for publication August 25, 2008.

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Editor: Toshinori Hoshi.

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0006-3495/08/12/5178/08 $2.00

doi: 10.1529/biophysj.108.140897

site and the AMP-binding site (23–25). Previous studies showed that Ap_5A also inhibits CFTR adenylate kinase activity and channel gating (17,18,20). We asked whether the inhibitory effect of Ap_5A would be reduced or eliminated by point mutations in the NBDs. We made a series of mutations, selecting residues based on the crystal structure of ATP bound to an NBD homodimer (7). Because ATP sits at the interface between the two NBDs, because both NBDs contribute to ATP binding, and because dimerization of the two NBDs may regulate gating, we chose residues in or near the dimer interface. Then, we tested the inhibitory effect of Ap_5A applied to the cytosolic surface of the variant CFTR channels.

EXPERIMENTAL PROCEDURES

Site-directed mutagenesis and cell expression systems

CFTR mutants were prepared in the pTM1-CFTR4 plasmid using QuikChange site-directed mutagenesis (Stratagene, LaJolla, CA). We determined the correct identity of each mutant by sequencing the entire cDNA. Wild-type and mutant CFTR proteins were transiently expressed in HeLa cells using the vaccinia virus/T7 expression system as described previously (26). For some studies, CFTR was transiently expressed in HeLa cells using the pIRES-EGFP vector (27) so that we could identify transfected cells by enhanced green fluorescent protein (eGFP) fluorescence.

Patch-clamp studies

We used excised, inside-out membrane patches. The pipette (extracellular) solution contained 140 mM *N*-methyl-D-glucamine, 100 mM L-aspartic acid, 3 mM $MgCl_2$, 5 mM $CaCl_2$, and 10 mM tricine (pH 7.3) with HCl; the final Cl^- concentration was ~51 mM. The bath (intracellular) solution contained 140 mM *N*-methyl-D-glucamine, 3 mM $MgCl_2$, 1 mM CsEGTA (cesium ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid), and 10 mM tricine (pH 7.3) with HCl; the final Cl^- concentration was 140 mM. After patch excision, channels were activated with the catalytic subunit of cAMP-dependent protein kinase (PKA, 80 U/mL; Promega, Madison, WI) and ATP. Unless otherwise specified, PKA was present in all cytosolic solutions that contained ATP. All nucleotides and inhibitors were purchased from Sigma-Aldrich (St. Louis, MO). ATP was added as the Mg^{2+} salt. The cytosolic surface of patches was continuously perfused with a multichannel rapid change perfusion system. Holding voltage was −40 mV for macropatch experiments and −50 to −100 mV for single-channel experiments. Experiments were performed at temperatures between 23 and 26°C.

An Axopatch 200B amplifier (Axon Instruments, Union City, CA) was used for voltage clamping and current recording, and the pCLAMP software package (version 9.1; Axon Instruments) was used for data acquisition and analysis. Data were digitized at 5 kHz. Macroscopic current recordings were low-pass filtered at 100 Hz using an 8-pole Bessel filter (Model 900; Frequency Devices, Haverhill, MA), and single-channel recordings were low-pass filtered at 500 Hz for analysis and at 10 or 50 Hz for display in the accompanying Figures. Single-channel analysis was performed as previously described (9,28), with a burst delimiter of 20 ms. Events ≤ 4 ms duration were ignored.

Data are presented as mean ± SE. p values <0.05 were considered statistically significant.

Ap_5A purification

We tested whether Ap_5A was contaminated with other nucleotides. High-performance liquid chromatography (HPLC) revealed low levels of ATP, ADP, and AMP (Fig. 1 *A*). Quantitative measurements indicated that 1 mM Ap_5A contained ~2 μM ATP and that the amount varied from lot to lot. Therefore, we purified Ap_5A for some experiments. Before HPLC purification, the Ap_5A preparation (10 mg/mL) was treated with hexokinase (100 U/mL) in the presence of 100 mM glucose/phosphorylate glucose using the γ-phosphate of any ATP present to generate glucose 6-phosphate and ADP. HPLC was performed on a System Gold microbore HPLC system with an anion exchange column-Q column (4.6 × 100 mm, 10 mm particle size) using 32 Karat software (Beckman Coulter, Fullerton, CA). Solution A was 10 mM TEAB (triethyl-ammonium bicarbonate), and solution B was 1 M TEAB (pH 7.5). A 2 mg sample was injected, detected at 254 nm, and collected. The collected sample was frozen at −80°C and then lyophilized. Purified Ap_5A (Fig. 1 *B*) was dissolved in double-deionized H_2O to ~50 mM, and the absorbance of the solution was measured at 260 nm to calculate the concentration. We obtained similar functional results with purified and nonpurified Ap_5A.

RESULTS

Ap_5A inhibited wild-type CFTR but stimulated CFTR-L1254A

We generated 29 variants in which alanine or another amino acid replaced a residue in NBD2 or NBD1. Fig. 2 shows locations of residues homologous to those we tested highlighted in the dimer structure of the ABC transporter MJ0796 (7). We used MJ0796 because it has a clear NBD dimer

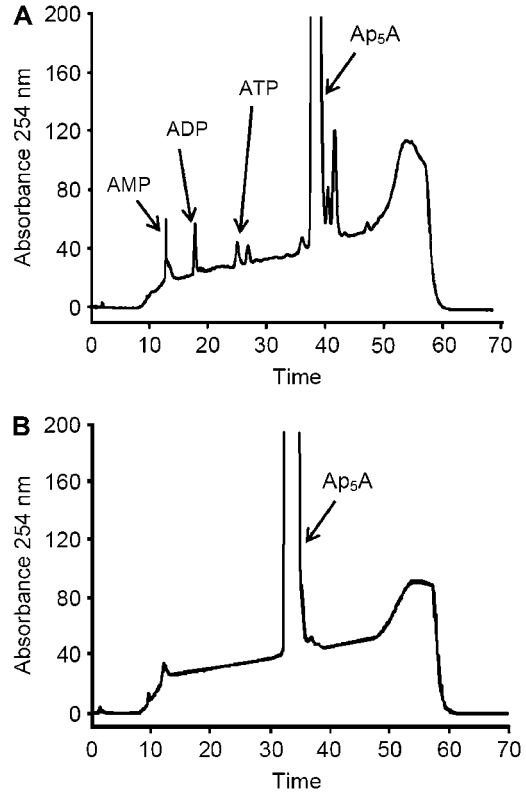

FIGURE 1 HPLC of Ap_5A before (*A*) and after (*B*) purification. Peaks were identified based on elution times of ATP, ADP, and AMP standards. The peaks around elution time 12 min and between 50 to 60 min are artifacts due to solution changes.

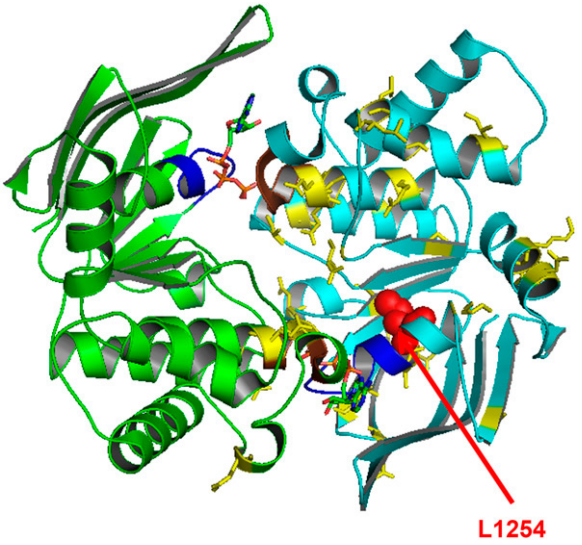

FIGURE 2 The dimer structure of the ABC transporter MJ0796 (7). For purposes of illustration, we represent the left side as NBD1 (*green*) and the right side as NBD2 (*blue*). Both Walker A motifs are in dark blue, and both LSGGQ motifs are in brown. Two ATP molecules are shown as stick shapes (oxygen is in *red* and phosphate in *orange*). The residue analogous to Leu-1254 in CFTR is Leu-48 in MJ0796; it is shown in red as a space-filling structure. Other tested residues are shown in yellow as stick structures.

structure, whereas the dimer structure of CFTR NBD1/NBD2 is not known.

We phosphorylated CFTR with PKA and studied current in the presence of 75 μM ATP; our previous work showed that 1 mM Ap_5A inhibited current by ~50% with this concentration of ATP (18). As we previously reported, adding 1 mM Ap_5A to the cytosolic surface of excised, membrane patches inhibited wild-type CFTR current (Fig. 3, *A* and *B*). In all but one of the variants, Ap_5A inhibited current, similar to what we observed in wild-type CFTR. Surprisingly, the L1254A mutation reversed the effect of Ap_5A, so that Ap_5A stimulated CFTR current. When added alone to phosphorylated wild-type CFTR (18) or CFTR-L1254A (Fig. 4), Ap_5A did not generate activity.

Ap_5A changed the ATP-dependence of CFTR-L1254A

To better understand ATP-dependent regulation of the L1254A variant, we examined the relationship between ATP concentration and current (Fig. 5, *A* and *B*). The L1254A mutation did not significantly change the shape of the curve; the Hill coefficient was 1.06 ± 0.08 for wild-type CFTR and 0.92 ± 0.06 for CFTR-L1254A, although the two lowest ATP concentrations were not well fit by the regression line. However, introduction of the L1254A mutation shifted the relationship between ATP concentration and current to the right, increasing the ATP EC50 from 34 ± 3 μM in wild-type CFTR to 392 ± 38 μM in CFTR-L1254A ($p < 0.05$). In

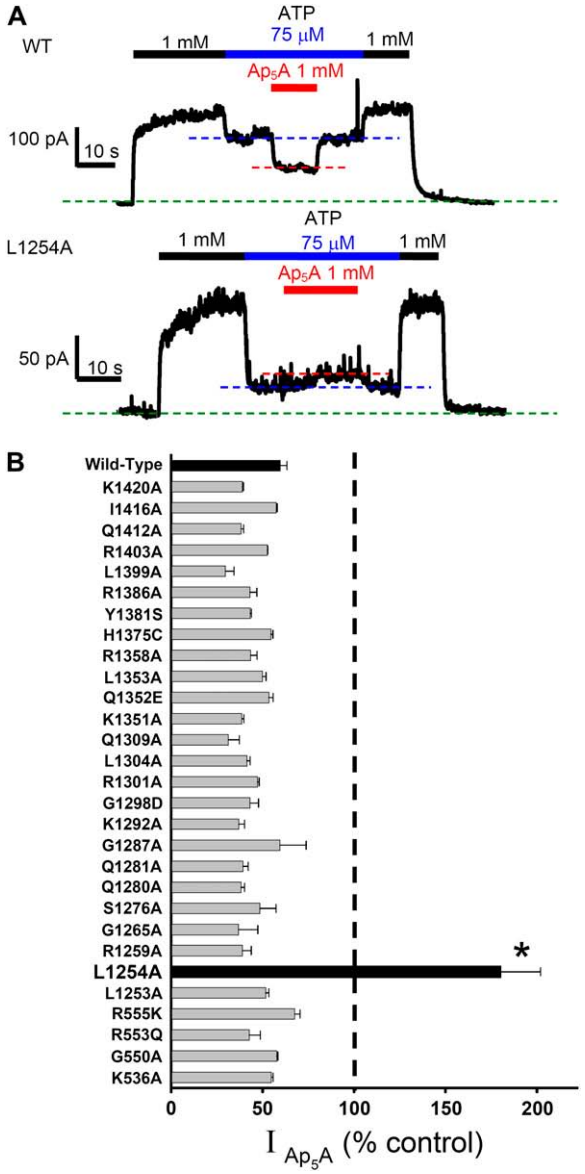

FIGURE 3 Effect of Ap_5A on wild-type and mutant CFTR Cl^- currents. (*A*) Representative time course of Cl^- current (*I*) in response to application of the indicated agents on wild-type CFTR and CFTR-L1254A. Data are from excised, inside-out patches containing multiple channels. The dashed lines indicate average current levels at the various conditions. (*B*) Data from all the tested mutations ($N \geq 3$ for each). The ATP concentration was 75 μM, and the Ap_5A concentration was 1 mM. Asterisk indicates $p < 0.05$ compared to wild-type CFTR using the Mann-Whitney rank sum test. It is possible that testing a larger number of membrane patches would show that Ap_5A inhibited some variants to a statistically greater degree than wild-type CFTR. Our goal, however, was to find mutations that reduced inhibition, and so we did not study most variants further.

addition, the activity of CFTR-L1254A was not completely saturated at 10 mM ATP. These results suggest that the L1254A mutation may have impaired the interaction with ATP.

In wild-type CFTR, Ap_5A did not alter the level of maximal current, but it increased the EC50 for ATP and the Hill

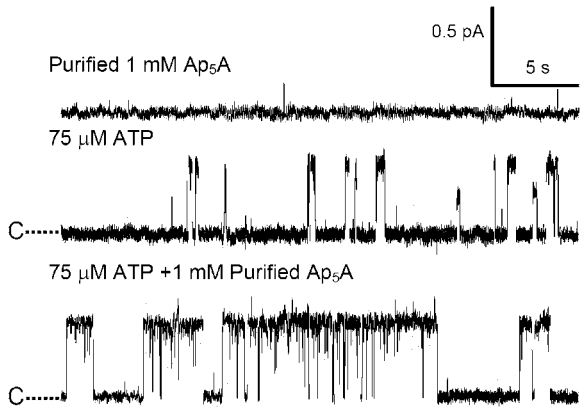

FIGURE 4 Effect of Ap_5A on current of CFTR-L1254A. Data are a tracing of a membrane patch perfused on the cytosolic surface with 1 mM purified Ap_5A alone, with 75 μM ATP alone, or with 75 μM ATP plus 1 mM purified Ap_5A. Channel was preactivated with PKA and 2 mM ATP. Adding Ap_5A alone yielded a failure to activate CFTR-L1254A in five other experiments.

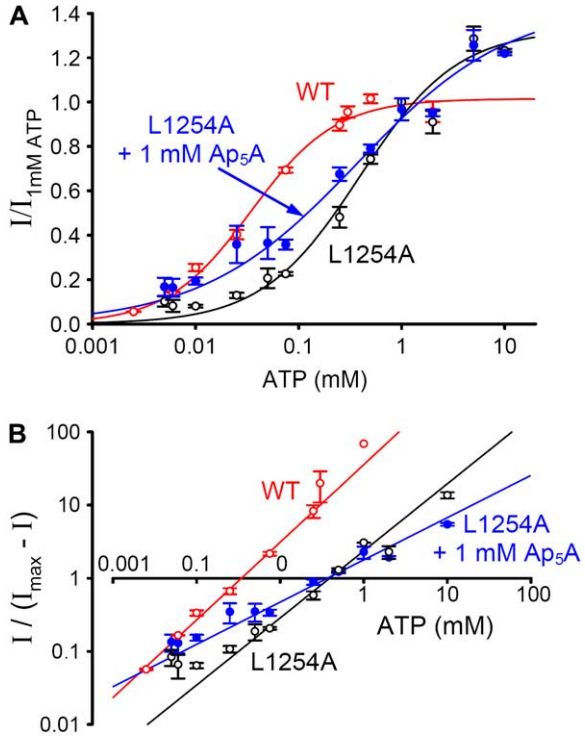

FIGURE 5 Effect of ATP concentration on current of wild-type CFTR (*WT*, *red*) and CFTR-L1254A in the absence (*black*) and presence (*blue*) of 1 mM Ap_5A. Because each patch contained a different number of CFTR channels, all currents were normalized to the current obtained with 1 mM ATP in the absence of Ap_5A. Data are plotted as ($I/I_{1mM\ ATP}$) in panel *A* or as a Hill plot in panel *B*. For wild-type CFTR, the Hill coefficient was 1.06 ± 0.08, the $K_m = 34 \pm 3\ \mu M$, and the $I_{max} = 1.01 \pm 0.02$. For CFTR-L1254A, the Hill coefficient was 0.92 ± 0.06, the $K_m = 392 \pm 38\ \mu M$, and the $I_{max} = 1.33 \pm 0.04$. For CFTR-L1254A studied with 1 mM Ap_5A data, the Hill coefficient was 0.58 ± 0.06, the $K_m = 369 \pm 100\ \mu M$, and the $I_{max} = 1.45 \pm 0.09$. Data are from 30 patches; n ≥ 3 for each ATP concentration.

coefficient decreased from 1.02 ± 0.04 to 0.63 ± 0.04 (18). With CFTR-L1254A, we found that 1 mM Ap_5A had no significant effect on the EC50 (369 ± 100 μM) or the predicted current at maximal ATP concentrations (Fig. 5, *A* and *B*). However, Ap_5A changed the shape of the curve; the Hill coefficient decreased to 0.58 ± 0.06 ($p < .05$). Thus, Ap_5A reduced the Hill coefficient to <1 in wild-type and L1254A CFTR suggesting conformational changes associated with negative cooperativity.

CFTR-L1254A interacts with AMP and other nucleotides

We tested several other agents to learn how the L1254A mutation affected function. In the presence of ATP, AMP induces adenylate kinase activity in isolated CFTR NBDs (17,18,20,21), and it induces gating that is dependent on adenylate kinase activity in CFTR (18). Previous studies also showed that ADP inhibits CFTR current (29,30), at least in part through its effect on adenylate kinase activity (19). We found that, as with wild-type channels, AMP stimulated and ADP inhibited current from the L1254A mutant (Fig. 6, *A* and *B*). These results suggest that the L1254A mutation did not completely disrupt the AMP-binding site in CFTR.

To further examine the functional consequences of the CFTR-L1254A mutation in the region around ATP-binding Site 2, we examined the effect of AMP-PNP and pyrophosphate (PPi). AMP-PNP is a nonhydrolyzable analog of ATP that increases current by delaying channel closure (9,15,31–33). Previous data showed that mutations that disrupt ATP-binding Site 2 prevented its stimulatory effect, although some biochemical data suggest that AMP-PNP may interact with ATP-binding Site 1 (13). Mutations around ATP-binding Site 2 can also prevent the stimulatory effects of PPi (14,34,35). We found that the L1254A mutation did not prevent the stimulatory effects of AMP-PNP or PPi (Fig. 6, *C* and *D*) and that stimulation was greater in CFTR-L1254A than in wild-type CFTR, perhaps because the basal activity before adding these agents was lower in CFTR-L1254A. These results suggest that the L1254A mutation did not completely disrupt the area around ATP-binding Site 2.

Ap_5A stabilized the open state of CFTR-L1254A

To determine how Ap_5A increased CFTR-L1254A current at low ATP concentrations, we removed ATP and measured the rate at which current decreased, that is, the relaxation rate. In wild-type CFTR, current decreased with a rate constant of 1195 ± 418 ms (Fig. 7, *A* and *B*). Ap_5A had no effect on the rate (1596 ± 430 ms; $p > 0.05$). In contrast, in CFTR-L1254A, Ap_5A nearly doubled the time constant for current decay (1657 ± 565 ms without Ap_5A, 3161 ± 948 ms with Ap_5A; $p < 0.01$). This result suggested that Ap_5A may have

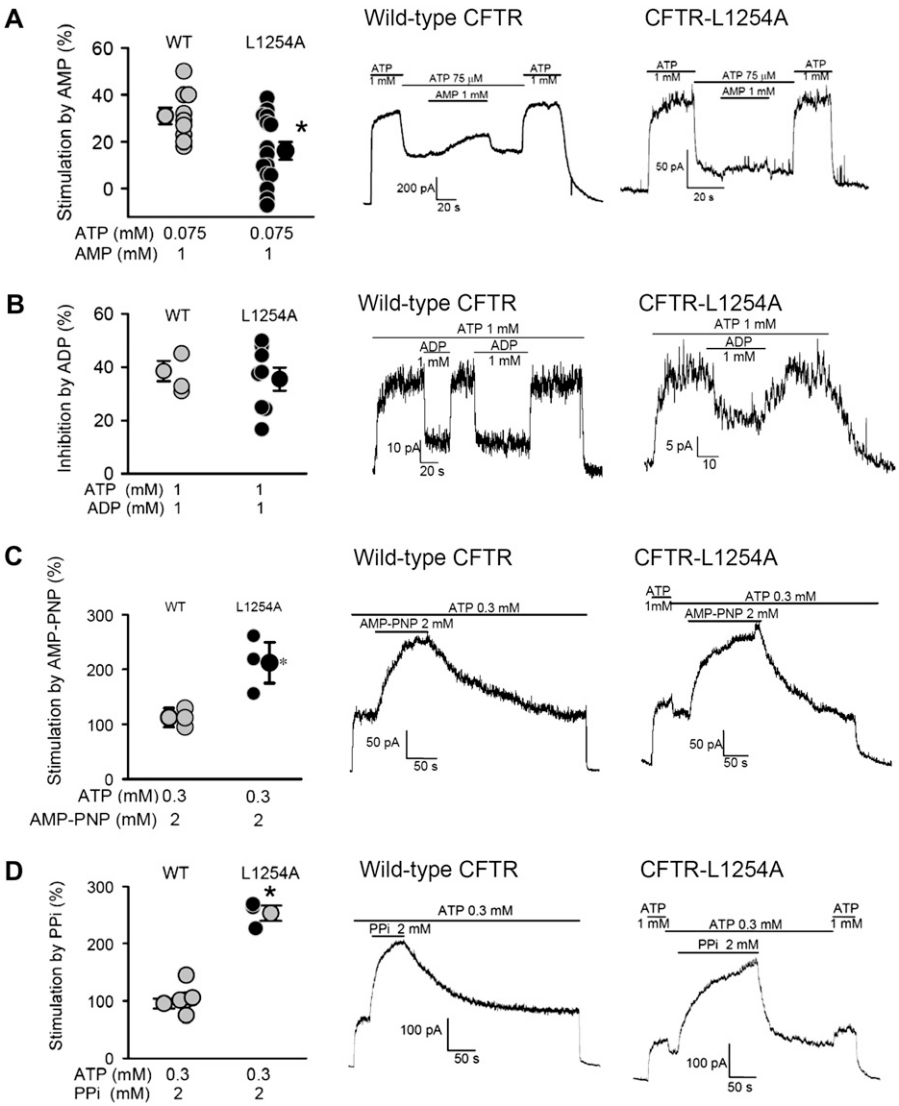

FIGURE 6 Effect of AMP (*A*), ADP (*B*), AMP-PNP (*C*), and PPi (*D*) on wild-type CFTR and CFTR-L1254A. Data are from excised, inside-out patches containing multiple CFTR channels. For each patch, data are normalized to current before addition of the test agent. Zero indicates no change. In *A*, N = 9 for wild-type CFTR and N = 15 for CFTR-L1254A; in *B*, N = 4 for wild-type and N = 8 for L1254A; in *C*, N = 3 for wild-type and L1254; and in *D*, N = 5 for wild-type and N = 3 for L1254A. Data are individual values and means ± SE. Asterisk indicates $p < 0.05$ by *t*-test. Tracings on right show examples.

stabilized the open state and predicted that Ap_5A would increase the burst duration of CFTR-L1254A.

In excised, inside-out patches of membrane, CFTR-L1254A showed reduced channel activity compared to wild-type CFTR. The P_o was 0.03 ± 0.01 (Fig. 8, *A* and *B*) compared with values of 0.15 ± 0.02 for wild-type CFTR obtained in recent studies from our laboratory under the same conditions (75 μM ATP) (18). A longer interburst interval (32.5 ± 11.6 s compared to 2.4 ± 0.27 s for wild-type CFTR (18)) reduced the P_o of CFTR-L1254A. However, the effect of the reduced opening rate on the P_o was partly countered by a prolonged burst duration (870 ± 204 ms compared to 427 ± 18 ms for wild-type CFTR (18)).

Adding 1 mM Ap_5A to CFTR-L1254A increased the P_o by increasing the burst duration ~40% (from 870 ± 204 ms to 1214 ± 281 ms; $p < 0.05$) without a significant effect on the interburst interval (from 32.5 ± 11.6 s to 32.9 ± 13.9 s; $p > 0.05$) (Fig. 8, *A* and *B*). These results support the conclusion that Ap_5A stabilized the open state. These findings are also very different from data obtained with wild-type CFTR in which Ap_5A had no significant effect on the burst duration but prolonged the interburst interval (18).

DISCUSSION

In all of the CFTR variants that we examined, Ap_5A altered current. However, we were surprised to observe that Ap_5A increased current from one of the variants, L1254A; this finding is the opposite of the effect of Ap_5A on wild-type CFTR (18).

Several observations suggest that the AMP-binding site remained available in CFTR-L1254A. First, Ap_5A altered CFTR-L1254A current. Our previous work on wild-type CFTR (18) and studies on other adenylate kinases (24,36) indicate that the ability of Ap_5A to alter function depends on

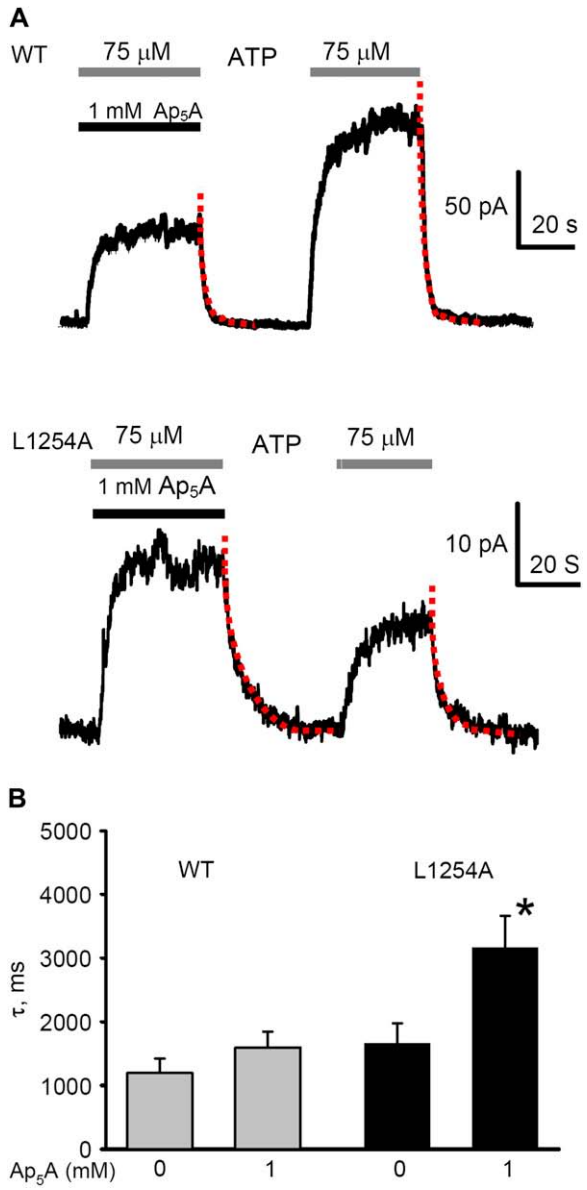

FIGURE 7 Current decay after removal of ATP or ATP plus Ap_5A. (*A*) Representative time course after removal of ATP and Ap_5A. Red dotted line shows the curves fit by an exponential function. (*B*) Mean decay rates after removal of ATP or ATP plus Ap_5A. N = 5 for both wild-type CFTR and CFTR-L1254A. As an additional test, we examined current decay after removing ATP but leaving Ap_5A in the solution. In those experiments, Ap_5A prolonged the relaxation rate for CFTR-L1254A (153 ± 57%; N = 5; $p < 0.05$) but not for wild-type CFTR (30 ± 42%; N = 5).

its simultaneous binding to the ATP- and AMP-binding sites. Second, AMP stimulated CFTR-L1254A current in the presence of ATP. This result is similar to the stimulation of wild-type CFTR (18). Third, ADP inhibited CFTR-L1254A current. Earlier studies showed that ADP inhibits CFTR function, at least in part via an effect on adenylate kinase activity involving ATP-binding Site 2 and the AMP-binding site (19).

The data also indicate that the L1254A variant altered the interaction with ATP. The observation most supportive of this conclusion was a 10-fold increase in EC50 for ATP-dependent stimulation. In addition, the mutation reduced the channel opening rate (increased the interburst interval); this step in the gating process is the one that is most dependent on ATP concentration (15,32,37). Studies of NBD crystal structures from related ABC proteins are also consistent with the conclusion that the L1254A mutation altered the interaction with ATP. Although residues corresponding to Leu-1254 itself do not interact directly with ATP, they do form hydrogen bonds to the Lys and Ser/Thr residues in the Walker A motif and to the Asp in the Walker B motif (6–8,38). These residues interact with ATP. In addition, these residues are important for gating (9,39–41) and for enzymatic activity (5,16,42). Our finding that CFTR-L1254A had a prolonged burst duration is consistent with a reduced enzymatic activity associated with mutating those residues.

How did Ap_5A stimulate CFTR-L1254A current? We speculate the following: 1), ATP binds ATP-binding Site 1 as it does in wild-type CFTR. Our data showing that Ap_5A only affects gating in the presence of ATP, combined with our earlier studies (18), suggest that the L1254A mutation does not disrupt ATP binding at Site 1. The predicted structure of the NBDs is also consistent with this conclusion because L1254 has hydrogen bonds only to other residues in NBD2; these hydrogen bonds are in the region of ATP-binding Site 2 and not in ATP-binding Site 1 or NBD1 (7,38). 2), The L1254A mutation partially disrupts ATP binding at Site 2. This speculation is supported by the reduced opening rate, the prolonged burst duration, and the 10-fold increase in ATP EC50 without a change in the shape of the relationship between ATP concentration and current. These changes are similar to those produced by other mutations thought to partially disrupt ATP-binding Site 2. For example, the K1250A and D1370N mutations also have a reduced opening rate, prolonged burst duration, and increased ATP EC50 (11,12,14,15,29,32,39,43). It seems unlikely that the L1254A mutation completely eliminates ATP binding at Site 2 because the mutation increased burst duration, whereas mutations that eliminate ATP binding at Site 2 do not increase burst duration (11,14,28). 3), Ap_5A binds to ATP-binding Site 2. We cannot exclude Ap_5A binding to ATP-binding Site 1; if that were the case, however, ATP would also have to bind to ATP-binding Site 2 because Ap_5A cannot open CFTR channels on its own. Why would the L1254A mutation reduce the affinity for ATP but allow Ap_5A to bind? We speculate that Ap_5A has a greater affinity in this mutant because it binds at two sites. Moreover, Ap_5A binding in adenylate kinases induces conformational changes that may enhance its binding affinity (23,44,45), and perhaps this occurs in CFTR-L1254A. 4), We speculate that Ap_5A binding to CFTR-L1254A generates a more stable open state because Ap_5A is not a substrate for ATPase or adenylate kinase activity. Similar behavior is observed with the nonhydrolyzable

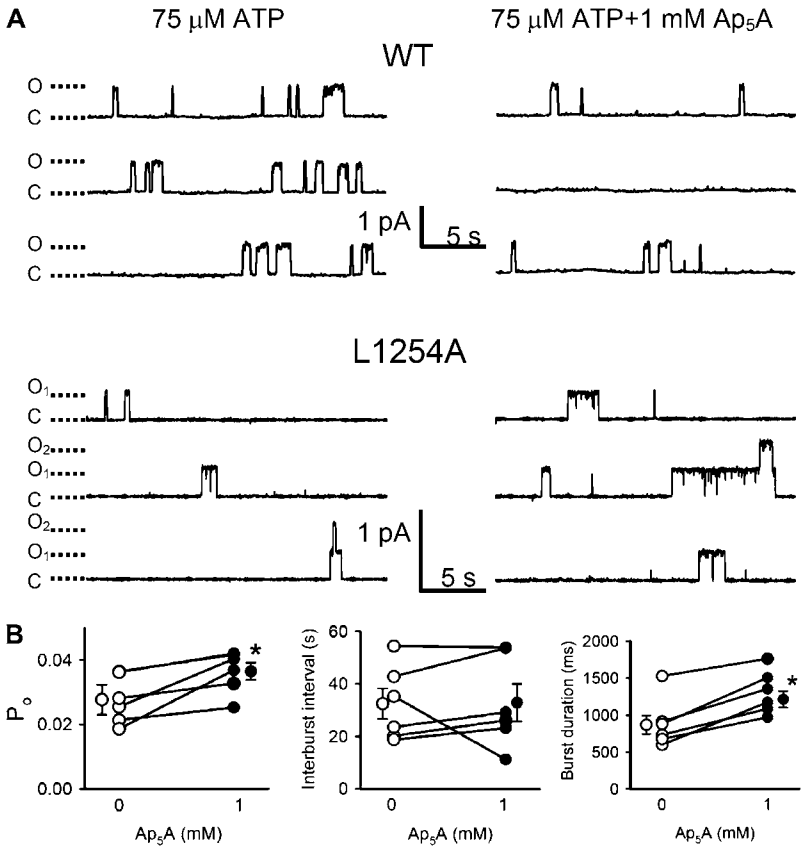

FIGURE 8 Effect of Ap_5A on single-channel activities of wild-type CFTR and CFTR-L1254A. (*A*) Example of a tracing of a patch perfused on the cytosolic surface with ATP alone or ATP and Ap_5A as indicated. For illustration purposes, traces were digitally filtered at 10 Hz with a Bessel filter. If there was more than one channel in a membrane patch, the total open time at all levels was divided by the number of channels to obtain P_o. Membrane patches with 1 to 3 channels were studied. Only single opening events were used to calculate burst duration. Voltage was −100 mV for wild-type CFTR and −50 mV for CFTR-L1254A. (*B*) Single-channel kinetic properties with 75 μM ATP. N = 6 membrane patches. Mean ± SD are also shown. Asterisks indicate $p < 0.02$ compared to 75 μM ATP alone using a paired *t*-test.

ATP analog AMP-PNP, which prolongs the burst duration (9,15,31–33). In addition, Ap_5A may have a slower off-rate than ATP if it binds two sites.

Although L1254A is not a mutation that causes disease, it does reduce CFTR function. The finding that Ap_5A enhances the function of this mutant, whereas it inhibits the function of wild-type CFTR, suggests the possibility that agents might be developed that could target unique cystic fibrosis-associated mutants. These data also suggest that using knowledge about the adenylate kinase activity of CFTR may be of value in developing new therapeutic strategies.

We thank Philip Karp, Tamara Nesselhauf, Pamela Hughes, Huiyu Gong, Yuhong Li, and Theresa Mayhew for excellent technical assistance. We thank Jeng-Haur Chen for help with patch-clamp methodology and helpful discussion. We also thank Lynda S. Ostedgaard and Dan Vermeer for helpful discussions. We thank Alexander R. Horswill, Jeffrey S. Kavanaugh, and Aaron Van Pelt for help with the HPLC purification. We thank Kevin P Campbell for use of his lyophilizer. We also thank Ziying Yan for use of his HPLC column.

This work was supported by the In Vitro Models and Cell Culture Core (supported in part by the National Heart, Lung, and Blood Institute (grant HL51670)), the Cystic Fibrosis Foundation (grants R458-CR02 and ENGLH9850), the National Institute of Diabetes and Digestive and Kidney Disease (grant DK54759); the National Institutes of Health (grant HL61234); the Cystic Fibrosis Foundation (grants RANDAK05F0 and RANDAK 04D0 to C.O.R.) and the Francis Family Foundation (to C.O.R.). M. J. W. is an Investigator of the Howard Hughes Memorial Institute.

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