Regulation of Channel Gating by AMP-activated Protein Kinase Modulates Cystic Fibrosis Transmembrane Conductance Regulator Activity in Lung Submucosal Cells*

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Cystic fibrosis transmembrane conductance regulator (CFTR) Cl− channel activity is important for fluid and electrolyte transport in many epithelia including the lung, the site of most cystic fibrosis-associated morbidity. CFTR is unique among ion channels in requiring ATP hydrolysis for its gating, suggesting that its activity is coupled to cellular metabolic status. The metabolic sensor AMP-activated kinase (AMPK) binds to and phosphorylates CFTR, co-localizes with it in various tissues, and inhibits CFTR currents in Xenopus oocytes (Hallows, K. R., Raghuram, V., Kemp, B. E., Witters, L. A. & Foskett, J. K. (2000) J. Clin. Invest. 105, 1711–1721). Here we demonstrate that this AMPK-CFTR interaction has functional implications in human lung epithelial cells. Pharmacological activation of AMPK inhibited forskolin-stimulated CFTR short circuit currents in polarized Calu-3 cell monolayers. In whole-cell patch clamp experiments, the activation of endogenous AMPK either pharmacologically or by the overexpression of an AMPK-activating non-catalytic subunit mutant (AMPK-y1-R70Q) dramatically inhibited forskolin-stimulated CFTR conductance in Calu-3 and CFTR-expressing Chinese hamster ovary cells. Plasma membrane expression of CFTR, assessed by surface biotinylation, was not affected by AMPK activation.

In contrast, the single channel open probability of CFTR was strongly reduced in cell-attached patch clamp measurements of Calu-3 cells transfected with the AMPK-activating mutant, an effect due primarily to a substantial prolongation of the mean closed time of the channel. As a metabolic sensor in cells, AMPK may be important in tuning CFTR activity to cellular energy charge, thereby linking transepithelial transport and the maintenance of cellular ion gradients to cellular metabolism.

The cystic fibrosis transmembrane conductance regulator (CFTR) is a plasma membrane Cl− channel expressed on the apical membranes in a wide variety of epithelial tissues, including the lung, intestine, pancreas, and reproductive tract. CFTR plays an important role in absorption and secretion of salt and water, with its channel activity often determining the rate of transepithelial transport in various tissues. The importance of native regulation of CFTR channel activity is underscored by pathological conditions, including cystic fibrosis (CF), where mutations in CFTR cause reduced activity (2), and secretory diarrhea, which results from excessive cellular stimulation of CFTR (3). CFTR is activated in cells by hormone- and neurotransmitter-induced CAMP signaling. The cAMP-dependent protein kinase A (PKA) phosphorylates several residues in the cytoplasmic R domain of CFTR, which activates gating by destabilizing channel closed states (4). Activated CFTR channels can be inactivated by phosphatases (5). PKA-mediated phosphorylation may also enhance Cl− transport through the insertion of additional CFTR channels into the plasma membrane (6). Whereas PKA-associated phosphorylation/dephosphorylation is the most thoroughly understood process regulating CFTR channel activity, other mechanisms may modulate the activity of CFTR under various cellular conditions. In this regard, AMP-activated protein kinase (AMPK) was discovered to interact with CFTR and inhibit its activity (7).

AMPK is a ubiquitous serine/threonine kinase with orthologues in all eukaryotes that exist as a heterotrimer with a catalytic α subunit and regulatory β and γ subunits (8). In response to metabolic stress and increasing intracellular AMP levels (9), AMPK phosphorylates and inhibits several important rate-limiting biosynthetic enzymes, thereby acting to preserve cellular ATP stores during metabolic depletion (8). Recently, mutations in AMPK have been linked to human diseases, including familial hypertrophic cardiomyopathy and Wolff-Parkinson-White syndrome (10). Furthermore, modulation of AMPK may also play an important role in the pathogenesis and treatment of type II diabetes mellitus (11) and obesity (12).

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** The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; CF, cystic fibrosis; PKA, cyclic AMP-dependent protein kinase; AMPK, AMP-activated protein kinase; CHO, Chinese hamster ovary; GFP, green fluorescent protein; HA, hemagglutinin; DMEM, Dulbecco’s modified Eagle’s medium; Isc, short circuit current; I-V, current-voltage; AICAR, 5-aminoo-4-imidazolecarboxamide riboside; PBS, phosphate-buffered saline.
AMPK-dependent Inhibition of CFTR Gating in Calu-3 Cells

K. R. Hallows and J. K. Foskett, unpublished results.

Calu-3 is unique among ion channels in its requirement for ATP binding and hydrolysis to support channel activity. We previously discovered that the COOH-terminal regulatory sequence of the AMPK α subunit directly interacts with the CFTR COOH-terminal tail. Our data indicate that this interaction could be of physiological significance, because AMPK and CFTR co-localize in epithelia, and AMPK phosphorylates CFTR in vitro and inhibits AMP-activated CFTR conductances in Xenopus oocytes (7) and polarized colonic T84 cell monolayers. However, the mechanisms underlying inhibition of CFTR activity by AMPK have not been determined.

The most serious CF-associated morbidity results from lung pathology. Defective Cl⁻ transport due to CFTR mutations results in abnormal composition and/or volume of airway surface liquid, which compromises airway clearance resulting in chronic infection and the destruction of lung tissue (13, 14). Because detailed insights into the mechanisms of CFTR regulation in the lung could provide new insights into the pathogenesis of CF lung disease and suggest possible novel therapeutics, here we have investigated AMPK inhibition of CFTR activity in Calu-3 human lung serous cells. Using pharmacological, biochemical, and electrophysiological approaches, we demonstrate that AMPK is an endogenous inhibitor of CFTR activity in lung submucosal gland serous cells. Furthermore, we have determined that this inhibition is mediated by effects on CFTR single channel gating.

**EXPERIMENTAL PROCEDURES**

**Reagents and Chemicals**—All reagents and chemicals used were purchased from Sigma unless otherwise noted.

**Cell Culture, Cloning and Transfection**—Calu-3 cells (ATCC HTT-55) were maintained as described (15). CHO cells stably transfected with CFTR (CHO-BQ2 cells), kindly provided by Dr. J. Riordan, were maintained as described (16).

AMPK-α-K45R and AMPK-β1-R70Q were cloned into the bicistronic pTracer-CMV2 vector (Invitrogen), which constitutively expresses a green fluorescent protein (GFP)-Zeocon fusion protein and the cloned gene insert under the control of separate promoters. This plasmid enabled transfected cells to be identified by their green fluorescence for patch clamp experiments, which was mandatory because of low transfection efficiency (~5%). Inserts were synthesized by high fidelity PCR (PFU Turbo, Stratagene) using the pMT2-α-K45R and pMT2-β1-R70Q plasmids (17, 18) as templates and specific primers (sequences available on request) that provided an NH₂-terminal hemagglutinin (HA) tag on each gene product. All final clones were verified by DNA sequencing. Transfections were performed using LipofectAMINE 2000 (Invitrogen) according to the manufacturer’s recommendations. For Calu-3 cells, 8–10 × 10⁵ cells were seeded onto a 25-mm circular sterile Petri dish, and grown in DMEM/F12 medium for 28 days in culture. The experimental bath solutions contained 120 mM NaCl, 2.7 mM KCl, 10 mM HEPES (pH 7.4), and 1 mM MgCl₂, 0.1 mM CaCl₂, 1 mM EGTA, 1 mM MgATP, 10 mM HEPES (pH 7.5), and 1 μM forskolin. The pipette solution was the same but without ATP and forskolin. Current traces were obtained using an Axopatch 200B amplifier at ±60 mV pipette potential, filtered at 100 Hz, digitized at 2 kHz, and recorded to hard disk using PULSE+PULSEFIT software (HEKA Electronics, Lambrecht/Pfalz, Germany) on a Power Macintosh computer. Liquid junction and tip potentials were corrected for each pipette after immersion into the bath solution. Corrections for cellular capacitance and series resistance were immediately after sealing and breaking into whole-cell mode. The plasma membrane was held at ~20 mV, and current-voltage (I-V) plots were obtained from currents recorded in response to a series of 850-ms voltage steps from ~100 to 100 mV in 20-mV increments at 1.5-s intervals. Whole-cell conductance was calculated as the slope at ~20 mV of a fifth order polynomial fit to the I-V data. Normalized conductance (gₛ/pf) was calculated as the mean calculated whole-cell conductance under a given condition divided by the whole-cell capacitance measured prior to the start of data collection for that cell. Forskolin (1 μM) was added to the bath solution to stimulate CFTR.

**Whole-cell Patch Clamp Experiments**—All experiments were performed at room temperature. Fire-polished borosilicate pipettes (catalog no. 1B150F-4, World Precision Instruments, Inc., Sarasota, FL) had resistances of ~3–7 MΩ. Bath and pipette solutions used are described previously (20). Recordings were made using an Axopatch 200A amplifier (Axon Instruments, Inc.) interfaced with PULSE+PULSEFIT software (HEKA Electronics, Lambrecht/Pfalz, Germany) on a Power Macintosh computer. Liquid junction and tip potentials were corrected for each pipette after immersion into the bath solution. Currents were filtered at 100 Hz, digitized at 2 kHz, and recorded to hard disk using PULSE+PULSEFIT software (HEKA Electronics, Lambrecht/Pfalz, Germany). Mean open and closed times for each condition were derived from the means of all of the mean open and closed times calculated for each individual experiment. To calculate mean open and closed times of data from patches containing >1 channel, we used the following formulas derived from Horton and Lange (21) as shown in Equations 1 and 2.

\[
\text{Mean open time} = \frac{(N \cdot \text{P})}{(T / \text{E}(2))}
\]

\[
\text{Mean closed time} = \frac{(N \cdot (1 - \text{P}) \cdot \text{E})}{(T / \text{E}(2))}
\]

where T is time and E is the total number of events (both opening and closing).

**AMPK Kinase Assays**—Polarized Calu-3 cells were grown on Costar Transwells (catalog no. 3460) as described above for short circuit current measurements. For the experiment, the medium was replaced with DMEM/F12 plus 1 mM 5-amino-4-imidazolecarboxamide riboside (AICAR; for controls) on the apical and basolateral sides and then incubated for 2 h at 37 °C with 5% CO₂. Before lysis, cells were washed twice on both sides with ice-cold PBS. Lysate buffer (LB) contained 20 mM Tris Cl, 50 mM NaCl, 50 mM NaF, 5 mM sodium pyrophosphate, 250 mM sucrose, and 1% Triton X-100 (pH 7.4, with NaOH). Complete protease inhibitor cocktail (1×, Roche Molecular Biochemicals), 1 mM phenylmethylsulfonyl fluoride, and 2 mM dithiothreitol were added to the LB just prior to addition of 120 μL of LB mixture to the apical side of each well. After rocking the samples for 15 min at 4 °C, lysates from each well were collected. For each condition (AICAR and control), three sets of lysates pooled from two wells each were pelleted at 14,000 × g at 4 °C for 10 min. Protein concentrations of lysates were estimated by the Bio-Rad kit. AMPK activity was measured against the SAMS peptide following immunoprecipitation of the kinase from cell lysates with an anti-α subunit antibody that recognizes both catalytic subunit isoforms (18).

**Surface Biotinylation Assays**—CHO-BQ2 cells transfected with chambers interfaced with a voltage-current clamp amplifier (Physiologic Instruments, San Diego, CA) and electronic chart recorder (PowerLab, ADInstruments, Grand Junction, CO). The monolayers were continuously voltage clamped to 0 mV after fluid resistance and asymmetry voltage compensation. Changes in transepithelial resistance were calculated using Ohm’s law from the current excursions resulting from periodic 5-mV bipolar voltage pulses. Under conditions of these experiments, Eₓ is equivalent to net Cl⁻ and/or HCO₃⁻ secretion via CFTR. Calu-3 cell monolayers used had baseline transepithelial resistance values ranging from 200–600 ohm cm².

**Cell-attached Patch Clamp Experiments**—Pipette resistances were ~7–20 megohms. The bath solution contained 140 NaCl m, 2 mM MgCl₂, 0.1 mM CaCl₂, 1 mM EGTA, 1 mM MgATP, 10 mM HEPES (pH 7.5), and 1 μM forskolin. The pipette solution was the same but without ATP and forskolin. Current traces were obtained using an Axopatch 200B amplifier at ±60 mV pipette potential, filtered at 100 Hz, digitized at 2 kHz, and recorded to hard disk using PULSE+PULSEFIT software (HEKA Electronics, Lambrecht/Pfalz, Germany). Because changes in the apparent open probability (P_open) and number of active channels (N) were observed in cell-attached patches during the first 5 min of recording, only subsequent current traces of at least 5 min duration were used for data analysis (TAC software, Bruxton, Seattle, WA). The number of channels in a patch was assumed to be the maximum number of open channel current levels observed during the experiment. The data were fitted and modeled using IGOR PRO 4.0 software (WaveMetrics, Lake Oswego, OR), as described (15). Mean open and closed times for each condition were derived from the means of all of the mean open and closed times calculated for each individual experiment. To calculate mean open and closed times of data from patches containing >1 channel, we used the following formulas derived from Horton and Lange (21) as shown in Equations 1 and 2.

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\]
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pTracer vector alone, pTracer-HA-wt-K45R, or pTracer-HA-y1-R70Q (two dishes per condition) were used 2 days after transfection. Biotinylation assays were performed based on a previously described protocol (22). Cells were exposed to 1 µM forskolin in growth medium at 37 °C for 10 min prior to washing twice in ice-cold PBS (pH 8.0) and then adding ice-cold 1 ng/ml suls-NHS-SS-biotin (Pierce) dissolved in PBS. Samples were rocked for 30 min at 4 °C, then washed once in ice-cold PBS plus 1% BSA, and then washed in PBS. Cells from two dishes were lysed in 250 µl of biotinylation lysis buffer that contained 150 mM NaCl, 20 mM HEPES, 2 mM EDTA, and 1% Nonidet P-40 (pH 7.5, at room temperature), with 1 mM phenylmethylsulfonyl fluoride and 1× complete protease inhibitor cocktail added just prior to lysis. After incubating dishes on ice for 10 min, cells were scraped using a cell lifter, and lysates were centrifuged at 14,000 × g at 4 °C. Supernatant protein concentrations were measured using the Bio-Rad assay, and the samples were diluted in lysis buffer to equalize all protein concentrations. 75 µl of a 50% streptavidin-agarose slurry (Pierce) was then added to each sample before rotating for 2 h at 4 °C. After gently pelleting the beads, 10% of the supernatant (~30 µl) was aliquoted into 10 µl of 4× Laemmli sample buffer (unbound fraction). The remainder of the supernatant was removed, and the beads were washed twice in cold lysis buffer and once in PBS. Then, 30 µl of 2× sample buffer containing 200 mM dithiothreitol was added to the beads and the mixture was heated to 65 °C for 15 min to elute all proteins (bound fraction). Samples were loaded onto a 4–12% gradient gel (NuPage, Invitrogen), electrophoresed, and transferred to nitrocellulose membranes. The membranes were cut into high molecular weight and low molecular weight portions and then immunoblotted using primary rabbit polyclonal anti-CFTR antibody (A2; kindly supplied by Dr. W. Skach) or mouse monoclonal anti-HA antibody (HA.11, Covance), followed by the appropriate secondary antibody as described (7). After exposure to film, band intensities were quantitated using a Molecular Dynamics Personal Densitometer SI scanner and ImageQuant software (Amersham Biosciences), with normalization of all bands to background levels on the film. To calculate the percentage of biotinylated CFTR for each condition, the intensity of the unbound fraction CFTR band was multiplied by 10 before comparison with the bound fraction band.

RESULTS

AMPK Activation Inhibits CFTR Currents in Polarized Calu-3 Cells—We have recently shown that AMPK modulates CFTR-mediated Cl– currents in both the Xenopus oocyte expression system (7) and polarized colonic T84 cell monolayers. However, the underlying mechanisms for AMPK-dependent inhibition of CFTR have not yet been determined. To examine whether AMPK regulates CFTR in polarized lung epithelial cells, the Calu-3 human lung serous epithelial cell line, which endogenously expresses both AMPK and CFTR (7, 23), was used. CFTR-dependent basal and forskolin-activated short circuit currents were measured in polarized cells grown on permeable supports and mounted in Ussing chambers. To test whether AMPK could modulate endogenous CFTR activity, cells were exposed for 2 h prior to and during the experiment to vehicle alone (control) or to 1 mM AICAR, a cell-permeant purine synthesis intermediate that activates endogenous AMPK in vivo (24) (Fig. 1A). After steady-state basal currents were measured, 4 µM forskolin was added to further stimulate the CFTR-mediated Cl– conductance at the apical membrane. To help ensure that the stimulated apical membrane conductance was not rate-limited by the basolateral membrane conductance, 2 µM thapsigargin, a Ca2+/ATPase inhibitor, was then added to increase cytosolic Ca2+ and thereby activate basolateral K+ conductances (25). Typical experimental traces are shown in Fig. 1A. Activation of endogenous AMPK with AICAR inhibited the baseline Isc (by 47 ± 11%) as well as the forskolin-stimulated Isc, measured in either the presence or absence of thapsigargin (by 41 ± 1% with forskolin alone and by 49 ± 3% with forskolin plus thapsigargin) as compared with untreated paired controls (Fig. 1B). To verify that AICAR treatment inhibited CFTR-mediated currents as a consequence of the activation of endogenous AMPK kinase activity, we employed an in vitro kinase assay using lysates prepared from AICAR-treated or control Calu-3 cells. AICAR pretreatment stimulated endogenous AMPK kinase activity to ~270% of control levels, as assessed by this assay (Fig. 1C). Therefore, AICAR treatment of Calu-3 monolayers stimulated AMPK activity, and this stimulation was associated with an inhibition of CFTR-mediated transepithelial currents. An independent experiment, described below, also indicated that AICAR mediates its effects on CFTR activity specifically by enhancing AMPK activity.

Whole-cell CFTR Conductance Is Inversely Modulated with Endogenous AMPK Activity Modulation—To confirm that the observed AMPK-dependent inhibition of Isc occurred via modulation of CFTR activity, whole-cell patch clamp measurements were made using different methods to modulate the activity of endogenous AMPK (Fig. 2). Forskolin increased the whole-cell conductance within 1–2 min and shifted the reversal potential to ~−20 mV, close to the Cl– reversal potential (ECl), consistent with activation of plasma membrane Cl– conductance (Fig. 2A, center panel). Glibenclamide (500 µM) substantially inhibited the stimulated conductance, especially at hyperpolarized voltages, suggesting that the forskolin-induced increase in whole-cell conductance was due to activation of CFTR (Fig. 2A, right panel). Consistent with the above results obtained from transepithelial measurements, AICAR treatment inhibited the forskolin-stimulated capacitance-normalized whole-cell conductance by 37% compared with untreated controls (Fig. 2B). To specifically modulate AMPK activity using distinct approaches, Calu-3 cells were transfected with mutant AMPK cDNAs. Two con-
structures were used that alter endogenous AMPK activity. AMPK-α1-K45R contains a Lys to Arg (K45R) point mutation at the active site of the AMPK α subunit kinase domain that renders the kinase catalytically inactive (17, 26). Overexpression of this mutant in cultured cells (27) and in transgenic mice (28) causes a down-regulation of the endogenous α subunit, presumably through competition for binding to the endogenous β and γ subunits. Therefore, overexpression of the α-K45R mutant has a dominant negative effect on AMPK activity in cultured cells and in vivo (17, 28). Conversely, a γ subunit mutant (γ1-R70Q) renders the AMPK heterotrimer constitutively active by causing hyperphosphorylation of the α subunit in the activation loop (at Thr-172) and rendering the enzyme relatively AMP-independent (18). Cells were transfected with either of these constructs in a vector that also contained the cDNA for the GFP, enabling transfected cells to be identified for patch clamp electrophysiology. In cells expressing the dominant negative α1-K45R mutant, the forskolin-activated conductance was not significantly different from that observed in control cells, although there was a trend toward activation (by 26% compared with mock-transfected cells and 8% compared with vector alone-transfected cells) (Fig. 2C). To ensure that the transfected cells expressed the α1-K45R mutant and that it was indeed exerting a dominant negative inhibitory effect on the endogenous AMPK activity, we examined the effects of AICAR in the transfected cells. Whereas AICAR treatment inhibited the forskolin-activated whole-cell conductance in vector alone-transfected cells, as described above, it had no inhibitory effect in cells that had been transfected with the dominant negative AMPK mutant (Fig. 2D). These results therefore suggest, first, that the AICAR inhibition of CFTR conductance observed (Figs. 1 and 2B) occurred as a specific consequence of the activation of endogenous AMPK and, second, that basal CFTR activity in Calu-3 cells is not significantly inhibited by AMPK under the conditions of our experiments so that the dominant negative inhibition of the kinase had either no effect or a slight stimulatory one (i.e. relief from AMPK inhibition).

More dramatic results were obtained from cells expressing AMPK-γ1-R70Q. The forskolin-activated whole-cell conductance was profoundly inhibited (by 69% compared with mock-transfected cells and 73% compared with vector alone-transfected cells) (Fig. 2C). Thus, specific activation of endogenous AMPK in lung serous cells strongly inhibited the activity of CFTR. In summary, these results show that modulation of endogenous AMPK activity in Calu-3 cells using both pharmacological and molecular expression approaches modulated endogenous CFTR activity in this cell type as assessed by two distinct electrophysiological approaches.

Cell Surface Expression of CFTR Is Unaffected by Modulation of Endogenous AMPK Activity—The inhibition of CFTR by AMPK observed in lung Calu-3 cells is in accord with observations made in the T84 colonic cell line,2 which also involved endogenous AMPK and CFTR as well as in Xenopus oocytes engineered to express both proteins (7). In all three cellular systems, activation of AMPK activity resulted in diminished CFTR activity, but the mechanisms that underlie this inhibition are unknown. Because total whole-cell or tissue CFTR-mediated anion currents have been measured in all of these cell systems, AMPK modulation of CFTR activity could conceivably occur through changes in the amount of CFTR expressed at the plasma membrane and/or through changes in CFTR single channel properties (Pn, and/or single-channel conductance). The activation of AMPK in skeletal muscle has been shown to modulate the amount of GLUT4 glucose transporters in the plasma membrane (29). To determine whether changes in CFTR cell surface expression could account for the observed modulation, biotinylation assays were performed to determine the amount of CFTR in the plasma membrane. For these experiments, CHO-BQ2 cells stably expressing CFTR were transiently transfected with either vector alone or HA-tagged dom-

![Fig. 2. Effects of modulating AMPK activity on whole-cell conductances in Calu-3 cells. A, whole-cell current sweeps (y axis and lower x axis) and superimposed I-V curves (y axis and upper x axis) from representative control experiment before (left) and after both the addition of 1 μM forskolin (middle) and then 500 μM glibenclamide (right). B, normalized conductances of forskolin-stimulated cells treated for 2 h with 1 mM AICAR versus untreated controls (*, unpaired t test). C, normalized conductances of mock (no DNA)-transfected cells and cells transfected with the pTracer vector alone or the dominant negative α1-K45R or constitutive-activating γ1-R70Q AMPK mutants (*, p < 0.001 for γ1-R70Q versus both mock- and α1-K45R-transfected cells, unpaired t tests). D, normalized conductances of cells transfected with pTracer alone or with the dominant negative α1-K45R AMPK mutant, with or without a 2-h AICAR pretreatment (*, p < 0.005 for pTracer + AICAR versus α1-K45R + AICAR, unpaired t test). Data shown are the mean (±S.E.) normalized conductances from 4 to 11 replicate experiments.](http://www.jbc.org/Downloaded from)
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Fig. 3. Lack of effects of modulating AMPK activity on CFTR surface expression. CHO-BQ2 cells were transfected with pTracer vector alone or the HA-tagged dominant negative α1-K45R or constitutive-activating γ1-R70Q AMPK mutants followed by surface labeling of plasma membrane proteins by biotinylation as described under “Experimental Procedures.” Cellular proteins were then adsorbed to streptavidin-agarose and bound (plasma membrane) and unbound (non-plasma membrane) fractions separated. A, representative immunoblots probed with antibodies to CFTR (top) or HA (bottom). For each transfection condition shown, 10% of the unbound fraction (U) and 100% of the bound fraction (B) was loaded onto the gel. B, scanning densitometric determinations of the mean (± S.E.) fraction of biotinylated CFTR from five replicate experiments. C, mean (± S.E.) normalized forskolin-stimulated conductance measurements from 3–5 replicate experiments for each transfection condition (*, p < 0.05 for HA-γ1-R70Q versus both pTracer vector alone-transfected and HA-α1-K45R-transfected cells, unpaired t-tests).

AMPK-dependent Inhibition of CFTR Gating in Calu-3 Cells

The preceding results suggested that the predominant mechanism by which AMPK modulates CFTR—predominant mechanism by which AMPK modulates CFTR—predominant mechanism by which AMPK modulates CFTR. To address this issue, we performed cell-attached patch clamp experiments on Calu-3 cells transfected with either pTracer vector alone (control) or the constitutive-activating AMPK-γ1-R70Q mutant. A, typical traces of single-channel patches under each condition. Arrows indicate the channel closed level. Data were recorded at −60-mV pipette potential. B, mean P0 from at least 10 experiments performed for each transfection condition with patches containing 1–6 active channels (*, p < 0.001 compared with control, unpaired t test). C, mean (± S.E.) open and closed times for each transfection condition (see “Experimental Procedures” for details; p values are shown). Because the P0 measured in the γ1-R70Q transfected cells was low, it is more likely that N was underestimated in them, which would tend to cause an overestimation of P0 under that condition.

Fig. 4. Effects of AMPK activation on single CFTR channels. Cell-attached patch clamp experiments were performed on Calu-3 cells transfected with either pTracer vector alone (control) or the constitutive-activating AMPK-γ1-R70Q mutant. A, typical traces of single-channel patches under each condition. Arrows indicate the channel closed level. Data were recorded at −60-mV pipette potential. B, mean P0 from at least 10 experiments performed for each transfection condition with patches containing 1–6 active channels (*, p < 0.001 compared with control, unpaired t test). C, mean (± S.E.) open and closed times for each transfection condition (see “Experimental Procedures” for details; p values are shown). Because the P0 measured in the γ1-R70Q transfected cells was low, it is more likely that N was underestimated in them, which would tend to cause an overestimation of P0 under that condition.

In contrast, the P0 was reduced by 64% (p < 0.001, unpaired t test) in the AMPK-γ1-R70Q-overexpressing cells (0.13 ± 0.03, n = 10) compared with vector alone-transfected cells (0.36 ± 0.03, n = 11) (Fig. 4B). Of note, this magnitude of P0 inhibition is comparable with that of the forskolin-activated whole cell conductance (Fig. 2C), suggesting that inhibition of single-channel gating can account for the AMPK-dependent inhibition of CFTR. The decreased P0 in the AMPK-γ1-R70Q-transfected cells was due primarily to a substantial prolongation of the mean channel closed time (Fig. 4C), suggesting that AMPK activation stabilizes the closed conformation of the CFTR channel.
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DISCUSSION

In the present study we have demonstrated that endogenous AMPK can modulate the activity of CFTR in lung serous epithelial cells (Figs. 1 and 2). This AMPK-dependent modulation of CFTR activity could not be accounted for by changes in the amount of CFTR expressed at the plasma membrane (Fig. 3). Rather, CFTR inhibition following constitutive AMPK activation was mediated through effects on CFTR single channel gating; i.e. AMPK activation significantly inhibited the Po of CFTR by stabilizing the closed conformation of the channel (Fig. 4). Until now, the most well established kinase regulation of CFTR activity was that associated with PKA and, to a lesser extent, protein kinase C, of which both act to stimulate gating (30). Whereas specific phosphorylation of CFTR is believed to underlie these effects, the molecular mechanisms involved in AMPK regulation of CFTR remain to be elucidated. Two general possibilities remain to be investigated. First, AMPK may exert its effects directly on CFTR. The catalytic subunit of AMPK directly binds to a region near the COOH terminus of CFTR, and interaction of CFTR with AMPK may provide a sensitive mechanism to couple its activity to cell metabolic state, fine-tuning AMP by adenylate kinase (33). Because AMPK responds to the ATP ratio due to the rapid interconversion of ATP, ADP, and AMP levels produce large changes in the intracellular AMP:ATP ratio (34). Therefore, AMPK may become engaged not only following severe reductions in intracellular ATP (e.g. that occur with ischemic or hypoxic injury) but also during more subtle, “physiological” fluctuations of intracellular ATP that may occur on a regular basis. Thus, the interaction of CFTR with AMPK may provide a sensitive mechanism to couple its activity to cell metabolic state, fine-tuning CFTR transport activity on a short time scale in response to metabolic conditions.

CFTR appears to mediate the activities of various membrane transport proteins and may thereby help to coordinate an ensemble of proteins involved in transepithelial salt and water transport (34). Thus, the AMPK-dependent regulation of CFTR may afford a mechanism to couple epithelial transport in general to cellular metabolic status. In addition, AMPK might directly modulate the activity of other important membrane transport proteins independently of CFTR. Indeed, AICAR-induced activation of AMPK appears to induce increased transcription and insertion of glucose transporters into the plasma membranes of muscle (29, 35, 36). Of note, the recent discovery that certain mutations in the γ subunit of AMPK cause the Wolff-Parkinson-White syndrome (10, 37), a disease that predisposes to fatal cardiac arrhythmias, may imply a possible functional link between AMPK and cardiac ion channels. Further, investigations into the effects of AMPK on various membrane transport proteins may provide important new insights into the pathophysiology of several disorders, including ischemic epithelial injury and cardiac arrhythmias.

Finally, the results of this study illustrate the potential utility of agents (e.g. AICAR or metformin (11)) that enhance endogenous AMPK activity in the treatment of diseases where inappropriately high CFTR activity plays a role, including certain secretory diarrheas (3) and autosomal dominant polycystic kidney disease (38). Conversely, if agents that could selectively inhibit AMPK are discovered, they might be of benefit in diseases associated with inappropriately low CFTR activity (i.e. CF). A further understanding of the detailed mechanisms involved in the AMPK-CFTR regulatory interaction may also provide additional insights into the pathogenesis of CF.

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