cAMP-activated anion conductance is associated with expression of CFTR in neonatal mouse cardiac myocytes

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Lader, Alan S., Yihan Wang, G. Robert J. Jackson, J. R., Steven C. Borkan, and Horacio F. Cantiello. cAMP-activated anion conductance is associated with expression of CFTR in neonatal mouse cardiac myocytes. Am. J. Physiol. Cell Physiol. 278: C436–C450, 2000.—In this study, patch-clamp techniques were applied to cultured neonatal mouse cardiac myocytes (NMCM) to assess the contribution of cAMP stimulation to the anion permeability in this cell model. Addition of either isoproterenol or a cocktail to raise intracellular cAMP increased the whole-cell currents of NMCM. The cAMP-dependent conductance was largely anionic, as determined under asymmetrical (low intracellular) Cl- conditions and symmetrical Cl- in the presence of various counterions, including Na+, Mg++, Cs++, and N-methyl-D-glucamine. Furthermore, the cAMP-stimulated conductance was also permeable to ATP. The cAMP-activated currents were inhibited by diphenylamine-2-carboxylate, glibenclamide, and an anticystic fibrosis transmembrane conductance regulator (CFTR) monoclonal antibody. The anti-CFTR monoclonal antibody failed, however, to inhibit an osmotically activated anion conductance, indicating that CFTR is not linked to osmotically stimulated currents in this cell model. Immunodetection studies of both neonatal mouse heart tissue and cultured NMCM revealed that CFTR is expressed in these preparations. The implication of CFTR in the cAMP-stimulated Cl- and ATP-permeable conductance was further verified with NMCM of CFTR knockout mice [cfr(-/-)] in which cAMP stimulation was without effect on the whole-cell currents. In addition, stimulation with protein kinase A and ATP induced Cl- permeable single-channel activity in excised, inside-out patches from control, but not cfr(-/-) NMCM. The data in this report indicate that cAMP stimulation of NMCM activates an anion-permeable conductance with functional properties similar to those expected for CFTR, thus suggesting that CFTR may be responsible for the cAMP-activated conductance. CFTR may thus contribute to the permeation and/or regulation of Cl- and ATP-permeable pathways in the developing heart.

cystic fibrosis; heart; adenosine 5'-triphosphate channels; chloride channels; adenosine 5'-triphosphate release

Cystic fibrosis is a genetic disease caused by mutations of the gene that encodes for the cystic fibrosis transmembrane conductance regulator (CFTR) (29, 30). CFTR is a member of the superfamily of transport proteins known as the ATP-binding cassette (ABC) transporters or traffic ATPase. ABC transporters have the ability to transport various substrates (13), and CFTR, in particular, is associated with the cAMP-dependent movement of both Cl- and ATP (4, 28).

Recent studies indicate that the cAMP-activated Cl-conductance of cardiac cells may be associated with the expression of CFTR (16, 17, 22). CFTR is present in various mammalian heart preparations including rabbit (39) and guinea pig (22). However, studies have suggested that adult rat and mouse cardiac myocytes lack a cAMP-activated Cl-conductance, consistent with the absence of a functional CFTR (8, 18). Similar findings have been reported in adult human cardiac myocyte preparations (24). Recently, we and others have provided evidence suggesting the presence of a functional CFTR in the neonatal rat cardiac myocyte (40, 42, 43). This raises the possibility of a developmentally controlled aspect of CFTR expression in different preparations.

In this report, primary cultures of neonatal mouse cardiac myocytes (NMCM) from control mice, cfr gene knockout [cfr(-/-)] mice, and mice heterozygous for the disrupted cfr gene [cfr(+/-)] were used to assess the effect of cAMP stimulation in the activation of anion-selective whole cell conductances. By applying voltage-clamp techniques, we functionally characterized a cAMP-inducible, time-independent, and Cl- and ATP-permeable conductance, which was absent in NMCM obtained from cfr(-/-) mice. The cAMP-activated anion currents were inhibited by diphenylamine-2-carboxylate (DPC), glibenclamide, and anti-CFTR antibodies. The results in this report are consistent with the presence of a functional cardiac CFTR in NMCM, which may play a relevant role in the developing heart.

MATERIALS AND METHODS

Primary cultures of NMCM. Primary cultures of NMCM were obtained with procedural modifications to a commercial isolation kit originally developed for neonatal rat cardiac myocytes (Worthington Biochemicals, Freehold, NJ). Pregnant control mice (C57BL/6 +/-, P100) were a kind gift of Drs. Richard L. Sidman and Aizhong Li (New England Regional Primate Research Center, Dept. of Neurology, Harvard Medical School). Briefly, beating hearts were harvested
from <24-h-old neonatal mice and immediately placed in a Ca\(^{2+}\)- and Mg\(^{2+}\)-free Hanks' balanced salt solution (HBSS; Worthington). The hearts were minced and subjected to trypsin digestion (100 µg/ml in HBSS) for 16–18 h at 4°C. Trypsin digestion was stopped by addition of trypsin inhibitor (Worthington). Further collagenase digestion (type I collagenase, 150 U/ml; Worthington) was conducted at 37°C on a shaking bath for 45 min. Cell clumps were filtered through a 70-µm nylon filter, centrifuged, and washed with fresh Lebovitz L-15 medium. Cell pellets were resuspended in Ham's F-10 medium with L-glutamine (BioWhittaker, Walkersville, MD) also containing 5% bovine serum and 10% horse serum (BioWhittaker). Cells were seeded onto glass coverslips and allowed to grow at 37°C in an incubator gassed with 5% CO\(_2\). Cells attached and spread after 1 day in culture and were usually used for the patch-clamping experiments within 1 wk with no electrical differences in the ion conductances studied.

CFTR knockout mice. Adult mice heterozygous for the disrupted cftr gene were obtained from Jackson Laboratories (Cftr\(^{tm1Unc}\); Bar Harbor, ME). These mice were originally generated by targeted gene disruption, involving insertion of a neomycin gene into the murine cftr gene (36). Mice homozygous for the disrupted gene display many features described (15). PCR screening for the cftr gene.

PCR was conducted with primers (Ransom Hill Bioscience, Ramona, CA) specific for either the wild-type or mutant cftr genes. The primers were 5′-TGA ACC TTA GTC CTA TGT TGC C-3′ (common), 5′-TGA AAT TCG CCA ATG ACA AGA C-3′ (mutant), and 5′-CTT TAG TAC CCG GTA TCA TC-3′ (wild type), which produce either a 500- or 300 bp product for the mutant and wild-type alleles, respectively. Amplification of 100 ng DNA was performed over 30 cycles in a 60-µl reaction volume (ABI 7700, Norwalk, CT). The primer sequences and reaction conditions were those specified by Jackson Laboratories, except that 3 mM MgCl\(_2\) was used in the reaction buffer to amplify the mutant sequence instead of the recommended 1.5 mM. The PCR amplification products were separated by electrophoresis in a 1.2% agarose gel.

Immunoprecipitation of CFTR. To assess the presence of wild-type CFTR in cultured myocytes, pooled batches of NMCM were grown as indicated in 25-cm\(^2\) (50-ml) culture flasks. WT-1 cells transfected to overexpress CFTR and used as a positive control (4) were grown on 10-cm petri dishes. WT-1 cells transfected to overexpress CFTR and used as a positive control (4) were grown on 10-cm petri dishes. CTL and WT-1 cells were washed (2 times) with PBS and then scraped with a rubber policeman. Cell pellets were obtained by differential centrifugation. Harvested cells in culture were resuspended in immunoprecipitation (IP) buffer [1% Triton X-100, 0.5% Nonidet P-40, 150 mM NaCl, 10 mM Tris·HCl pH 7.5, 1 mM EGTA, 1 mM EDTA, 0.25 mM NaVO\(_4\), 10 µg/ml phenylmethylsulfonyl fluoride (PMSF), and 10 µg/ml aprotinin] and then sonicated on ice. Sonicated samples were centrifuged at 10,000 g for 10 min at 4°C. The supernatant was transferred and then incubated for 16–18 h with a monoclonal antibody (MAb; 2 µg antibody · mg protein\(^{-1}\)· ml IP buffer\(^{-1}\)) either directed against the COOH terminus (MAb 24–1; Genzyme) or directed against the R-domain of CFTR (MAb 13–1; Genzyme). Immobilized protein A agarose was added to the solution during the final 2 h of incubation. An agarose pellet was obtained by centrifugation in a table microcentrifuge and washed three times with ice-cold IP buffer (1 ml). Agarose samples containing 300 µg total protein were mixed with sample buffer and heated to 60°C for 5 min before loading. After being separated by 6% SDS-PAGE, samples were transferred to a polyvinylidene fluoride membrane. The membrane was blocked with 5% nonfat dry milk for 1 h at room temperature and then probed with the anti-CFTR antibody (MAb 24–1; Genzyme) for 18 h at 4°C, followed by a horseradish peroxidase-based enzyme-linked chemiluminescence system.

The presence of CFTR was also determined directly from myocardial tissue obtained from neonatal mice. Hearts were harvested from neonatal mice (<24 h old and immediately placed in Ca\(^{2+}\)- and Mg\(^{2+}\)-free PBS containing 20 µM PMSF. The hearts were minced, homogenized, and stored as described above. Tissue samples contained 1 mg total protein.

Whole cell currents. Currents and command voltages were obtained and driven as previously described (28). Actual currents and step potentials were obtained and driven with a Dagan 3900 (Dagan, Minneapolis, MN). Signals were obtained at 4 kHz, filtered at 1.5 kHz with an eight-pole Bessel filter (Frequency Devices, Haverhill, MA), and stored in a hard disk of a personal computer to be analyzed with pCLAMP 6.0.3 (Axon Instruments, Foster City, CA). The patch pipette contained (in mM) either 30 KCl, 120 potassium aspartate, 1.0 KH\(_2\)PO\(_4\), 1.0 MgCl\(_2\), 10 NaCl, 5.0 ATP, 5.0 EGTA, and 5.0 Hepes, pH adjusted to 7.4 with KOH, or 140 NaCl, 5.0 KCl, 5.0 ATP, 1.0 MgCl\(_2\), and 10 Hepes, pH adjusted to 7.4 with NaOH. The bathing solution contained (in mM) 140 NaCl, 5.0 KCl, 1.0 CaCl\(_2\), 1.0 MgCl\(_2\), and 10 Hepes, pH adjusted to 7.4 with NaOH. In some experiments, whole cell currents were measured in symmetrical Cl\(^{-}\) with Cs\(^{+}\), N-methyl-D-glucamine (NGM), or Mg\(^{2+}\) as the counterion where both the bath and pipette solutions contained (in mM) either 140 CsCl, 140 NGM-Cl, or 70 MgCl\(_2\), along with 10 Hepes (pH adjusted to 7.4 with NGM). CaCl\(_2\) (1 mM) was added to the bath solution to facilitate the formation of the seals between the pipette and cell membrane. Asymmetrical ATP/Cl\(^{-}\) currents were assessed by filling the patch pipette with a solution containing MgATP (100 mM, adjusted to pH 7.4 with NaMGO) and then backfilled with one of the solutions above, as previously described (1, 28). Whole cell current–voltage relationships were obtained by applying 20-mV voltage steps for 500 ms between +100 and −100 mV from a holding potential of 0 mV. The whole cell conductance was calculated from currents measured at 490 ms after applying the voltage steps. This voltage protocol effectively eliminates the contribution of time-dependent Na\(^{+}\) and Ca\(^{2+}\) currents.

NMCM beat spontaneously, in agreement with other neonatal mouse cardiac myocyte preparations (23). Only single, spontaneously beating cells were chosen for electrophysiological recordings. However, in the few cases (∼10%) where a cell in a cluster was selected instead, the whole cell currents obtained were consistent with those of single cells under the same experimental conditions, thus ruling out possible electrical coupling with neighboring cells. After the whole cell patch was obtained, the maximum repolarization potential (maximum diastolic potential) for the cardiac myocytes still beating was −61.3 ± 6.1 mV, whereas the overshoot of the action potential was 35.5 ± 5.1 mV (n = 5). The average beating rate was 54 ± 11 beats per min (n = 5). The resting potential measured using the zero-current-clamp technique was −76.3 ± 1.0 mV (n = 6). NMCM are largely round in shape, and the whole cell capacitance of wild-type and cftr−/− were similar [42.5 ± 6.9 pF, n = 11, vs. 31.8 ± 10.0 pF, n = 6, for...
wild-type and cfr(−/−) NMCM, respectively, P < 0.3; therefore, no correction for whole cell current was conducted.

Single-channel currents. The excised, inside-out patch-clamp configuration was carried out as previously described (10). Currents and command voltages were obtained and driven with a Dagan 3900 with the use of sampling and filtering frequencies as described for the whole cell experiments. Data were stored in a hard disk of a personal computer and analyzed with pCLAMP 6.0.3. Data were further filtered at 200 Hz for display purposes. These experiments were performed in the symmetrical Cl− solutions described previously. Data from the excised, inside-out patches with upward and downward deflections indicating the channel open states for positive and negative holding potentials, respectively, were obtained between +100 and −100 mV.

ATP release assay. ATP release from NMCM was assessed with the luciferin-luciferase assay using a monolight 2010 luminometer (Analytical Luminescence Laboratory, San Diego, CA) and methods previously described (1, 26). Briefly, extracellular and total cellular ATP were measured from confluent NMCM grown on glass coverslips. At the time of the experiment, the coverslips were placed in 12 × 75 mm plastic cuvettes containing 0.1 ml of the luciferin-luciferase assay mix and 0.5 ml of a Ca2+ -free solution containing (in mM) 140 NaCl, 5 KCl, 0.8 MgCl2, and 10 HEPES, pH 7.4. The purified luciferin-luciferase solution (2005/2003; Analytical Luminescence Laboratory) contained 3 µg/ml luciferase and 400 µM luciferin. The HEPES buffer also contained 10 mM MgCl2, a cofactor of the luciferin-luciferase reaction. The final pH of the mixture was 7.40. Photon release was continuously measured in a luminometer (Monolight 2010; Analytical Luminescence Laboratory). The HEPES buffer contained 3 µg/ml luciferase and 400 µM luciferin-luciferase solution (2005/2003; Analytical Luminescence Laboratory) (26). The ATP release assay was followed by the photon release of the luciferin-luciferase assay for ∼2 min. To determine the amount of ATP released from cells, known concentrations of ATP in solution were also measured to construct a calibration curve.

To activate the cAMP-dependent stimulatory pathway of NMCM, cells were incubated with cholera toxin (CT; 1 µg/ml, Sigma) for 12 h, which does not interfere with the luciferin-luciferase assay (26). Neither cell volume nor cell count changed significantly after CT treatment. Both control and CT-treated cells excluded trypan blue, an indication that cell permeability was not impaired by the experimental conditions imposed. This was further supported by the ability of CT-treated cells to spontaneously beat.

Drugs and chemicals. The cAMP-stimulatory cocktail contained 8-bromo adenosine 3′,5′-cyclic monophosphate (8-Br-cAMP; 500 µM; Sigma Chemical, St. Louis, MO), IBMX (200 µM; Sigma), and forskolin (10 µM, Sigma). The cAMP analog 8-Br-cAMP was used from a 25 mM stock in a 1:1 (vol/vol) ethanol/DMSO solution. Forskolin was used from a 100% ethanol, 50 mM stock solution. The phosphodiesterase inhibitor IBMX was used from a 20 mM solution in ethanol. Use of this cocktail, which results in a maximum concentration of 1.6% ethanol and 0.3% DMSO in the bathing solution, was without effect on any NMCM electrical parameters (data not shown). The CFTR channel blocker DPC (Fluka Chemical, Ronkonkoma, NY) was kept in a 100-fold stock solution (20 mM) in 50% water and ethanol. Gibenclamide (Research Biochemicals International, Natick, MA) was kept in a 10 mM stock solution in 100% DMSO. Isoproterenol (Sigma) was freshly prepared in water (0.1 mM) and used at a final concentration of 1 µM. The monoclonal antibody raised against the R-domain of CFTR (MAb 13–1; Genzyme) was directly diluted 1:100 times in the intracellular solution from a stock solution (292 µg/ml).

Statistical analysis. Average data values were expressed as means ± SE for each group tested. Statistical significance was obtained by Student’s t-test for paired data (35) unless otherwise specified in the results. Data were considered significantly different when P < 0.05.

The perm-selectivity ratio PATP/PCl was calculated with a derivation of the Goldman-Hodgkin-Katz equation (14) from the cAMP-stimulated Cl− and ATP currents obtained under asymmetrical conditions, such that

\[
P_{\text{ATP}/\text{Cl}} = \frac{|z_A^2| \times C_{\text{Cl}} \times (1 - \exp(\alpha z_A E_{\text{Cl}}))}{|z_P^2| \times A_i \times \exp(\alpha z_A E_{\text{Cl}}) \times (1 - \exp(\alpha z_c E_{\text{Cl}}))}
\]

where \( \alpha = -F/RT \), \( z_A \) is the valence of ATP, \( A_i \) is the concentration of ATP (100 mM), and \( E_{\text{Cl}} \) is the reversal potential under asymmetrical conditions.

RESULTS

Whole cell currents of NMCM in low intracellular Cl−. To assess the presence of a CAMP-dependent anion conductance in neonatal cardiac myocytes, whole cell Cl− currents were recorded with the voltage-clamp technique (10) under physiological conditions, namely, the presence of low intracellular (42 mM) and high extracellular (149 mM) Cl− concentrations. Addition of isoproterenol (1 µM) induced a 499% increase in the whole cell conductance for positive holding potentials (1.36 ± 0.23 vs. 8.14 ± 0.45 nS/cell, n = 4, P < 0.001; Fig 1A) and a 161% increase in whole cell conductance for negative holding potentials (1.09 ± 0.16 vs. 2.84 ± 0.63 nS/cell, n = 4, P < 0.025; Fig 1A). Although the isoproterenol induced whole cell currents in asymmetrical Cl−-rectified (785 ± 66 pA/cell, n = 3, vs. −251 ± 46 pA/cell, n = 4, P < 0.005, at ±100 mV, respectively; Fig 1A) in the direction expected for activation of an anion-permeable conductance, the reversal potential (\( E_{\text{Cl}} \)) following isoproterenol was only −23.0 ± 1.0 mV (n = 4), thus different from the predicted \( E_{\text{Cl}} \) of −33 mV if the currents were carried exclusively by Cl−, but consistent with previous reports (9, 11).

Activation of an anion conductance was further explored by direct stimulation of the cAMP pathway. Addition of a stimulatory cocktail containing 8-Br-cAMP, IBMX, and forskolin also induced whole cell currents that rectified in the direction of the Cl− chemical gradient. A 1,143% stimulation (0.70 ± 0.18 vs. 8.70 ± 1.79 nS/cell, n = 7, P < 0.001; Fig 1B) was observed at positive holding potentials, also consistent with inwardly directed Cl− movement. cAMP stimulation at negative potentials, in contrast, only amounted to 237% (0.99 ± 0.22 vs. 3.34 ± 0.88 nS/cell, n = 7, P < 0.05; Fig 1B). The \( E_{\text{Cl}} \) following cAMP stimulation was −18.0 ± 3.3 mV (n = 7), again somewhat lower than the expected −33 mV predicted by the Nernst potential for Cl−. The cAMP-induced currents were inhibited by 72% (2.85 ± 1.88 vs. 10.0 ± 2.83 nS/cell, n = 3, P < 0.05;
Fig. 1. Effect of isoproterenol and cAMP and diphenylamine-2-carboxylate (DPC) on whole cell currents of neonatal mouse cardiac myocytes (NMCM) in asymmetrical Cl\(^-\). A: whole cell currents were obtained in the presence of low-intracellular (42 mM) and high-extracellular (149 mM) Cl\(^-\) before (○) and after (△) addition of isoproterenol (1 µM). Data are means ± SE for 4 experiments. B: whole cell currents in the same conditions as A before (○), after addition of the cAMP-stimulatory cocktail (△), and after further addition of DPC (400 µM, ▽). Data are means ± SE for 7 experiments under control and stimulated conditions, respectively, and 3 experiments after addition of DPC. C: representative whole cell tracings under control conditions (top) and after isoproterenol (bottom). D: representative whole cell tracings under control conditions (top), after the addition of the cAMP-stimulatory cocktail (middle), and after the addition of DPC (bottom).
(0.33 ± 0.11 vs. 8.32 ± 2.31 nS/cell, n = 8, P < 0.005; Fig. 2A) in the linear whole cell conductance. Similar findings were obtained in symmetrical NMG-Cl (0.28 ± 0.17 vs. 7.97 ± 2.38 nS/cell, n = 4, P < 0.02; Fig. 2B), MgCl₂ (3.45 ± 1.48 nS/cell, n = 8, vs. 15.0 ± 3.60 nS/cell, n = 6, P < 0.02; Fig. 2C), and CsCl (1.33 ± 0.24 vs. 8.74 ± 1.60 nS/cell, n = 6, P < 0.005; Fig. 2D). The cAMP-stimulated whole cell currents in symmetrical NaCl were inhibited with DPC (400 µM, n = 3, P < 0.05, Fig 3), but not DIDS (400 µM, P < 0.8, n = 3, Fig. 3).

Also interesting was the finding that, in symmetrical MgCl₂, the basal and cAMP-induced whole cell conductances were substantially higher than those observed

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**Fig. 2.** Effect of cAMP on symmetrical Cl⁻ whole cell currents (I) of NMCM. A: whole cell currents were obtained in symmetrical NaCl (149 mM) under control conditions (○) and after addition of the cAMP-stimulatory cocktail (△). Data are means ± SE of 8 experiments. B: whole cell currents were obtained in symmetrical N-methyl-D-glucamine chloride (NMG-Cl; 140 mM) before and after addition of cAMP-stimulatory cocktail. Data are means ± SE of 4 experiments. C: whole cell currents were obtained in symmetrical MgCl₂ (70 mM) before and after addition of cAMP-stimulatory cocktail. Data are means ± SE of 6 experiments. D: whole cell currents were obtained in symmetrical CsCl (140 mM) before and after addition of cAMP-stimulatory cocktail. Data are means ± SE of 6 experiments. Vₜ, holding potential.
with the other counterions. However, the data are in agreement with a cAMP activation of a Cl
conductance in all cases. The cAMP-stimulated whole cell currents in symmetrical MgCl\textsubscript{2} were inhibited by both DPC (400 µM, n = 3, data not shown), and glibenclamide (600 µM, n = 3; Fig. 4), a CFTR blocker (32).

Anion selectivity of cAMP-stimulated whole cell currents in NMCM. To further investigate the nature of the cAMP-dependent currents in NMCM, whole cell currents were measured in the presence of high intracellular Cl\textsuperscript{−} (149 mM) with either bromide (Br\textsuperscript{−}), iodide (I\textsuperscript{−}), or gluconate (Glu) as the extracellular anion. The anion permeability sequence of the cAMP-stimulated currents was Br\textsuperscript{−} > Cl\textsuperscript{−} > I\textsuperscript{−} > Glu. The perm-selectivity ratio for Br\textsuperscript{−}/Cl\textsuperscript{−} was 1.15 ± 0.12 (n = 3), for I\textsuperscript{−}/Cl\textsuperscript{−} was 0.54 ± 0.13 (n = 4), and for Glu/Cl\textsuperscript{−} was 0.40 ± 0.27 (n = 2). These data are consistent with halide selectivity data previously reported for epithelial CFTR (2).

Effect of anti-CFTR antibody on the whole cell currents of NMCM. The functional properties of the cAMP-dependent Cl\textsuperscript{−} conductance, including lack of rectification, time independence, and inhibition by DPC and glibenclamide were most consistent with previous reports implicating a functional CFTR. Thus the effect of an anti-CFTR antibody (MAb 13–1, Genzyme), previously reported to block CFTR-mediated Cl\textsuperscript{−} currents (27), was also tested on cAMP-stimulated whole cell currents of NMCM. In the presence of asymmetrical Cl\textsuperscript{−} (as in Fig. 1) and also a 1:100 dilution of the anti-CFTR antibody in the pipette, the whole cell conductance did not vary for at least 15 min after cell breaking (0.77 ± 0.12 vs. 0.89 ± 0.23 nS/cell, n = 5, P < 0.7). The basal whole cell currents were also similar to those in the absence of the monoclonal antibody (0.86 ±

![Fig. 3](http://ajpcell.physiology.org/) Effect of Cl\textsuperscript{−} channel inhibitors on cAMP-stimulated Cl\textsuperscript{−} conductance of NMCM. Whole cell currents were obtained in symmetrical NaCl (149 mM) before (control) and after addition of cAMP-stimulatory cocktail. Whole cell conductance was calculated as slope of the linear current-voltage relationship between +100 and -100 mV. Addition of DIDS (400 µM, P < 0.8) was without effect on the cAMP-activated currents. However, addition of DPC (400 µM) produced a 75% inhibition (P < 0.05). Data are means ± SE of 5 control and cAMP experiments and 3 DIDS and DPC experiments. *P < 0.05, with respect to control conditions; **P < 0.05, with respect to cAMP-stimulated conditions.

![Fig. 4](http://ajpcell.physiology.org/) Effect of glibenclamide on symmetrical MgCl\textsubscript{2} currents of NMCM. Whole cell currents were obtained in symmetrical MgCl\textsubscript{2} (70 mM) before (control), after addition of cAMP-stimulatory cocktail, and after further addition of glibenclamide (600 µM). Data are means ± SE of 8 experiments under control conditions, 6 experiments after addition of the stimulatory cocktail, and 3 experiments after addition of glibenclamide. B: representative whole cell tracings for control conditions (top), after addition of cAMP-stimulatory cocktail (middle), and after glibenclamide (bottom).
0.02 nS/cell, n = 6, P < 0.5). Furthermore, addition of the cAMP-stimulatory cocktail under these conditions was without effect since the whole cell conductance remained unchanged (0.85 ± 0.09 nS/cell, n = 5, P < 0.8) for 20 min after adding the cocktail.

Similar results were obtained in symmetrical MgCl2 (140 mM Cl−), where dialysis of NMCM with the active antibody also prevented the cAMP-activated Cl− conductance (0.80 ± 0.13 vs. 2.83 ± 1.11 nS/cell, n = 5, P < 0.8) for 20 min after adding the cocktail. Addition of the cAMP-stimulatory cocktail to cells dialyzed with heat-inactivated antibody, however, resulted in a 151% increase in conductance (5.54 ± 0.94 nS/cell, n = 3, vs. 13.87 ± 2.51 nS/cell, n = 3, P < 0.025; Fig. 5B). Nevertheless, the NMCM dialyzed with heat-inactivated antibody displayed a significantly higher basal whole cell conductance compared with cells dialyzed with the intact antibody (5.54 ± 0.94 vs. 0.80 ± 0.13 nS/cell, P < 0.02 by unpaired t-test; Fig. 5B). The cAMP-stimulated conductance was inhibited by 79% with glibenclamide (600 µM, 7.31 ± 2.35 nS/cell, n = 3, P < 0.001; Fig. 5B).

Osmotically activated whole cell currents of NMCM. To determine whether cAMP activation was actually inducing an osmotically activated Cl− channel and not CFTR (24, 38), the effect of the MAb 13–1 antibody, which prevented the cAMP-stimulated Cl− currents, was tested on osmotically activated whole cell currents. In the presence of a 1:100 dilution of the antibody in the pipette under symmetrical NaCl conditions, the control conductance was 0.14 ± 0.03 nS/cell (n = 3; Fig. 6). Osmotically activated whole cell currents were induced by a 33% decrease in the osmolarity of the bathing solution. The osmotically activated conductance was highly rectifying (5.94 ± 0.79 and 1.76 ± 0.18 nS/cell, respectively, for positive and negative holding potentials, respectively, n = 3; Fig. 6) and significantly greater than the control conductance (P < 0.01; Fig. 6). The Er following osmotic activation was -6.8 ± 2.5 mV, which was statistically similar to the predicted Er of -10.5 mV (P < 0.4). Similar results were observed in the absence of the anti-CFTR antibody (Fig. 6), thus indicating that the antibody was unable to inhibit the osmotically activated Cl− conductance and suggesting that the

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**Fig. 5.** Effect of anti-CFTR antibody on whole cell currents of NMCM in symmetrical Cl−. A: whole cell currents were obtained in the presence of MgCl2 (70 mM) and 1:100 dilution of the anti-R-domain CFTR antibody in the pipette. Bathing solution also contained MgCl2 (70 mM). Data were obtained immediately after whole cell breaking (○) and at least 15 min after addition of the cAMP-stimulatory cocktail (▼). Data are means ± SE of 6 experiments under each condition. B: whole cell currents were obtained in the presence of MgCl2 (70 mM) and 1:100 dilution of heat-inactivated anti-R-domain CFTR antibody in the pipette. Bathing solution also contained MgCl2 (70 mM). Data were obtained immediately after whole cell breaking (○), after addition of cAMP-stimulatory cocktail (▼), and after addition of glibenclamide (600 µM, ▼). Data are means ± SE of 3 experiments under each condition.

**Fig. 6.** Effect of anti-CFTR antibody on osmotically activated whole cell currents of NMCM. Osmotically activated currents were obtained in the presence of symmetrical NaCl (140 mM) in NMCM with a 1:100 dilution of anti-R-domain CFTR antibody in the pipette before (○) and after (▼) addition of deionized water. Also shown are whole cell currents in absence of the antibody before (○) and after (▼) osmotic activation. Data are means ± SE of 2 experiments under control conditions and 3 experiments in the presence of the antibody.
osmotic currents are not associated with CFTR. This is further suggested by recent evidence indicating that osmotically activated currents may involve channel structures other than CFTR (6, 7).

Immunoprecipitation of CFTR in NMCM. The presence of CFTR in NMCM was examined using immunoprecipitation with the monoclonal antibody MAb 24–1 (Genzyme) raised against the COOH terminus of CFTR. Immunoprecipitation of WT-1 cells transfected to overexpress CFTR (used as a positive control) revealed immunoreactive band(s) in the range of 160–180 kDa (Fig. 7, lane 1), consistent with a previous report demonstrating mature (i.e., glycosylated) CFTR in this cell line (4). A band representing CFTR was observed at ~180 kDa in both wild-type [cfr(+/+)] cardiac myocytes (Fig. 7, lane 2) and in neonatal heart tissue (Fig. 7, lane 4). This band is within the range of sizes reported for mature CFTR in rat cardiac myocytes (40). In contrast, no immunoreactive band was detected at this molecular weight in cfr(−/−) cardiac myocytes (Fig. 7, lane 3). Similar results were obtained with the monoclonal antibody MAb 13–1 directed against the R-domain of CFTR (data not shown).

Whole cell currents of NMCM in asymmetrical ATP/Cl−. Expression of CFTR is associated with a cAMP-activated pathway that is permeable to both Cl− and ATP (4, 28). Thus, to assess the presence of ATP-permeable pathways in mouse cardiac myocytes, whole cell currents were obtained in the presence of intracellular MgATP (100 mM) and bathing Cl− (149 mM). Addition of the cAMP-stimulatory cocktail (149 mM). Addition of the cAMP-stimulatory cocktail induced a 1,058% (0.43 ± 0.05 vs. 5.41 ± 0.40 nS/cell, n = 5, P < 0.001; Fig. 8) stimulation of highly linear whole cell currents, indicative of an electrodiffusional pathway permeable to both Cl− and ATP. A 38-mV shift (54 ± 8 vs. 16 ± 5 mV, n = 5, P < 0.001) in the E0, was observed after cAMP stimulation, in agreement with activation of an anionic conductive pathway. The change

![Figure 7: Immunoprecipitation of CFTR in NMCM. CFTR-expressing WT-1 cells, used as positive controls (lane 1) displayed immunoreactive bands between 160 and 180 kDa, consistent with the presence of CFTR (4). Immunoreactive bands between 160 and 180 kDa were observed in cfr(+/+) NMCM (lane 2) and in isolated neonatal mouse heart tissue from cfr(+/+) mice (lane 4). However, no immunoreactive bands were observed at this molecular weight in cfr(−/−) NMCM (lane 3).](http://ajpcell.physiology.org/)

![Figure 8: Effect of cAMP on asymmetrical ATP vs. Cl− currents of NMCM. Whole cell currents were obtained in the presence of intracellular MgATP (100 mM) and bathing Cl− (149 mM) before (●), after addition of cAMP-stimulatory cocktail (◇), and after addition of cocktail + DPC (400 µM, ▼). Data are means ± SE of 5 experiments under each condition. B: voltage dependence of DPC effect in the presence of either intracellular Cl− or ATP at different holding potentials. Data were obtained by dividing the difference between the DPC-blocked currents and cAMP-stimulated currents by the cAMP-stimulated currents. Fraction (ordinate axis) therefore represents the fractional inhibition by DPC, using averaged data in Fig. 8 (intracellular ATP, filled circles) and data used in Fig. 3 (149 mM intracellular Cl−, n = 3, filled squares). Values at 0 mV were omitted from the graph. A lower fractional inhibition was observed in the presence of intracellular ATP at negative potentials, thus suggesting a competition between the intracellular nucleotide and DPC.)
in $E_r$ was also consistent with a perm-selectivity ratio for ATP/Cl$^-$ of 0.42 or 0.37 for $z_{ATP} = -2$ (MgATP) or $-4$ (free ATP), respectively. Thus the ATP/Cl$^-$ perm-selectivity ratio of the cAMP-activated anion-selective pathway of NMCM was in agreement with our previous report on cells expressing the epithelial isoform of CFTR (28) and with our previous report on CFTR reconstituted into lipid bilayers (4). Both cAMP-stimulated ATP and Cl$^-$ currents were simultaneously inhibited by DPC (65.7%; Fig. 8), thus further suggesting a permeation of Cl$^-$ and ATP through the same anionic pathway. Inhibition was slightly higher at positive potentials, however, suggesting a competition between Cl$^-$ and ATP (Fig. 8B) for the blocking effect of DPC. Interestingly, a similar effect was also observed in the presence of low Cl$^-$ (Fig. 1B), where the change in slope conductance was 76% and 57% for positive and negative potentials, respectively.

Whole cell currents in NMCM from CFTR knockout mice in symmetrical Cl$^-$. To further assess the functional role of CFTR in the cAMP-activated Cl$^-$ current of NMCM, whole cell currents were also obtained in NMCM from mice lacking the gene for CFTR (36). Whole cell currents from cfr$^{(-/-)}$ NMCM were obtained in symmetrical Cl$^-$ under conditions similar to those reported above. In the presence of the NaCl solution on both sides of the membrane, the basal whole cell conductance was $1.18 \pm 0.20$ nS/cell ($n = 6$). Addition of the cAMP-stimulatory cocktail was without effect on the whole cell conductance ($1.63 \pm 0.32$ nS/cell, $n = 6$, $P < 0.4$; Fig. 9A).

Similar results were obtained in symmetrical MgCl$_2$, since basal whole cell currents rendered a conductance of $0.15 \pm 0.07$ nS/cell ($n = 3$) that remained stable 15 min after the addition of the stimulatory cocktail ($0.24 \pm 0.09$ nS/cell, $n = 3$, $P < 0.5$, data not shown). Thus cAMP stimulation was without effect on the whole cell conductance of the cfr$^{(-/-)}$ NMCM.

The heterozygous (+/−) littermates responded with a 157% increase in the whole cell conductance ($1.45 \pm 0.37$ vs. $3.73 \pm 1.03$ nS/cell, $n = 6$, $P < 0.05$; Fig. 9B) following cAMP stimulation. These data suggest that a copy of the wild-type cfr gene is sufficient for eliciting a cAMP-dependent whole cell activation in NMCM.

Whole cell currents in NMCM from CFTR knockout mice in asymmetrical ATP/Cl$^-$. To further assess the potential role of cardiac CFTR in the regulation of an ATP-permeable pathway, cAMP-activated whole cell currents were also obtained under asymmetrical ATP/Cl$^-$ conditions in both cfr$^{(+/-)}$ and cfr$^{(-/-)}$ NMCM. The cAMP-stimulated whole cell currents from heterozygous cardiac myocytes were significantly larger than those from CFTR-knockout myocytes ($7.94 \pm 0.1$ nS/cell, $n = 2$, vs. $0.34 \pm 0.16$ nS/cell, $n = 4$, $P < 0.001$ by unpaired t-test, data not shown). The data are consistent with a role of CFTR expression in the cAMP-activated Cl$^-$ and ATP currents.

Single-channel currents from control NMCM in symmetrical Cl$^-$. The nature of the electrodiffusional pathway for Cl$^-$ was further investigated at the single-channel level. Excised patches from control NMCM were obtained in either symmetrical NaCl (140 mM, $n = 5$), or MgCl$_2$ (70 mM, $n = 3$) solutions. Channel activity was observed after addition of MgATP (5 mM) and protein kinase A (PKA; 50 ng/ml) in four of five experiments in NaCl and three of three experiments in MgCl$_2$, but no channel activity was observed with either MgATP or PKA alone. The single-channel conductance of the PKA-activated channels was $17.7 \pm 2.2$ pS/cell ($n = 7$).

Similar experiments were performed in symmetrical NMG-Cl where channel activity was observed in 9 of 11 experiments after the addition of both MgATP and PKA (Fig. 10A). The conductance of these channels was $16.1 \pm 3.1$ pS/cell ($n = 9$; Fig. 10B). Replacement of the 140 mM NMG-Cl bath solution with a 1:10 NMG-Cl solution also containing 300 mM sucrose to maintain

\[ I_p = g_{Cl} \cdot (E_C - V) \]

\[ g_{Cl} = \frac{z_{ATP} \cdot F \cdot R \cdot T \cdot z_{Cl} \cdot C_{Cl}}{R^2 \cdot T} \]

\[ I_{ATP} = g_{ATP} \cdot (E_{ATP} - V) \]

\[ g_{ATP} = \frac{z_{ATP} \cdot F \cdot R \cdot T \cdot z_{ATP} \cdot C_{ATP}}{R^2 \cdot T} \]
Fig. 10. Single-channel currents from NMCM in NMG-Cl. A: single-channel currents were obtained by excising membrane patches in symmetrical NMG-Cl (140 mM). Addition of MgATP (5 mM) and protein kinase A (PKA; 50 ng/ml) stimulated single-channel currents at both positive and negative holding potentials. Total point histograms (right) show a single channel at +60 mV (top) and 2 distinct channels at -60 mV more clearly observed in the expanded tracing (bottom). Tracings are representative of 9 experiments. B: current-voltage relationship obtained from single-channel experiments. C: single-channel currents were obtained in symmetrical NMG-Cl (140 mM, •). Bath was then switched to a bath containing a 1:10 dilution of NMG-Cl (140 mM in pipette and 14 mM in bath) to maintain osmolarity (○). Shown are current-voltage relationships under symmetrical and asymmetrical conditions. Also shown are current-voltage relationships fitted with the Goldman-Hodgkin-Katz equation for symmetrical (dashed line), and asymmetrical (solid line) conditions, respectively.
osmolarity modified the single-channel conductance for positive and negative holding potentials. Further, the \( E_r \) shifted by +38 mV, from an \( E_r \) of 0 mV observed in symmetrical NMG-Cl (Fig. 10C).

To further confirm the nature of the cAMP-stimulated Cl\(^-\) conductance, the effect of the anti-CFTR antibody used to block the cAMP-stimulated whole cell currents was also tested on PKA- and ATP-stimulated single-channel currents of cftr\((+/+)\) NMCM. Similar to the whole cell currents, the anti-CFTR antibody (2.92 \( \mu \)g/ml) inhibited PKA- and ATP-activated single-channel activity (n = 3; Fig 11).

Single-channel currents from cftr\((-/-)\) NMCM in symmetrical Cl\(^-\). The presence of Cl\(^-\) channel currents in cftr\((-/-)\) NMCM was also sought. In symmetrical MgCl\(_2\) (70 mM), addition of MgATP (5 mM) and PKA (50 ng/ml) to the cytosolic side of the patch failed to stimulate channel activity in five of five patches. However, MgATP and PKA stimulated single-channel activity in six of seven patches obtained from the cftr\((+/+)\) littermate NMCM (Fig. 12). These channels had a conductance of 11.4 ± 2.0 pS/cell (n = 6) that was statistically similar to the single-channel conductance observed in the control NMCM (P < 0.1).

ATP release in NMCM. The possibility that NMCM express ATP release mechanisms similar to those previously reported in CFTR-expressing cells (26) was also assessed. ATP released to the extracellular milieu was determined directly from the supernatant of CT-treated cells obtained from wild-type and cftr\((-/-)\) mice. Cells were stimulated with CT (1 \( \mu \)g/ml) for 12 h to increase intracellular cAMP without interfering with the ATP assay. The ATP released from wild-type NMCM was 86% higher following CT treatment compared with basal conditions (9.67 ± 1.38 mol^{-15}/cell, n = 15, vs. 18.0 ± 1.96 mol^{-15}/cell, n = 15, P < 0.005; Fig. 13).

Fig. 11. Effect of anti-CFTR antibody on single-channel currents of NMCM. Single-channel currents were obtained in the presence of MgCl\(_2\) (70 mM). Currents observed after addition of MgATP (5 mM) and PKA (50 ng/ml) (top) were inhibited after addition of anti-R-domain CFTR antibody (MAb; 2.92 \( \mu \)g/ml) (bottom). Data are representative of 3 experiments.

Fig. 12. Single-channel currents from heterozygous cftr\((+/-)\) NMCM. Single-channel currents were obtained by excising membrane patches from cftr\((+/-)\) NMCM in symmetrical MgCl\(_2\). Addition of MgATP (5 mM) and PKA (50 ng/ml) stimulated currents at both positive and negative holding potentials. Data are representative of 6 experiments.
Cl− channels have been reported in this model, the presence of CFTR has been, heretofore, largely unknown. The presence of a cAMP-dependent anion conductance in NMCM was assessed by addition of either isoproterenol or a cAMP-stimulatory cocktail. Both cAMP-stimulating maneuvers increased rectifying currents under asymmetrical Cl− conditions (low intracellular Cl−) consistent with a Cl− conductance, which was also DPC inhibitable. The shift in the ER after cAMP stimulation, however, was not unequivocally consistent with the sole activation of an anionic conductance. Nevertheless, the observed ER was in agreement with the report by Harvey et al. (11), where the ER was −23.4 mV, with 42 mM Cl− inside and 151 mM Cl− outside the cell (the predicted ER in their study was −33 mV).

Ehara and Ishihara (9) also reported on an epinephrine-dependent Cl− conductance in guinea pig ventricular myocytes, where discrepancies between the predicted and measured ER were observed. The conditions in both of these previous studies favored the possibility of contaminating cation conductances (9, 11) that could partially account for the change in ER in the present study. Nevertheless, in symmetrical Cl− with either Na+, NMG, Cs+, or Mg2+ salts, cAMP stimulation induced a highly linear and time-independent whole cell current, thus further indicating that the cAMP-stimulated whole cell current was largely accounted for by Cl− movement.

The cAMP-activated anion conductance in NMCM was permeable to both Cl− and ATP. Thus, although this electrodiffusional anion pathway may be associated with the expression of an as yet unidentified anion channel, these data are most consistent with the expression of a functional CFTR, which was confirmed by immunodetection. Expression of CFTR by Western blots of either neonatal hearts or cultured NMCM were found to contain mature CFTR. Furthermore, the cAMP-activated currents were also consistent with a functional CFTR phenotype, namely the activation of highly linear, time-independent currents in symmetrical Cl−, and a halide perm-selectivity of Br− > Cl− > I− > gluconate. In addition, the cAMP-induced Cl− currents were insensitive to DIDS but were inhibited by DPC and glibenclamide, both known blockers of CFTR (20, 32). However, the blocking effect(s) of DPC and glibenclamide showed little voltage dependence compared with previous reports on epithelial CFTR (20, 33).

Nevertheless, the possibility exists for a stronger competition between intracellular Cl− and DPC in this preparation, since voltage dependence was actually observed in low intracellular Cl− (Fig. 1B) and high intracellular ATP (Fig. 8B), but not symmetrical Cl− (Fig. 8B).

Further evidence suggesting that the cAMP-activated Cl− currents are associated with a functional expression of cardiac CFTR was provided by the blocking effect of intracellular dialysis with an anti-CFTR antibody recently shown to block human epithelial CFTR (27). Most consistent with the presence of functional CFTR (4, 28), however, was the finding that cAMP stimulation elicited DPC-inhibitable Cl− and
ATP currents under asymmetrical ATP/Cl\(^-\) conditions. The ATP/Cl\(^-\) perm-selectivity ratio of 0.42 and 0.37 for \(z_{ATP} = -2\) and \(-4\), respectively, was in agreement with values previously reported for CFTR-expressing cells (28) and purified CFTR (4). Furthermore, cAMP-activated Cl\(^-\) and ATP-permeable currents have also been observed in neonatal rat cardiac myocytes (43) known to express CFTR (40).

Although the findings above are consistent with CFTR function in the heart, cAMP stimulation has also been associated with the activation of an osmotically activated Cl\(^-\) conductance likely distinct from CFTR (24, 38). This was ruled out in the present study, however, as cAMP-stimulated Cl\(^-\) currents of NMCM were blocked by the anti-CFTR antibody, while the osmotically activated currents were not. These results suggest, therefore, that the osmotically stimulated and cAMP-stimulated Cl\(^-\) currents of NMCM are likely reflections of two distinct electrodiffusional pathways.

To further confirm the role of CFTR in the cAMP-activated anion conductance of NMCM, we took advantage of the recently developed cftr\((+/−)\) mouse (36) that lacks a functional gene for CFTR. As expected from the lack of CFTR, cAMP stimulation in symmetrical NaCl and MgCl\(_2\) solutions was without effect on the cftr\((+/−)\) NMCM. This was confirmed by the inability to stimulate single-channel activity with ATP and PKA in excised patches of NMCM. Likewise, cAMP stimulation did not elicit whole cell currents under asymmetrical ATP/Cl\(^-\) conditions in the cftr\((+/−)\) NMCM. These results are in agreement with the absence of immunodetectable CFTR in cftr\((+/−)\) NMCM (Fig. 7). However, the cAMP cocktail was able to activate a Cl\(^-\) and ATP-permeable conductance in heterozygous cftr\((+/+)\) NMCM, suggesting that one copy of the CFTR gene may be sufficient to elicit a response. This may account for the lack of a cystic fibrosis phenotype in the heterozygous littermates (36). In addition, the results of the single-channel experiments confirmed that a normal gene, and thus a functional CFTR, is necessary to observe the whole cell and single-channel currents reported in this manuscript.

The role of CFTR in the cAMP-stimulated anion conductance in NMCM was further verified with the single-channel data, which revealed that addition of PKA and ATP to excised patches stimulated Cl\(^-\)-permeable channels only in cftr\((+/+)\) and cftr\((+/−)\) NMCM but not in the cftr\((−/−)\) NMCM. These Cl\(^-\)-channels displayed single-channel conductances between 11 and 17 pS, similar to those previously reported for cardiac CFTR (9, 22). Ehara and Ishihara (9) reported an epinephrine-activated 13-pS Cl\(^-\) channel in ventricular myocytes. In a later study, Nagel et al. (22) reported a PKA- and ATP-dependent Cl\(^-\) channel from guinea pig ventricular myocytes with a single-channel conductance of 12 pS, which was identified as CFTR. The single-channel conductance for the channels reported here, therefore, are distinctly different from the single-channel conductance of 28 pS reported for the outwardly rectifying Cl\(^-\) channels (7) and 1.3 pS for the Ca\(^{2+}\)-activated Cl\(^-\) channel (5). Thus the present data are in agreement with the contention that CFTR is likely responsible for the cAMP-stimulated Cl\(^-\) conductance of NMCM.

Besides the data with the cftr\((−/−)\) mice and the immunodetection of CFTR in cftr\((+/+)\) NMCM, a strong functional indication of the presence of CFTR is the cAMP-activated electrodiffusional movement of ATP, thus far only elicited by CFTR (4, 28). Further evidence in this regard, therefore, was the cellular ATP release under CT-stimulated conditions, which is also consistent with expression of CFTR, as previously reported (26). The CT-stimulated steady-state ATP release was \(-18.0 \times 10^{-15}\) mol/cell, in agreement with the ATP release of CFTR-expressing mouse mammary carcinoma cells (26). The lack of a CT-stimulated release of cellular ATP from cftr\((−/−)\) NMCM also implicates CFTR in the CT-stimulated ATP release in the wild-type NMCM.

The ability of CFTR to conduct ATP has been a subject of previous debate (34). However, a recent study from our laboratory has finally demonstrated that purified CFTR is indeed able to conduct ATP (4). Furthermore, other studies have demonstrated that other organic anions are able to permeate CFTR (19). A paradigm is emerging that suggests that the functional role of CFTR is indeed related to its function as an ATP-permeable pathway (4, 28).

The physiological role of CFTR in the human heart still remains largely unclear. Despite recent reports indicating the presence of mRNA for CFTR in human cardiac myocytes (41), functional assays have failed to demonstrate a cAMP-stimulated Cl\(^-\) conductance (24). However, children with cystic fibrosis present symptoms of cardiac disease associated with acute heart failure (21). Furthermore, it has also been suggested that the ventricular diastolic reserve is diminished in cystic fibrosis patients (25). Thus the possibility exists for a dysfunctional CFTR to be implicated in the onset of cardiac disease in cystic fibrosis. Conversely, wild-type CFTR may itself play an important but as yet undefined functional role in the human heart.

Current dogma suggest that CFTR is a Cl\(^-\)-permeable channel. However, Cl\(^-\) channel activation has failed to modify the maximal repolarization potential or the shape of the action potential in preparations known to contain CFTR (11, 22). Thus it could be argued that CFTR activation in the heart may not be largely associated with a Cl\(^-\) conductance, providing a likely scenario for experimental conditions where functional CFTR has failed to be detected. CFTR-mediated release of cellular ATP, which can act as an autacoid by binding to purinergic receptors, may be part of a regulatory mechanism that is necessary for regulating myocyte function in the developing heart where autonomic regulation is not fully developed (31). Although the source of extracellular ATP has not been positively identified, cardiac extracellular ATP has been associated with afterdepolarizations linked to the perilucelular ATP concentration (37). Thus it is tempting to postulate that mechanisms such as CFTR function may be implicated.
In conclusion, the data in this report are most consistent with the presence of a functional CFTR in NMCM, where it is associated with the activation of an anion-selective electrodifusional pathway permeable to both Cl⁻ and ATP. The presence of CFTR in neonatal cardiac tissue, and the absence of a CAMP-activated conductance in the cfr(−/−) NMCM, further indicate that CFTR is responsible for CAMP-activated anion conductance in NMCM. Although other explanations are still feasible, the fact that CFTR has not been detected in cardiac tissue of adult mice (18) may suggest a developmental role for cardiac CFTR. This functional role of CFTR requires further investigation.

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