Up-regulation of Acid-gated Na\textsuperscript{+} Channels (ASICs) by Cystic Fibrosis Transmembrane Conductance Regulator Co-expression in Xenopus Oocytes*

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Cystic fibrosis transmembrane conductance regulator (CFTR) functions as both a chloride channel and an epithelial transport regulator, interacting with Na\textsuperscript{+} (epithelial sodium channel), Cl\textsuperscript{-}, renal outer medullary potassium channel, and H\textsubscript{2}O channels and some exchangers (i.e. Na\textsuperscript{+}/H\textsuperscript{+} and co-transporters (Na\textsuperscript{+}/HCO\textsubscript{3}\textsuperscript{-}, Na\textsuperscript{+}/K\textsuperscript{+}/2Cl\textsuperscript{-}). Acid-sensitive ion channels (ASICs), members of the epithelial sodium channel/degenerin superfamily, were originally cloned from neuronal tissue, and recently localized in epithelia. Because CFTR has been immunocytochemically and functionally identified in rat, murine, and human brain, the regulation of ASICs by CFTR was tested in oocytes. Our observations show that the proton-gated Na\textsuperscript{+} current formed by the heteromultimeric ASIC1a2a channel was up-regulated by wild type but not by ΔF508-CFTR. In contrast, the acid-gated Na\textsuperscript{+} current associated with either the homomultimeric ASIC1a or ASIC2a channel was not influenced by wild type CFTR. The apparent equilibrium dissociation constant for extracellular Na\textsuperscript{+} for ASIC1a2a was increased by CFTR, but CFTR had no effect on the gating behavior or acid sensitivity of ASIC1a2a. CFTR had no effect on the pH activation of ASIC1a2a. We conclude that wild type CFTR elevates the acid-gated Na\textsuperscript{+} current of ASIC1a2a in part by altering the kinetics of extracellular Na\textsuperscript{+} interaction.

Hyperactivated Na\textsuperscript{+} absorption through epithelial sodium channel (ENaC)\textsuperscript{1} is important in the pathophysiology of cystic fibrosis (CF (1–5)). The idea that the cystic fibrosis transmembrane conductance regulator (CFTR) may function as a regulator of ENaC has been tested in systems co-expressing CFTR and ENaC (6–8). Those studies demonstrated that wild type CFTR up-regulates the ENaC current (8–10). Also, defective Na\textsuperscript{+} absorption is restored by CFTR replacement therapy (11). Mutagenesis studies show that the cystic fibrosis transmembrane conductance regulator domain of CFTR and the cystolic C termini of β- and γ-ENaCs contribute to the functional and physical inter-molecular interactions between CFTR and ENaC (8, 10). Because the sulfonylurea receptors, a branch of the family of ATP binding cassette (ABC) transporter proteins, show a high degree of homology with CFTR, Konstas et al. (12) studied the interaction between sulfonylurea receptors and ENaC. They concluded that sulfonylurea receptors inhibit Na\textsuperscript{+} transport by reducing surface expression of ENaC, but there is no protein-protein interaction at the level of the plasma membrane. Although several mechanisms have been proposed to explain the interaction between CFTR and ENaC, they have not yet been systematically tested (13–16).

A new subfamily of the ENaC/DEG superfamily, the acid-sensing ionic channels (ASICs), has recently been identified (17). Like other family members, ASICs are thought to have a single, large extracellular loop, and two short transmembrane domains with both the C and N termini located intracellularly (17). ASICs are ligand-gated ion channels activated by extracellular acidification and perhaps mechanical stimulation. The biophysical and pharmacological characteristics of ASICs are similar to those of ENaC, albeit with a lower sensitivity to amiloride (K\textsubscript{i} = 10 μM versus 0.1 μM) and a different cation permeability pattern (17). However, the acid-gated current transient is transiently (seconds) activated and inactivated, whereas ENaC current decreases at extracellular pH 6.4 after a transient increase (18). Likewise, the native amiloride-sensitive Na\textsuperscript{+} conductance is activated by slightly acidic pH, (5.5 or 6.4) in toad bladder and A6 cells (18, 19). ASIC members, like ENaC, may assemble as a tetramer or as a higher order heteromultimeric channel (20). The biophysical features of heteromultimeric channels constructed with ASIC1a and ASIC2a (ASIC1a2a) or ASIC2b with ASIC3 (ASIC2b3) can be distinguished from those built from homomultimeric individual channels (21, 22). ASIC1a (23, 24), ASIC2a (21, 23, 25–28), and ASIC2b (21) show a wide-spread distribution in the brain. ASIC1a, ASIC1b (29), ASIC2b, and ASIC3 (30, 31) have been localized to dorsal root ganglion sensory neurons. However, CFTR expression has not been reported in the peripheral neuronal tissues. Human ASIC3 is distributed in both neuronal tissues and epithelial tissues (32), and a splice variant (hT-NaCl) was cloned from human testis (33). The most recently discovered homolog, ASIC4, has been found in the spinal cord, the pituitary gland, and the inner ear (34, 35). The distribution of ASIC members thus extends beyond the central nervous system.

CFTR has been immunocytochemically and electrophysi-
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experimentally identified in rat brain (36–40), human hypothalamus (41), and a murine neuronal cell line established from the hypothalamus (42). In these same groups of neurons where CFTR is found, ASIC1a and ASIC2a are also expressed (17). The function of neuronal CFTR remains unknown. It is proposed that neuronal CFTR may regulate membrane trafficking processes of cytoplasmic ion transporters and secretion of neuropeptides (42). Decreased CFTR expression in GT1-7 hypothalamic neurons produced by antisense against CFTR mRNA inhibits gonadotropin-releasing hormone secretion (42). Gonadotropin-releasing hormone has been postulated to be related to the infertility and delayed sexual maturation of CF patients in a developmentally sensitive pattern (42, 43). ASICs may act as pain sensors, mechanical receptors, and/or components of a signal transduction pathway responding to extracellular acidity.

To investigate the possible functional regulation by neuronal CFTR on ASIC1a/2a, the objective of the present study was to use Xenopus oocytes as an expression model to study the effect of human CFTR on the channel activity of ASIC1a/2a. Our results show that CFTR up-regulates proton-gated Na+ currents by altering the kinetics of extracellular Na+ interaction with ASIC1a/2a.

**EXPERIMENTAL PROCEDURES**

**cRNA Preparation**—Full-length human ASIC1a (BnaC2) and ASIC2a (BnaC1) cDNAs and their “degenerin” mutants were kind gifts of Drs. D. Corey and J. García-Añoveros (Harvard Medical School (23)). DNA samples were in vitro transcribed using either SP6 or T3 mMessage Machine kits (Ambion, TX) as appropriate. The quality and size of the cRNAs were confirmed by denaturing formaldehyde-agarose gel electrophoresis. RNA concentration was estimated by UV spectrophotometry at a wavelength of 260 nm.

**Immunofluorescence of Rat Hypothalamus**—Expression of ASIC2a and CFTR in the rat hypothalamus was stained by indirect immunofluorescence. Rats were anesthetized with halothane, perfused transcardially with 60 ml of ice-cold phosphate-buffered saline, and followed by 80 ml of ice-cold 4% paraformaldehyde. This protocol was reviewed and approved by the University of Alabama at Birmingham Institutional Animal Use and Care Committee. The brains were then removed from the skull, post-fixed in the same fixative for 2–4 h on ice, and cryoprotected by overnight immersion in 30% sucrose in phosphate-buffered saline. Tissue samples were embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA) medium, then 5-µm frozen sections were obtained. The sections were incubated with the primary antibody solution for 72 h at 4 °C with 5% normal goat serum and 0.1% Triton X-100. Rat brain tissue sections were labeled with guest on July 26, 2018

**Data Analysis**—All macroscopic currents presented in this paper are proton-activated Na+ currents. The maximal inward and outward current activated by low pH was measured and referred to as the transient peak current (I transient, NA). I transient usually occurred within 500–1000 ms after the change in pH. The relatively stable current was measured 8 s after the application of acidic pH, and is referred to as the sustained current (I sustained, NA). The inactivation time constant (τ inactivation) was calculated by fitting the time course of current decay subsequent to peak current according to the equation,

\[ f(t) = \sum_{n=1}^{\infty} A_n e^{-t/\tau_n} + C \]  

(Eq. 1)

where \( A \) stands for the current amplitude, \( \gamma \) is the inactivation time constant, \( C \) is the basal current recorded at pH 7.5 for each component \( i \), and \( t \) is the time period of the recording. The inactivating current (from the peak current to the transient peak) was fit to a first order exponential. Determination of \( \gamma \) is depicted in Fig. 2B.

Computation of the EC50 of the external proton concentration (pH50/Na+) was performed by fitting both the acid-gated peak current and the sustained currents as a function of external pH with the Hill equation,

\[ I_{Na} = I_{max} \left( \frac{[H]^+}{EC50 + [H]^+} \right)^n \]  

(Eq. 2)

where \( I_{Na} \) is the proton-gated currents, \( I_{max} \) is the maximal acid-gated current, [H+] stands for the extracellular pH, \( EC50 \) represents the value of [H+] which results in half of the maximal current of the ASIC channel and \( n \) is the Hill coefficient. To further verify the calculation, the Hill coefficient and \( EC50 \) were also computed by fitting the normalized acid-gated Na+ currents with the equation,

\[ I_{max}J_{Na} = \left( \frac{1}{1 + \frac{EC50}{[H]^+}} \right) \]  

(Eq. 3)

where [H+] is the proton concentration in the bath solution.

To analyze the steady-state kinetics for proton-activated Na+ current as a function of bath solution [Na+], superaustes containing variable concentrations of Na+ (ranging from 0 to 180 mM) were sequentially superfused into the chamber. \( K_s \) and \( I_{max} \) were calculated by fitting the proton-gated Na+ currents against the concentration of extracellular Na+ with the Michaelis-Menten equation,

\[ I_{Na} = I_{max} \left( \frac{[Na]}{K_s + [Na]} \right) \]  

(Eq. 4)

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**RESULTS**

**CFTR Co-localizes with ASIC2a—**ASIC2a and CFTR were detected in rat anterior hypothalamus by indirect immunofluorescence (Fig. 1). Control sections labeled with nonimmune IgG and secondary antibodies did not reveal any staining (not shown). CFTR is expressed in long cellular processes, most likely dendrites (Fig. 1, top panel). ASIC2a is expressed in punctate regions, most likely cell bodies of neurons (Fig. 1B, middle panel). Double-labeling with anti-CFTR and anti-ASIC2a antibodies overlap in sub-population of neuronal cell bodies and processes.

where \( K_m \) is the concentration required for activating half of the maximal current, \( I_{\text{max}} \), and \( I_{\text{h}} \) are the proton-gated currents, and [Na] represents the extracellular concentration of sodium. A nonlinear curve fitting subroutine was applied to fit the data.

All results are presented as the mean ± S.E. Student’s t test was used to compare means. A probability level of 0.05 or less was considered significant.

**ASII2a antibodies revealed that CFTR is primarily expressed in a sub-population of neuronal cells expressing ASIC2a (Fig. 1, bottom panel). Some co-localization is also observed in neuronal cell bodies. Similar patterns of co-localization have also been observed in the hippocampus (not shown).**

**CFTR Activates Acid-gated Currents—**To avoid any current from endogenous hyperpolarization-activated ion channels, which are activated at membrane potentials more hyperpolarized than -140 mV (47), the exogenous acid-gated Na⁺ currents associated with ASICs were obtained while oocytes were clamped at -60 mV. Reduction of pH₈ (4.0) had no effect on \( I_{\text{p}} \). The voltage for \( I_{\text{s}} \) was also unchanged (Fig. 3).

**The current-voltage (I-V) relationships of both \( I_{\mu} \) and \( I_{\text{s}} \) for the heteromultimeric ASIC1a/2a channel displayed a linear conductance (Fig. 3, A and B). CFTR co-expression increased the slope conductance of the I-V curves for \( I_{\mu} \) (Fig. 3A). The reversal potential for \( I_{\mu} \) was also
unchanged by CFTR (Fig. 3B). $I_p$ of ASIC1a/2a was $-6772 \pm 985$ nA ($n = 20$) at a holding potential of $-60$ mV (Fig. 3C), significantly greater than the current in uninjected oocytes ($-14 \pm 17$ nA, $p < 0.001$). ASIC1a/2a-associated $I_p$ was up-regulated in oocytes co-expressing CFTR ($-15,554 \pm 2,880$ nA, $p < 0.01$) compared with oocytes expressing ASIC1a/2a alone. $I_p$ in the presence of CFTR also markedly increased to $-2217 \pm 506$ ($n = 19$) from $-624 \pm 124$ nA ($n = 20$; $p < 0.01$, Fig. 3D). In contrast to the up-regulatory effect of CFTR on ASIC1a/2a, $\Delta F508$-CFTR co-expression did not show a significant alteration in $I_p$ or $I_s$ (Fig. 2C).

**CFTR Co-expression with either ASIC1α or ASIC2α**—Our previous studies demonstrated that $\beta$- and $\gamma$-ENaCs were involved in the cross-talk between CFTR and ENaC (8). In an attempt to identify which isoform of the heteromultimeric ASIC1α/2α channel was involved in the intermolecular interaction with CFTR, we co-expressed CFTR with either ASIC1α or ASIC2α in oocytes. As shown in Fig. 4, CFTR did not increase $I_p$ or $I_s$ produced by ASIC1α alone or by ASIC2α alone. However, the time to peak current was $1851 \pm 414$ ms ($n = 9$), and $\tau_s$ was $3613 \pm 17$ ($n = 9$) for ASIC1α, slower than those of ASIC2α or ASIC1α/2a.

To examine the effect of CFTR activation on ASICs, we first tested the effect of a cAMP-elevating mixture on ASIC1α/2α. After application of this cAMP-elevating mixture, the normalized $I_p$ decreased to $92 \pm 5.1\%$ ($n = 5$, $p > 0.05$) of its value before mixture application. In oocytes co-expressing ASIC1α/2α with CFTR, ASIC1α/2α $I_p$ was increased by $220 \pm 31\%$. Application of the cAMP-elevating mixture produced a saturation of the CFTR current and did not significantly influence the protonated ASIC1α/2α current ($-1665 \pm 974$ nA without mixture versus $-1502 \pm 842$ nA with mixture, $n = 6$, $p > 0.05$).

**CFTR Co-expression on pH Sensitivity of ASIC1α/2α**—To determine whether CFTR co-expression modified the protonation of ASIC1α/2α channels, solutions of different external pH were applied to calculate the pH required for half-activation of the maximal conductance ($EC_{50}$). Current transients for ASIC1α/2α, in the presence and absence of CFTR, were measured at different solution pH values (Fig. 5A). CFTR had no effect on the $EC_{50}$ for acid activation of ASIC1α/2α (Fig. 5B). The Hill coefficient was $20.2 \pm 3.7$ and $22.2 \pm 3.7$ for ASIC1α/2α alone and ASIC1α/2α + CFTR, respectively, indicating that protonation of multiple sites is required for activating ASIC1α/2α. To verify the above findings, we applied the modified Hill function to the same data (Fig. 5C). The computed apparent bath pH values at the midpoint of the maximal Na$^+$ current were $5.87$, $5.95$, and $5.99$ in oocytes expressing ASIC1α/2α alone, ASIC1α/2α with CFTR, and ASIC1α/2α with

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**Fig. 3.** Up-regulation of acid-gated conductance associated with the heteromultimeric ASIC1α/2α channel by CFTR co-expression. A, I-V relationship of peak current. The proton-gated transient Na$^+$ currents ($I_p\text{\textsc{max}}$) for the heteromultimeric ASIC1α/2α channel alone (circles) and co-expressed with CFTR (squares) were evoked at membrane potentials ($V_m$) ranging from $-100$ mV to $+100$ mV. The macroscopic conductances are linear for both groups with reversal potentials of about $+30$ mV. B, corresponding I-V curve of sustained current ($I_{s\text{\textsc{max}}}$). Reversal potentials were $-7$ mV for ASIC1α/2α and $10$ mV for CFTR and ASIC1α/2α as indicated by the arrows. C, average macroscopic currents of the transient Na$^+$ currents ($I_p\text{\textsc{max}}$) in water-injected oocytes (H$2$O) with (ASIC1α/2α squares) and without CFTR co-expression (ASIC1α/2α). D, corresponding whole-cell sustained Na$^+$ currents ($I_{s\text{\textsc{max}}}$) evoked by pH 4.0 at a holding potential of $-60$ mV for H$2$O, ASIC1α/2α, and ASIC1α/2α + CFTR. $n$ stands for the number of cells.
The same computation was also applied to the sustained \( \text{Na}^+ \) currents, and nearly identical EC\(50\) values as those for the peak currents were computed (data not shown). Thus, there was no difference in ASIC1a/2a sensitivity to external pH in the presence or absence of CFTR (\( p > 0.05 \)).

**CFTR Co-expression on Gating Behavior of ASIC1a/2a**—A further question concerns the influence of CFTR on the kinetics of the ASIC1a/2a channel. To address this issue, the time to peak current and the inactivation time constants of ASIC1a/2a as functions of holding potential and bath solution pH were examined. Fig. 6A demonstrates that there is no difference in the time to peak current as a function of holding potential from -100 mV to +100 mV in the presence or absence of CFTR. Although the average time to peak current of ASIC1a/2a was decreased from 408 ± 34 to 364 ± 17 ms by CFTR (between -60 mV and +100 mV), this difference was not significant (\( p > 0.05 \)). The summarized results for the inactivation time constants are depicted in Fig. 6B. No significant change at any applied voltage was observed.

The absence of acid-gated \( \text{Na}^+ \) currents in oocytes at pH\( _e \) 7.5 made it impossible to measure both the time to \( I_p \) and inactivation time constant. Therefore, the time to peak currents from the most acidic pH\( _e \) of 4.0 to the least acidic pH\( _e \) of 6.1 were plotted in Fig. 6C (\( n = 6 \)). At pH\( _e \) 4.0, the time to peak current was 319 ± 22 and 308 ± 24 ms for ASIC1a/2a and ASIC1a/2a co-expressed with CFTR, respectively. The time to peak current was not changed significantly by CFTR co-expression at any pH\( _e \) (\( p > 0.05 \)). The inactivation time constant of the ASIC1a/2a channel exhibited a minimal pH\( _e \) dependence and was unchanged in the presence of CFTR (Fig. 6D).

It appears that in oocytes co-expressing ASIC1a/2a and CFTR, \( \tau_i \) was pH\( _e \)-dependent and that this was not the case for the heteromultimeric ASIC1a/2a channels expressed in the absence of CFTR (whereas the inactivation time constant was uninfluenced by CFTR co-expression). The inactivation time constants were 938 ± 161 and 1222 ± 86 ms for ASIC1a/2a and co-expressed with CFTR, respectively, at pH\( _e \) 4.0 (\( p > 0.05 \)).

**Kinetics of Extracellular Sodium Interaction with Heteromultimeric Channel**—The homomultimeric ASIC1a and ASIC2a channels and the heteromultimeric ASIC1a/2a channel are permeant to Na\(^+\), Li\(^+\), and Ca\(^{2+}\) (17, 22, 51). The heteromultimeric ASIC1a/2a channel has distinctly different biophysical and pharmacological features from homomultimeric ASIC1a and ASIC2a channels (22). Thus, we tested the hypothesis that the kinetics of extracellular sodium interaction with the ASIC1a/2a channel could be affected by CFTR co-expression. This was examined by sampling acid-gated Na\(^+\) currents from oocytes expressing ASIC1a/2a plus and minus CFTR at various extracellular Na\(^+\) concentrations. As shown in Fig. 7, the peak of the acid-activated transient Na\(^+\) current (\( I_p \)) levels are dependent on the external Na\(^+\) concentration. The estimated \( K_m \) obtained by fitting the peak current (Fig. 7A) with the Michaelis-Menten function was 105 ± 13 and 166 ± 19 mM for ASIC1a/2a and ASIC1a/2a +
CFTR, respectively. These values are statistically different (p < 0.05). Similarly, the $K_m$ values calculated from the sustained Na$^+$ currents were 102 ± 13 and 164 ± 8 mV for ASIC1a/2a and ASIC1a/2a + CFTR, respectively, again significantly different from one another (Fig. 7B, p < 0.05). The time to peak and $\tau$ were not affected by CFTR at various external [Na$^+$] (Fig. 7C).

**Intermolecular Cross-talk Requires Normal Structure**—To address the issue of a possible intracellular sodium dependence of the regulation of ASICs by CFTR, we co-expressed CFTR with a gain-of-function mutant of ASIC2a, namely, G430F. As shown in Fig. 8, expression of G430F-ASIC2a alone exhibited a constitutively activated inward Na$^+$ current at pH 7.5. Application of acidic pH (4.0) unmasked a novel additional acid-sensitive Na$^+$ conductance in 6 of 7 oocytes expressing G430F-ASIC2a. The acid-sensitive Na$^+$ current associated with G430F-ASIC2a took up to 8 s to reach its maximal activity and displayed no tendency to inactivate over the observation period (10 s). The influence of CFTR on both the constitutively activated basal Na$^+$ current and the acid-sensitive component ($\Delta I_{\text{em}}$) are summarized in Fig. 8, C and D. In oocytes expressing G430F-ASIC2a alone, the basal current (at a holding potential of −60 mV and pH$_e$ of 7.5) was $-7490 \pm 1018$ nA (n = 14). CFTR co-expression had no significant effect on this current (mean, $-9643 \pm 1050$ nA, n = 11; p > 0.05). Likewise, CFTR had no effect on the acid-sensitive component of G430F-ASIC2a (−3825 ± 648 nA versus −3541 ± 2158 nA, +CFTR; n = 6). Time to peak of the acid-gated current (for those without peak current, it was calculated as time to stable) increased slightly to 6059 ± 1078 ms from 5278 ± 1169 ms (p > 0.05).

**Depolarization of Membrane Potential Induced by Acidic pH$_e$**—If CFTR co-expression increased the rate of positive charge flowing through the heteromultimeric ASIC1a/2a channel, the membrane potential should also be regulated by acidic pH$_e$. This idea was tested in the current clamp mode using the same protocol for acidic pH$_e$ application. The average resting membrane potential of un.injected oocytes at pH$_e$ 7.5 was $-43 \pm 5$ mV (n = 10, 3 frogs) and $-35 \pm 2$ mV (n = 57, 11 frogs) for ASIC1a/2a-injected oocytes and $-42 \pm 2$ mV (n = 38, 6 frogs, Fig. 9) for ASIC1a/2a + CFTR-injected oocytes. As shown in Fig. 9C, the net change in the maximal depolarized membrane potential ($\Delta E_m$) at pH$_e$ 4.0 in oocytes co-expressing ASIC1a/2a with CFTR was 39 ± 3 mV (n = 6), significantly greater (p < 0.05) than in oocytes expressing only ASIC1a/2a (24 ± 1 mV, n = 6). The decay rate (2 s) of the membrane potential, calculated by fitting with a first order exponential, paralleled the $\tau$ of the ASIC1a/2a-associated, acid-gated Na$^+$ currents (1.5 s). These observations indicate that there was a
coupling between the proton-gated Na\(^+\)/H\(^+\) current and the proton-induced depolarization of the membrane potential, suggesting that the depolarization of membrane potential resulted from the entry of positive charge carried by Na\(^+\) through ASICs.

**DISCUSSION**

The purpose of the present study was to examine the hypothesis that CFTR may interact with other members of the ENaC/DEG superfamily, the ASICs. We co-expressed CFTR with ASIC1a and/or ASIC2a in *Xenopus* oocytes, and the proton-gated Na\(^+\)/H\(^+\) currents were evaluated with the conventional two-electrode voltage clamp technique. Our results demonstrated that steady-state CFTR up-regulates ASIC1a/2a, which was opposite to the effect of CFTR on rat ENaC (8). Taken together, our studies strongly suggest that the cross-talk between CFTR and these ENaC/DEG family members is very specific.

Our immunolocalization results revealed that both CFTR and ASIC2a channel proteins are co-expressed in hypothalamic neurons. These observations are completely in agreement with previous studies demonstrating that mRNA for ASIC2a and CFTR are present in hypothalamus (17, 37, 38). Although we did not immunolocalize CFTR and ASIC1a in the hypothalamus due to the lack of antibodies against ASIC1a, ASIC1a and ASIC2a are co-expressed in hypothalamus (for review, see Ref. 17).

CFTR co-expression does not influence the ionic selectivity of the ASIC1a/2a channel (Fig. 3). The reversal potentials (+30 mV) measured in the presence or absence of CFTR co-expression were similar. The measured reversal potentials are in agreement with the prediction of the Goldman-Hodgkin-Katz equation for a highly Na\(^+\)-permeable cation channel (\(P_{Na} / P_K = 8.8\)).

Based on the assumption of a physical protein-protein interactions between CFTR and ASIC1a/2a, we tried to identify the ASIC subunit involved in the functional cross-talk of CFTR and ASIC1a/2a. Our results demonstrated that neither the homomultimeric ASIC1a channels nor the homomultimeric ASIC2a channels functionally interact with CFTR.

The opening of acid-gated channels has been proposed to be a consequence of protonation of the channel protein. In the case of ASIC channels, possible multiple protonation domains would be histidine residues located in the extracellular loops. CFTR may alter this process within the ASIC1a/2a channel by pH \(e\).

However, our study does not support this idea. No changes were observed in the pH \(e\) activation of ASIC1a/2a with CFTR co-expression (Fig. 6, B and C). Furthermore, CFTR co-expression did not regulate the kinetics of the heteromultimeric ASIC1a/2a channel. Apart from the activation rate, which was regulated in a pH \(e\)-dependent manner, the inactivation time constant and the time to peak current were not voltage-depend-
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Although CFTR co-expression did not alter the protonation of ASICs, analysis of the kinetics of external Na$^+$ interaction with the heteromultimeric ASIC1a/2a channel revealed a decrease in Na$^+$ concentration required for activating half of the maximal current (Fig. 7). The currents did not saturate at Na$^+$ concentrations between 0 and 100 mM. We did not measure currents at higher Na$^+$ concentrations because the increase in solution osmolarity will change cell volume, which is likely to activate ASICs, which are sensitive to mechanical stimuli (17). The reason why CFTR increased Na$^+$ interaction with the ASIC1a/2a channel is unknown. CFTR may increase open probability and ASIC expression.

ENaC expression in oocytes leads to an increased cytoplasmic sodium concentration accompanied by a depolarized membrane potential due to constitutive channel activity. We hypothesized that the crucial determinant to the contrasting effects of CFTR on ENaCs and on ASICs could be the resultant changes in membrane potential and cytosolic salt concentration. However, the noninteraction of G430F-ASIC2a, and CFTR ruled out this possibility (Fig. 8). These results also suggested, because the active Na$^+$ transport pathway (Na$^+$/K$^+$-ATPase) could not keep pace with passive Na$^+$ leak (Na$^+$ channel, Na$^+$/H$^+$ antiporter, etc.), leading to accumulation of cytosolic Na$^+$ and depolarization of membrane potential possibly accompanied by acidosis, CFTR would not up-regulate ASICs to protect neuronal function. Little is known about the mechanism of the gating behavior of the modified channel.

Recently, Nagel et al. (52) suggested that CFTR inhibition of ENaC was nonspecific. They argue that the apparent interaction results from electrical artifacts produced by a variable access resistance (because of overexpression of CFTR) and an uncompensated electrode resistance. Large changes in membrane resistance caused by CFTR and ASIC expression did not occur in our experiments because the membrane resistance of injected oocytes was close to that of uninjected oocytes in the absence of acidic pH ($\leq$ 5.5). Thus, the whole cell currents measured in our experiments were much less than the minimal 50 $\mu$A/oocyte Nagel et al. (52) calculated that would make voltage clamping problematic. Moreover, the series resistance of the reference electrode, the agar bridges, and bath solution totaled less than 1 $k\Omega$, again less than the 20 $k\Omega$ assumed by Nagel et al. (52). Thus, the total series resistance was much less (4%) than that of the membrane and could therefore not introduce a significant error in voltage measurements. Even assuming that overexpression of CFTR resulted in a change in the access resistance and, thus, produced a significant voltage drop in the bath, it cannot account for the opposite effects of CFTR on ENaCs and ASICs. Therefore, the interaction between CFTR and ENaC/DEG is specific and not due to an artificial error resulting from an uncompensated series and membrane resistance.

Divalent cations such as Ca$^{2+}$ and Mg$^{2+}$ can modulate both ENaC and ASIC activity (17, 29, 49, 53–55). Recently, de Weille et al. (52) showed that extracellular Ca$^{2+}$ regulated ASIC1a and ASIC2a differently. Furthermore, intracellular Ca$^{2+}$ only affected ASIC1a. CFTR significantly up-regulates ASICs at a pH more acidic than 5.5 (Fig. 5B) in contrast to the effects of Ca$^{2+}$ on ASICs, which is only evident at pH 7.0 (55). Therefore, the up-regulation of ASICs by CFTR is unlikely due to an increase in cytoplasmic Ca$^{2+}$ because the effects of cytoplasmic Ca$^{2+}$ and the regulation of CFTR on the homomultimeric ASIC1a and ASIC2a channels and the heteromultimeric ASIC1a/2a channel are not the same (49).

Even in the case of CFTR regulation of ENaC, diverse observations have been documented in epithelial tissues. The epi-

![Fig. 7. CFTR co-expression on kinetics of extracellular Na$^+$ interaction with ASIC1a/2a channel](image)

The holding potential is $-60\, mV$. Extracellular proton concentration is $10^{-4}\, M$. A, normalized acid-gated transient Na$^+$ currents evoked in the presence of a series of extracellular Na$^+$ concentrations ranging from 0 to 96 mM in oocytes expressing heteromultimeric ASIC1a/2a channel (squares) and co-expressed with CFTR (circles). The normalized $I_p$ was calculated as $I_p^{\text{norm}} = I_p(0) - I_p(\text{Na})$, where $I_p(0)$ is the peak current in the absence of sodium, and $I_p(\text{Na})$ is the peak current sampled in the presence of different extracellular sodium. The data points are connected with fitting curves drawn by the Michaelis-Menten equation (see “Experimental Procedures” for details). B, the normalized sustained Na$^+$ currents obtained with a range of extracellular Na$^+$. Calculation of normalized $I_p$ and representation of the symbols are identical to panel A. C, gating kinetics of ASIC1a/2a channel as a function of external sodium ([Na$^+$]). Both times to peak (circles) and inactivation time constants (squares) for ASIC1a/2a and ASIC1a/2a + CFTR (inverted triangles) for time to peak and up-triangles for $\tau_i$ are presented in panel C.
The principal Na\(^{+}\) channel located in the apical membrane is activated in airway, lung, intestinal, renal epithelia, and in heterologous expression systems (for review, see Refs. 13–27). In contrast, ENaC function depends critically on the state of CFTR in a purely salt-absorbing epithelium, and ENaC cannot be activated in cystic fibrosis sweat gland ducts where CFTR is defective or absent (56). Moreover, the effect of ENaC overexpression on CFTR in the *Xenopus* oocytes (8, 10) is different from that observed in mouse endometrial epithelium (57). One explanation for these diverse observations between CFTR and ENaC is that it is due to variant forms of ENaC and CFTR. Alternatively, different accessory components that operate in different cellular systems may underlie these differential effects (56). But the puzzle is still unresolved. Although ASICs are members of the ENaC/DEG family, there are differences in the biophysical and pharmacological characteristics between ENaC and ASIC, including their molecular sequence, cation selectivity, the unitary conductance, gating behavior, sensitivity to amiloride, etc. Furthermore, their responses to regulators or modulators (for example, protons, Ca\(^{2+}\), Mg\(^{2+}\), Na\(^{+}\), neuropeptides, monocarboxylic acids, inflammation, global ischemia, etc.) are different, even opposite (for review, see Refs. 17 and 58). The possibility that the diverse function and regulation between ASICs and ENaC may also contribute to their different interactions with CFTR has not been tested.

Our observations that wild type but not ΔF508-CFTR up-regulated the ASIC1a/2a channel augment the idea that CFTR, functioning as a regulator of a plethora of plasma membrane transporters, may also interact with neuronal membrane proteins. In the central nervous system (CNS), the function of ASICs is still hypothetical. The primary function of ASICs in the central nervous system may be to detect pH drops in brain tissue produced by release of neurotransmitters under physiological conditions participating in the regulation of synaptic activity and in pathophysiological conditions, such as cerebral ischemia, seizure, epilepsy, and trauma, where a reduction of tissue pH occurs. Up-regulation of ASICs by CFTR implies that in cystic fibrosis, the relative attenuation of ASICs in brain is less sensitive to pH changes in the absence of normal CFTR. In addition, CFTR itself regulates proton transmembrane transport and, therefore, modulates synaptic activity (13, 16). Because ASICs may also respond to other stimuli, including Phe-Met-Arg-Phe-amide and other neuromodulatory substances (32), up-regulation of ASICs by CFTR can contribute to the motor and sensory neuronal function, for example, modulation of nociceptive synaptic transmission (i.e. pain, a most common

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**Fig. 8. Co-expression of CFTR with the gain-of-function mutant of ASIC1a, G430F.**

- **A**, constitutively activated Na\(^{+}\) current at neutral pH (pH\(_{e}\) 7.5), proton-activated Na\(^{+}\) current (pH\(_{e}\) 4.0), and the net proton-sensitive Na\(^{+}\) conductance (Δ\(I_{Na}^{p}\)) in a single oocyte expressing G430F-ASIC1a. Δ\(I_{Na}^{p}\) is the difference of proton-induced Na\(^{+}\) current at pH\(_{e}\) 7.5 and that at pH\(_{e}\) 4.0. The scale for the abscissa is 2 s and for the ordinate is 2 μA. These currents were recorded by switching the holding potential from 0 to −60 mV while pH\(_{e}\) 4.0 was being superfused. **B**, whole-cell proton-gated Na\(^{+}\) currents in an oocyte co-expressing ASIC1a/2a with CFTR. The experimental protocol, Δ\(I_{Na}^{p}\) measurement, and symbols are same as in panel A. **C**, constitutively activated Na\(^{+}\) currents. Basal currents (\(I_{Na}^{basal}\)) with (G430F/CFTR) or without (G430F) at neutral pH\(_{e}\). **D**, proton-sensitive currents (\(I_{Na}^{p}\)). \(I_{Na}^{p}\) was calculated based on the values of Δ\(I_{Na}^{p}\).
symptom of cystic fibrosis) by altering central sensitization (59). ASICs may function as one component of oxygen sensors in combination with $K_{\text{Cl}}^{-}$ channels in $O_2$-sensing and acid-sensing neurons based on the hypothesis that the response to oxygen requires multiple sensors and they work together to shape the overall sensory response of brain neurons over a wide range of arterial oxygen tensions (50). Thus, the interactions of CFTR and ASICs indicate that brain neuropathophysiology in cystic fibrosis patients may be related to a depression of ASICs function in the presence and absence of extracellular acidosis.

Our observations demonstrate that activity of the heteromultimeric ASIC1a/2a channel is up-regulated in the presence of CFTR when co-expressed in $\text{Xenopus}$ oocytes at least in part by modifying the interaction with extracellular sodium. The physiological relevance in the context of native tissues remains to be explored.

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Up-regulation of Acid-gated Na⁺ Channels (ASICs) by Cystic Fibrosis Transmembrane Conductance Regulator Co-expression in *Xenopus* Oocytes

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