J. Membrane Biol. 214, 1–8 (2006)
DOI: 10.1007/s00232-006-0064-8

The Journal of
Membrane
Biology

Interplay between Cystic Fibrosis Transmembrane Regulator and Gap Junction Channels Made of Connexins 45, 40, 32 and 50 Expressed in Oocytes

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Received: 15 August 2006/Revised: 23 October 2006

Abstract. The cystic fibrosis transmembrane regulator (CFTR) is a Cl^- channel known to influence other channels, including connexin (Cx) channels. To study the functional interaction between CFTR and gap junction channels, we coexpressed in *Xenopus* oocytes CFTR and either Cx45, Cx40, Cx32 or Cx50 and monitored junctional conductance (G_j) and its sensitivity to transjunctional voltage (V_j) by the dual voltage-clamp method. Application of forskolin induced a Cl^- current; increased G_j approximately 750%, 560%, 64% and 8% in Cx45, Cx40, Cx32 and Cx50, respectively; and decreased sensitivity to V_j gating, monitored by a change in the ratio between G_j steady state and G_j peak (G_jSS/G_jPK) at the pulse. In oocyte pairs expressing just Cx45 in one oocyte (#1) and both Cx45 and CFTR in the other (#2), with negative pulses applied to oocyte #1 forskolin application still increased G_j and decreased the sensitivity to V_j gating, indicating that CFTR activation is effective even when it affects only one of the two hemichannels and that the G_j and V_j changes are not artifacts of decreased membrane resistance in the pulsed oocyte. COOH-terminus truncation reduced the forskolin effect on Cx40 (Cx40TR) but not on Cx32 (Cx32TR) channels. The data suggest a cross-talk between CFTR and a variety of gap junction channels. Cytoskeletal scaffolding proteins and/or other intermediate cytoplasmic proteins are likely to play a role in CFTR-Cx interaction.

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Key words: Chloride channel — Cystic fibrosis transmembrane regulator — Cell communication — Connexin — Gap junction — Channel gating — *Xenopus* oocyte

Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride (Cl^-) channel that mediates fluid diffusion across the luminal membrane of a number of epithelial cells (Sheppard & Welsh, 1999). CFTR gene mutations result in cystic fibrosis, an autosomal recessive disease caused by defective regulation of CFTR channels by cyclic adenosine monophosphate (cAMP). In normal subjects, CFTR phosphorylation by cAMP-dependent protein kinases activates CFTR-mediated Cl^- transport by increasing the CFTR affinity to adenosine triphosphate (ATP) (Gadsby & Nairn, 1999). In patients with cystic fibrosis, absence of CFTR activation impairs the normal absorptive and secreting function of epithelia, causing multiple organ failure (Greger et al., 2001). However, there is evidence that the mechanisms by which CFTR mutations result in cystic fibrosis may not depend entirely on altered Cl^- transport because CFTR is known to influence the function of other ion channels and transporters (*reviewed in* Kunzelmann, 1999; Schwiebert et al., 1999; Wang & Li, 2001).

Chanson, Scerri & Suter (1999) reported that in pancreatic duct cells CFTR activation by cAMP increases the Cl^- current as well as the electrical conductance of gap junctions made of connexin45 (Cx45), suggesting that CFTR influences the function of connexin channels. This is an important finding because gap junction channels are highly expressed in tissues most severely affected by cystic fibrosis. Therefore, a better understanding of the relationship between gap junctional communication and CFTR

function may provide novel insights into the pathogenesis of cystic fibrosis.

Recently, we have confirmed the data of Chanson et al. (1999) in *Xenopus* oocyte pairs coexpressing CFTR and Cx45 (Kotsias & Peracchia, 2005); in addition, we have reported a decrease in transjunctional voltage (V_j) sensitivity of Cx45 channels (Kotsias & Peracchia, 2005). For testing the potential interplay between CFTR and other connexin channels, in the present study we coexpressed in *Xenopus* oocytes CFTR and either Cx45, Cx40, Cx32 or Cx50. The *Xenopus* oocyte is an expression system easily accessible to genetic manipulation, which has been shown to express exogenous CFTR channels (Bear et al., 1991; Cunningham et al., 1992; Drumm et al., 1991). The data indicate that activation of CFTR channels by cAMP induces a typical Cl^- outward current, increases junctional conductance (G_j) and reduces the V_j sensitivity of these connexin channels without significantly altering the kinetics of junctional current decay.

Materials and Methods

Oocyte Preparation And Microinjection

Oocytes were prepared as previously described (Peracchia, Wang & Perrachia, 1996). Briefly, adult female *X. laevis* frogs were anesthetized with 0.3% tricaine (MS-222) and the oocytes surgically removed from the abdominal incision. The oocytes were placed in ND96 medium, containing (in mM) NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1 and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 5 (pH 7.6 with NaOH). Oocytes at stage V or VI were subsequently defolliculated in 2 mg/ml collagenase (Sigma, St. Louis, MO) for 80 min at room temperature in nominally Ca^{2+}-free OR2 solution, containing (in mM) NaCl 82.5, KCl 2, $MgCl_2$ 1 and HEPES 5 (pH 7.6 with NaOH). The defolliculated oocytes were injected with 46 nl (0.25 μg/μl) of antisense oligonucleotide complementary to endogenous *Xenopus* Cx38: 5′-GCTTTAG-TAATTCCCATCCTGCCATGTTTC-3′ (commencing at nt -5 of Cx38 cDNA sequence; Barrio et al., 1991) by means of a Drummond nanoject apparatus (Drummond, Broomall, PA). The antisense oligonucleotide blocks the endogenous junctional communication within 24 h. At 24–72 h, 46 nl of human CFTR cRNA (~0.2 μg/μl) were injected into oocytes at the vegetal pole, and 6–24 h later the oocytes were reinjected with 46 nl of cRNA (~0.4 μg/μl) of either mouse Cx45, rat Cx40, rat Cx32, mouse Cx50, rat Cx40TR or rat Cx32TR. cRNA of Cx40TR, a Cx40 mutant whose COOH terminus was truncated beyond residue 248, was prepared as previously described (Peracchia, 2004) by introducing a stop codon in the cDNA of Cx40 wild-type in the same location used by Stergiopoulos et al. (1999). Cx32TR, a rat Cx32 mutant whose COOH terminus was truncated beyond residue 225, was prepared by introducing a stop codon by polymerase chain reaction (PCR). Cx45TR, a mouse Cx45 mutant whose COOH terminus was truncated beyond residue 274 (Stergiopoulos et al., 1999), was prepared by introducing a stop codon by PCR. The oocytes were incubated overnight at 18°C. The oocytes were mechanically stripped of their vitelline layer in hypertonic medium (Peracchia et al., 1996) and paired at the vegetal poles in conical wells of culture dishes (Falcon Becton Dickinson Labware, Franklin Lakes, NJ) filled with ND96. All oocyte pairs were studied electrophysiologically 2–3 h after pairing. The human CFTR cDNA used in this study was provided by Dr. D. C. Devor (Department of Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, PA).

Measurement of Junctional Conductance

The oocyte chamber was continuously perfused at a flow rate of 0.6 ml/min by a peristaltic pump (Dyamax RP-1; Rainin Instrument, Woburn, MA). The superfusion solution was ejected by a 22-gauge needle placed near the edge of the conical well containing the oocyte pair. The level of the solution in the chamber was maintained constant by continuous suction. All experiments were performed using the standard double voltage-clamp procedure for measuring G_j (Spray, Harris & Bennett, 1981). Following the insertion of a current and a voltage microelectrode in each oocyte, both oocytes were individually clamped by two oocyte clamp amplifiers (OC-725C; Warner Instrument, Hamden, CT) to the same holding potential, $V_{m1} = V_{m2}$ (−40 mV), so that no junctional current would flow at rest ($I_j = 0$). For measuring G_j in the presence and absence of forskolin (20 μM), a V_j gradient was created by imposing a negative voltage step (V_1) of 12-s duration at 30-s intervals to oocyte 1, while maintaining V_2 at V_m; thus, $V_j = V_1$. The following V_j steps were used: −40 mV for Cx45 and Cx40, −100 mV for Cx32 and −60 mV for Cx50. The negative feedback current (I_2), injected by the clamp amplifier in oocyte 2 for maintaining V_2 constant at V_m, was used for calculating G_j as it is identical in magnitude to the junctional current (I_j) but of opposite sign ($I_j = -I_2$); $G_j = I_j/V_j$. Pulse generation and data acquisition were performed by means of pCLAMP software (version 8.2.0.232; Axon Instruments, Foster City, CA) and DigiData 1322A interface (Axon). I_j and V_j were measured with Clampfit (Axon) and the data plotted with SigmaPlot (SPSS Inc., Chicago, IL). The time constant (τ) of G_j decay and the ratio G_j steady-state over G_j peak (G_jSS/G_jPK), in the presence and absence of forskolin, were calculated by fitting each I_j curve to a single exponential function, following baseline correction (Clampfit, Axon). G_jSS was obtained from the exponential fit (parameter C of Clampfit).

Results

Effect of Forskolin On G_j And Sensitivity To V_j Gating In Oocyte Pairs Coexpressing CFTR And Either Cx45, Cx40, Cx32 or Cx50

In the present study, forskolin was applied for significantly shorter times (2 min) than in the previous study (10 min; Kotsias & Peracchia, 2005). Therefore, for properly comparing the effect of CFTR activation on the function of Cx40, Cx32 and Cx50 to that of Cx45 channels, experiments on CFTR-Cx45 with shorter forskolin application time (2 min) were carried out as well. Application of 20 μM forskolin to oocyte pairs coexpressing CFTR and Cx45 significantly increased G_j and decreased the sensitivity of V_j gating. This is obvious in low-speed chart records of currents elicited by application of −40 mV square voltage pulses (12-s duration) to one oocyte of the pair (Fig. 1A). The oocytes were individually voltage-clamped at V_m = −40 mV. Application of 20 μM

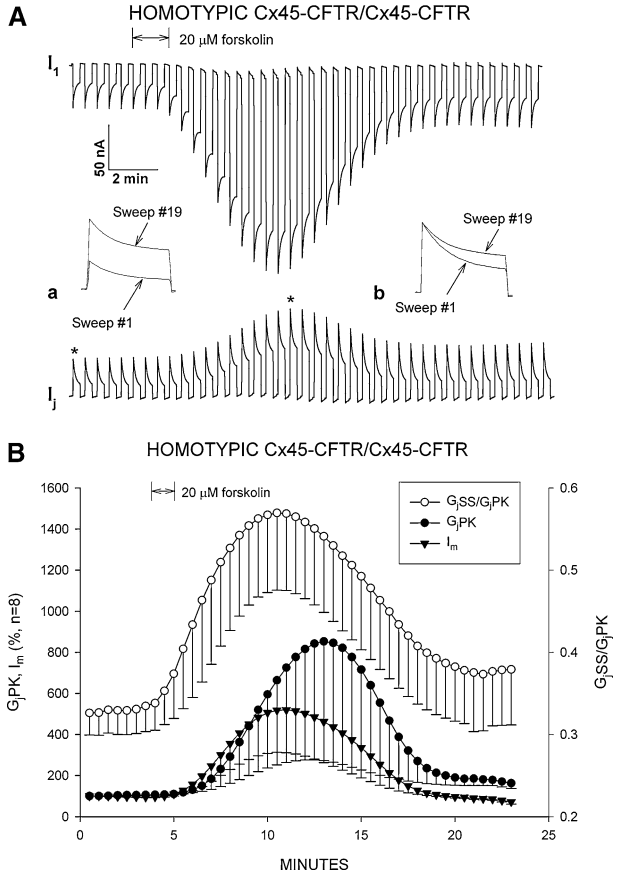

Fig. 1. Effect of forskolin on membrane current (I_m), peak junctional conductance (G_jPK) and V_j sensitivity, estimated by measuring the ratio G_jSS/G_jPK, in *Xenopus* oocyte pairs coexpressing Cx45 and CFTR. (*A*) Low-speed chart recording of junctional current (I_j) generated by applying –40 m*V* pulses (12-s duration) to oocyte 1 while maintaining oocyte 2 at control V_m. Application of 20 μM forskolin reversibly increases both I_1 and I_j ($I_j = -I_2$). The increase in I_1 partially reflects an increase in I_m ($I_m = I_1 - I_j$) caused by activation of CFTR channels in the pulsed oocyte (*upper trace*). The increase in I_j reflects an increase in G_j (*lower trace*). In addition, the ratio I_jSS/I_jPK at the pulse (*lower trace*) reversibly increases, reflecting a drop in V_j sensitivity. The *insets* show representative I_j traces sampled before and during forskolin application (sweeps 1 and 19, *asterisks*). *Inset a* shows the superimposed I_j curves at their actual amplitudes, whereas *inset b* shows the I_j curves normalized to peak amplitude, to demonstrate the increase in G_jSS/G_jPK. (*B*) Average values of G_jPK, I_m and the ratio G_jSS/G_jPK before, during and after application of 20 μM forskolin. Forskolin causes a significant and reversible increase in I_m and in both G_jPK and G_jSS. G_jSS increases by a greater fraction than G_jPK, resulting in a significant increase in G_jSS/G_jPK.

forskolin resulted in a reversible increase in I_1 and I_j. The increase in I_1 reflects an increase in both membrane current (I_m) and junctional current (I_j) as $I_1 = I_m + I_j$. The increase in I_m is due to cAMP-induced activation of CFTR in the pulsed oocyte (Fig. 1A, upper trace), whereas the increase in I_j reflects an increase in G_j (Fig. 1A, lower trace and insets). In addition, the ratio of I_jSS/I_jPK reversibly increased, reflecting a sizable drop in V_j sensitivity (Fig. 1A, lower trace). This is clearly seen in representative I_j traces normalized to peak amplitude (Fig. 1A, inset b) and sampled before (sweep 1) and during (sweep 19) forskolin application (marked by asterisks in Fig. 1A, lower trace).

The effects of forskolin on I_m, G_j and G_jSS/G_jPK are summarized in Figure 1B. In oocyte pairs coexpressing CFTR and Cx45, 2-min forskolin applications increased I_m by 420 ± 208% (mean ± standard error [SE], $n = 8$) and G_jPK by 753 ± 580% (mean ± SE, $n = 8$; Fig. 1B). G_jSS increased by a greater fraction than G_jPK (Fig. 1A, inset b), resulting in a 74.7% increase of G_jSS/G_jPK (Fig. 1B). Interestingly, the increase in G_jSS/G_jPK preceded the increase in both I_m and G_jPK (Fig. 1B). In addition, G_jSS/G_jPK did not fully recover (Fig. 1B).

Oocytes coexpressing Cx40 and CFTR behaved similarly to Cx45-CFTR oocytes. Two-minute applications of forskolin increased I_m by 405 ± 146% (mean ± SE, $n = 6$) and G_jPK by 561 ± 243% (mean ± SE, $n = 6$; $V_j = -40$ m*V*; Fig. 2A). G_jSS increased by a greater fraction than G_jPK, resulting in a 43.2% increase of G_jSS/G_jPK (Fig. 2A). Similar to Cx45, the increase in G_jSS/G_jPK preceded the increase in both I_m and G_jPK (Fig. 2A) and both G_jPK and G_jSS/G_jPK recovered less than in Cx45 (Fig. 2A).

In oocytes coexpressing Cx32 and CFTR, 2-min applications of forskolin increased I_m by 405 ± 146% (mean ± SE, $n = 12$) and G_jPK by 64 ± 23% (mean ± SE, $n = 12$; $V_j = -100$ m*V*; Fig. 2B). G_jSS/G_jPK increased by 43.2% (Fig. 2B). Similar to Cx45 and Cx40, the increase in G_jSS/G_jPK preceded the increase in both I_m and G_jPK (Fig. 2B) but to a lesser extent. G_jSS/G_jPK did not recover completely (Fig. 2B).

Oocytes coexpressing CFTR and Cx50 were much less sensitive to forskolin than Cx45, Cx40 and Cx32. Two-minute applications of forskolin increased I_m by 269 ± 139% (mean ± SE, $n = 11$) and G_jPK by only 8.3 ± 9.8% (mean ± SE, $n = 11$; $V_j = -60$ m*V*; Fig. 3A). G_jSS/G_jPK increased by 19.4% (Fig. 3A). With Cx50, the increase in G_jSS/G_jPK did not precede the increase in both I_m and G_jPK but rather was slightly delayed (Fig. 3A). In none of the connexins tested was the time constant (τ) of single exponential I_j decay at the pulse significantly affected by forskolin application (Fig. 3B).

Effect of Forskolin on G_j and Sensitivity to V_j Gating in Oocyte Pairs Coexpressing CFTR and COOH-Terminus Truncated Cx40 or Cx32

Truncation of the COOH terminus of Cx40 significantly reduced the effect of forskolin on Cx40 (Fig. 4A) but did not inhibit the forskolin effect on Cx32 (Fig. 4B). With Cx40TR, 4-min applications of forskolin increased I_m by 201 ± 82% (mean ± SE,

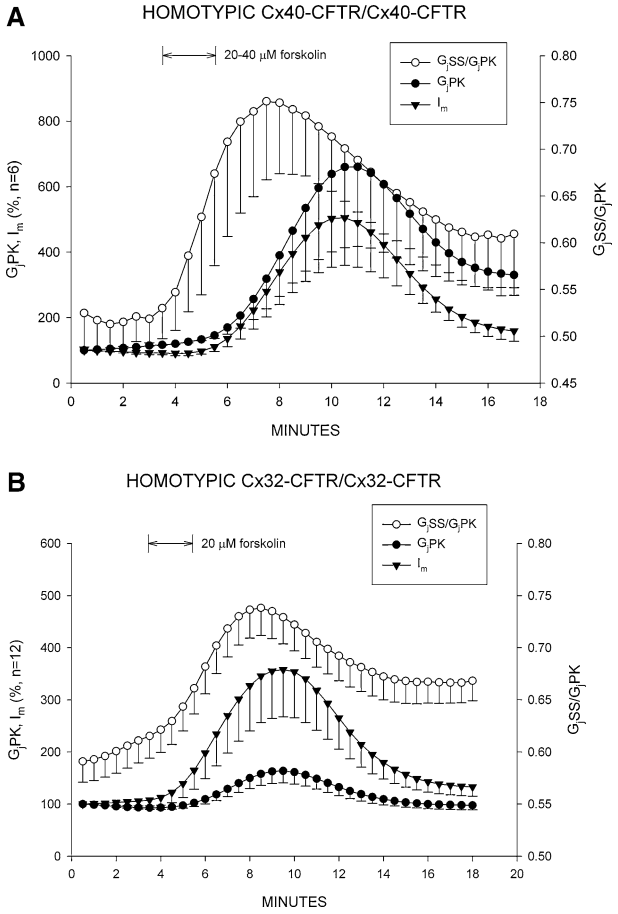

Fig. 2. Effect of forskolin on I_m, G_jPK and V_j sensitivity, estimated by measuring the ratio G_jSS/G_jPK, in *Xenopus* oocyte pairs coexpressing Cx40 and CFTR (*A*) or Cx32 and CFTR (*B*). Application of 20 μM forskolin causes a significant and reversible increase in membrane current (I_m) and in both peak (G_jPK) and steady-state (G_jSS) junctional conductances. As with Cx45, G_jSS increases by a greater fraction than G_jPK, resulting in a significant increase in G_jSS/G_jPK.

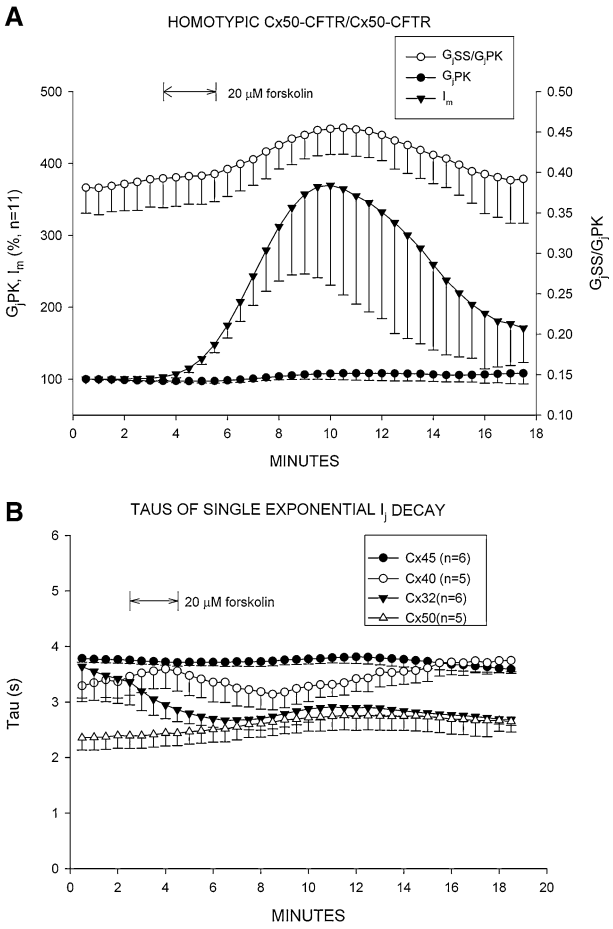

Fig. 3. (*A*) Effect of forskolin on I_m, G_jPK and V_j sensitivity, estimated by measuring the ratio G_jSS/G_jPK in *Xenopus* oocyte pairs coexpressing Cx50 and CFTR. Forskolin causes a reversible increase in I_m and in both G_jPK and G_jSS. As with Cx45, Cx40 and Cx32 channels, G_jSS increases by a greater fraction than G_jPK, resulting in a significant increase in G_jSS/G_jPK (n = 11). (*B*) Forskolin application (20 μM) does not significantly change the time constant (τ) of I_j inactivation at the pulse in oocytes coexpressing CFTR and Cx32, Cx40, Cx45 or Cx50.

n = 9) and G_jPK by only 35 ± 30% (mean ± SE, n = 9; Fig. 4A). G_jSS/G_jPK increased by only 13% (Fig. 4A). In contrast, with Cx32TR 4-min applications of forskolin increased I_m by 448 ± 217% (mean ± SE, n = 5) and G_jPK by 285 ± 149% (mean ± SE, n = 5; Fig. 4B). G_jSS/G_jPK increased by 58.4% (Fig. 4B). The effect of forskolin on Cx45TR could not be tested because this connexin did not express functional channel in sufficient amount even with injections of high concentrations of cRNA.

Effect of Forskolin on G_j and Sensitivity to V_j Gating of Heterotypic Cx45/Cx45-CFTR Oocyte Pairs

The effect of forskolin was also tested on oocyte pairs in which CFTR was only expressed in one of the two oocytes. In Cx45/Cx45-CFTR oocytes, −40-mV square voltage pulses (12-s duration) were applied to the oocyte expressing only Cx45. The application of 20 μM forskolin increased G_j and slightly decreased the sensitivity to V_j gating (Fig. 5A). The increase in I_j reflects an increase in G_j (Fig. 5A, lower trace and insets). In addition, the ratio of I_jSS/I_jPK reversibly increased, reflecting a small drop in V_j sensitivity (Fig. 5A, lower trace). This is seen in representative I_j traces normalized to peak amplitude (Fig. 1A, inset b) and sampled before (sweep 1) and during (sweep 17) forskolin application (marked by asterisks in Fig. 5A, lower trace). The effects of forskolin on I_m, G_j and G_jSS/G_jPK are summarized in Figure 5B. Forskolin reversibly decreased I_m in the pulsed oocyte to 82.8% ± 11% (mean ± SE, n = 14), a drop of ~17% from control values, and increased G_jPK by 43.5 ± 10.4% (mean ± SE, n = 14; Fig. 5B). G_jSS/G_jPK increased by 29% (Fig. 5B). The

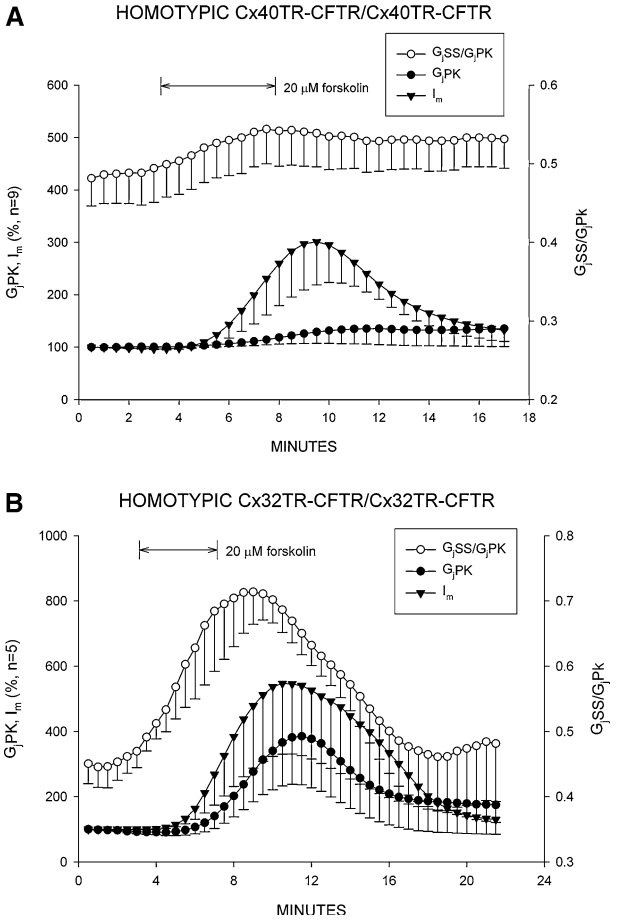

Fig. 4. Effect of forskolin on I_m, G_jPK and V_j sensitivity, estimated by measuring the ratio G_jSS/G_jPK, in *Xenopus* oocyte pairs coexpressing CFTR and either COOH-terminally truncated Cx40 (Cx40TR, *A*) or COOH-terminus truncated Cx32 (Cx32TR, *B*). Truncation of the COOH terminus of Cx40 significantly reduces the effect of forskolin on Cx40 channels (*A*) but has no effect on Cx32 channels.

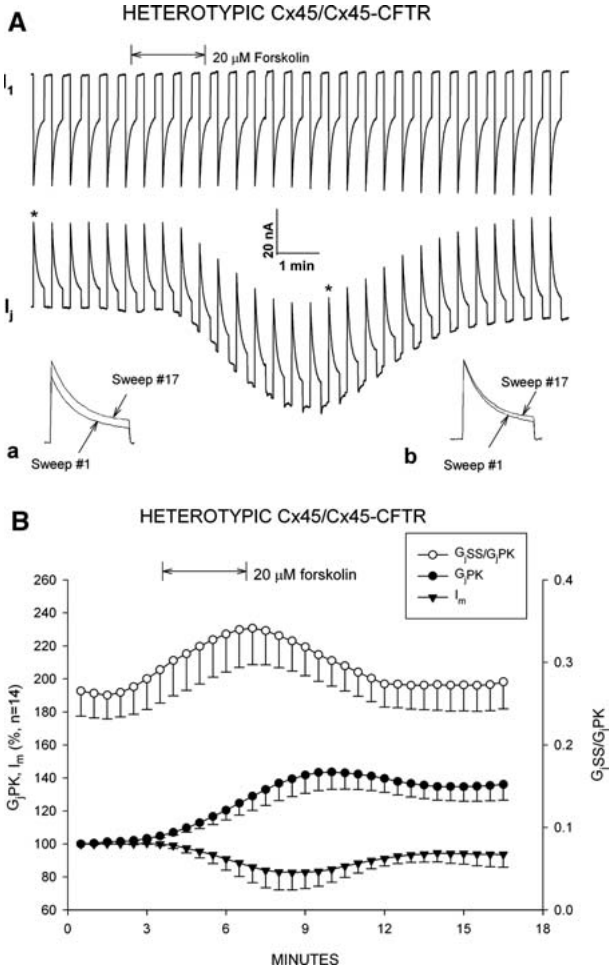

Fig. 5. Effect of forskolin on I_m, G_jPK and V_j sensitivity, estimated by measuring the ratio G_jSS/G_jPK, in heterotypic Cx45/Cx45-CFTR oocyte pairs. (*A*) Low-speed chart recording of junctional current (I_j) generated by applying –40-mV pulses (12-s duration) to oocyte 1 (Cx45) while maintaining oocyte 2 (Cx45-CFTR) at control V_m. Application of 20 μM forskolin reversibly increases I_j ($I_j = -I_2$). The increase in I_j reflects an increase in G_j (*lower trace*). In addition, the ratio I_jSS/I_jPK at the pulse (*lower trace*) reversibly increases, reflecting a drop in V_j sensitivity. The *insets* show representative I_j traces sampled before and during forskolin application (sweeps 1 and 17, *asterisks*). *Inset a* shows the I_j curves at their actual amplitudes, whereas *inset b* shows the I_j curves normalized to peak amplitude, which demonstrates the increase in G_jSS/G_jPK. (*B*) Average values of G_jPK, I_m and G_jSS/G_jPK before, during and after forskolin application. In oocyte pairs in which CFTR is only expressed in oocyte 2, with V_j pulses applied to oocyte 1 there is still an increase in both G_jPK and G_jSS/G_jPK. This suggests that CFTR activation is effective even when it affects only one of the two hemichannels. In addition, this indicates that the changes in G_j and V_j sensitivity are not artifacts of decreased membrane resistance in the pulsed oocyte. Indeed, I_m actually decreases slightly, reflecting an increase, rather than a decrease, in membrane resistance.

increase in G_jSS/G_jPK fully recovered, whereas that of G_jPK recovered only partially (Fig. 5B).

Discussion

This study shows that in *Xenopus* oocyte pairs coexpressing CFTR and Cx45, Cx40, Cx32 or Cx50, the forskolin-induced activation of CFTR results in a significant increase in junctional conductance (G_j) and a decrease in transjunctional voltage (V_j) sensitivity. The effect of forskolin is much less pronounced in cells expressing Cx50. Interestingly, the drop in V_j gating sensitivity is not accompanied by changes in the time constant (τ) of I_j decay. Truncation of the COOH terminus sizably reduces the forskolin effect on Cx40 but does not alter the response of Cx32. Forskolin is also effective on heterotypic Cx45/Cx45-CFTR oocyte pairs, although to a lesser degree.

The increase in G_jPK is likely to reflect a reversible increase in open channel probability, as previously demonstrated in small cells (Chanson et al., 1999), whereas the increase in G_jSS/G_jPK could be explained by different mechanisms. One possibility is

that it results from reduced V_j sensitivity of either fast or slow V_j-sensitive gates. Gap junction channels are gated by V_j gradients and increased $[Ca^{2+}]_i$ or $[H^+]_i$ via mechanisms still poorly understood (reviewed in Harris, 2001; Peracchia, 2004). Changes in G_j induced by cytosolic acidification are more closely related to $[Ca^{2+}]_i$ than to $[H^+]_i$ (Peracchia, 1990a, b; Lazrak & Peracchia, 1993), and there is evidence that in some cells gating is sensitive to nearly physiological $[Ca^{2+}]_i$, probably via calmodulin activation (reviewed in Peracchia, 2004). At least two V_j-sensitive gates have been identified: fast and slow. Fast V_j gate and chemical gate are believed to be distinct (Bukauskas & Peracchia, 1997), whereas slow V_j gate and chemical gate are likely to be the same (Bukauskas & Peracchia, 1997; Peracchia, Wang & Peracchia, 1999, 2000). Slow and fast V_j gates are in series, and each hemichannel has both gates. The slow gate closes at the negative side of V_j presumably in all connexin channels, whereas the polarity of fast V_j gating varies among connexin channels (reviewed in Harris, 2001; Bukauskas et al., 2002). In the absence of single-channel data, one can only guess which of these gates might be influenced by CFTR activation.

Alternatively, the reduction in G_jSS/G_jPK could result from the loss of V_j sensitivity in some of the channels. Perhaps, the activation of CFTR adds to a population of V_j-sensitive channels a population of V_j-insensitive channels. This would increase both G_jPK and G_jSS by the same amplitude and, consequently, decrease G_jSS/G_jPK; the latter would not reflect an actual drop in V_j sensitivity but rather the addition of a number of V_j-insensitive channels. Indeed, looking at the I_j curves, it is obvious that the I_j decay follows the same kinetics before and after CFTR activation, but both peak and steady-state amplitudes are elevated to higher values. One would expect that a change in V_j sensitivity, e.g., a shift in current-voltage relationship to higher V_j values, would be accompanied by a change in the kinetics of I_j inactivation. The same would be expected to follow a drop in V_j sensitivity of one of the two V_j-sensitive gates since fast and slow gates have different kinetics. In contrast, this is not the case because no significant change in I_j kinetics was observed. An increase in the number of operational channels with the same V_j sensitivity of the preexisting operational channels is also unlikely because in this case the increase in G_jPK would not be accompanied by a decrease in G_jSS/G_jPK; this ratio would remain the same.

G_jPK increases in synchrony with I_m or with a small delay, whereas the increase in G_jSS/G_jPK usually precedes the changes in both I_m and G_jPK. This is especially true for Cx45 and Cx40, whereas for Cx50 the change in G_jSS/G_jPK follows slightly those in I_m and G_jPK. The reason for the asynchrony among the three phenomena is unclear. A possibility is that the activation of CFTR initiates more than one cascade of events. Perhaps the mechanisms that affect G_jSS/G_jPK are set in motion before the actual opening of Cl^- channels. This asynchrony, however, is not due to an independent effect of the increase in cAMP because no changes in I_m, G_jPK and G_jSS/G_jPK were observed in oocytes not expressing CFTR (Kotsias & Peracchia, 2005).

Significantly, COOH-terminus truncation inhibits the effect of forskolin on junctional conductance and V_j sensitivity in Cx40 channels but has no effect on Cx32 channels. The reason for this difference is unclear. In terms of V_j sensitivity, one of the differences between Cx40 and Cx32 is the gating polarity of the fast V_j gates as Cx40 and Cx32 are activated at the positive and negative sides of V_j, respectively. However, whether the V_j gating polarity of the connexin is relevant to this phenomenon will need to be investigated by testing other positive and negative connexin channels.

Interestingly, experiments on heterotypic Cx45/Cx45-CFTR channels show that CFTR activation still affects, although to a lesser extent, both G_jPK and G_jSS/G_jPK in spite of the fact that CFTR is expressed in only one of the two oocytes of the pair. This indicates that modification of one of the two hemichannels (connexons) that form a cell-cell channel is sufficient to slightly increase the open channel probability and the ratio G_jSS/G_jPK. In these heterotypic pairs, I_m, measured in the oocyte expressing only Cx45, decreased slightly with forskolin application. A possibility is that CFTR activation in the Cx45-CFTR oocyte generates some soluble intermediates that cause a drop in membrane conductance by diffusing via gap junctions. One may question why, if this were the case, this would not curb the increase in I_m observed in homotypic Cx-CFTR oocytes. This may very well occur, but the effect would be expected to be quite small because of the much larger effect on I_m by Cl^- channel opening. Indeed, with heterotypic Cx45/Cx45-CFTR channels pulsed at the CFTR side, we did not observe an I_m increase significantly greater than that seen with homotypic channels (*data not shown*). However, this is not surprising because with heterotypic channels I_m dropped by a mean value of only 17%, whereas with homotypic channels I_m increased by a mean value of 420%. Due to the sizable standard error, a large number of experiments would be needed to detect potential differences in the I_m change between homotypic and heterotypic channels.

The change in G_jPK seems to roughly match that of G_jSS/G_jPK among the connexins tested, such that if the percent increase in G_jSS/G_jPK were plotted as a function of the percent increase in G_jPK, one would generate a roughly sigmoidal curve with Cx50 at the lower end and Cx45 at the upper end. While this trend could be real and potentially important, due to the small number of connexins tested it could be

entirely coincidental. Indeed, Cx40 and Cx32 show the same percent increase in G_jSS/G_jPK in spite of a drastically different increase in G_jPK.

The mechanism by which CFTR activation affects the function of gap junction channels is unclear. However, CFTR, aside from being a Cl^- channel, is also known to be a regulator of other ion channels, such as outwardly rectifying Cl^- channels, Ca-activated Cl^- channels and ROMK2 K^+ channels, among others (reviewed in Kunzelmann, 1999, 2001; Schwiebert et al., 1999). The regulation of the epithelial Na^+ channel ENaC by CFTR remains the subject of debate (Nagel et al., 2005; Bachhuber et al., 2005). In addition to this, it was suggested that CFTR also regulates bicarbonate transport (Ko et al., 2002; Park et al., 2002) and the expression of inflammatory mediators (Donaldson & Boucher, 2003).

There is evidence that CFTR interacts with other proteins via PDZ domains as it contains a classic PDZ1 binding domain. Significantly, Cx45 also contains a classic PDZ binding motif (SVWI, Res. 293–296), which is known to interact with the PDZ domain of ZO-1 (Kausalya, Reichert & Hunziker, 2001). However, Cx32 neither has PDZ binding motifs nor interacts with ZO-1. The functional interaction between CFTR and other channels and transporters appears to involve scaffolding proteins containing multiple PDZ domains, such as the Na^+/H^+ exchanger regulatory factor, also known as EBP50 (ezrin binding protein of 50 kDa; Reczek, Berryman & Bretscher, 1997), the CFTR-associated protein (CAP70; Wang et al., 2000) and the CFTR-associated ligand (CAL; Cheng & Guggino, 1998; Cheng, Wang & Guggino, 2004). Therefore, it is possible that CFTR interacts with Cx45, Cx32, Cx40 and Cx50 via cytoskeletal scaffolding proteins (actin?) and/or other intermediate cytoplasmic proteins. Chanson et al. (1999, 2001) and Huang et al. (2003) provided evidence that the tyrosine kinase c-Src links mediators of inflammation to Cx43 gap junction channels and acts as a central element in connecting CFTR to Cx43 regulation in airway epithelial cells. According to their results, expression in non-cystic fibrosis cells of a dominant negative construct of c-Src prevents gap junction channel closure by tumor necrosis factor-α and Cx43 channel closure is not observed by expressing a connexin mutant lacking tyrosine phosphorylation sites for c-Src.

In conclusion, the data show that forskolin-induced activation of CFTR expressed in oocytes increases the electrical conductance of gap junctions made of Cx45, Cx40, Cx32 and Cx50 and decreases their transjunctional voltage gating sensitivity. The COOH terminus of C40 may play a role in these phenomena, whereas that of Cx32 does not seem to be relevant. The drop in voltage sensitivity is not accompanied by changes in the kinetics of voltage-dependent inactivation of junctional current. This may suggest that CFTR activation renders some channels voltage-insensitive, possibly generating two populations of channels: V_j-sensitive and V_j-insensitive. The data suggest a functional interaction between CFTR and a variety of gap junction channels.

This study was supported by the National Institutes of Health (grant GM20113). The authors thank Dr. D. C. Devor (University of Pittsburgh, Pittsburgh, PA) for the generous gift of human CFTR cDNA.

References

Bachhuber, T., Konig, J., Voelcker, T., Murle, B., Schreiber, R., Kunzelmann, K. 2005. Cl^- interference with the epithelial Na^+ channel ENaC. *J. Biol. Chem.* **280:**31587–31594

Barrio, L.C., Suchyna, T., Bargiello, T., Xu, L.X., Roginski, R.S., Bennett, M.V.L., Nicholson, B.J. 1991. Gap junctions formed by connexins 26 and 32 alone and in combination are differently affected by applied voltage. *Proc. Natl. Acad. Sci. USA* **88:**8410–8414

Bear, C.E., Duguay, F., Naismith, A.L., Kartner, N., Hanrahan, J.W., Riordan, J.R. 1991. Cl^- channel activity in *Xenopus* oocytes expressing the cystic fibrosis gene. *J. Biol. Chem.* **268:**19142–19145

Bukauskas, F.F., Bukauskiene, A., Verselis, V.K., Bennett, M.V.L. 2002. Coupling asymmetry of heterotypic connexin 45/connexin 43-EGFP gap junctions: Properties of fast and slow gating mechanisms. *Proc. Natl. Acad. Sci. USA* **99:**7113–7118

Bukauskas, F.F., Peracchia, C. 1997. Two distinct gating mechanisms in gap junction channels: CO_2-sensitive and voltage-sensitive. *Biophys. J.* **72:**2137–2142

Cunningham, S.A., Worrell, R.T., Benos, D.J., Frizzell, R.A. 1992. cAMP-stimulated ion currents in *Xenopus* oocytes expressing CFTR cRNA. *Am. J. Physiol.* **262:**C783–C788

Chanson, M., Berclaz, P.Y., Scerri, I., Dudez, T., Wernke-Dollries, K., Pizurki, L., Pavirani, A., Fiedler, M.A., Suter, S. 2001. Regulation of gap junctional communication by a proinflammatory cytokine in cystic fibrosis transmembrane conductance regulator-expressing but not cystic fibrosis airway cells. *Am. J. Pathol.* **158:**1775–1784

Chanson, M., Scerri, I., Suter, S. 1999. Defective regulation of gap junction coupling in cystic fibrosis pancreatic duct cells. *J. Clin. Invest.* **103:**1677–1684

Cheng, J., Guggino, W.B. 1998. Molecular cloning and characterization of a novel PDZ domain containing protein that interact with CFTR. *Pediatr. Pulmonol.* **26:**213

Cheng, J., Wang, H., Guggino, W.B. 2004. Modulation of mature cystic fiobrosis transmembrane regulator protein by the PDZ domain protein CAL. *J. Biol. Chem.* **279:**1892–1898

Donaldson, S.H., Boucher, R.C. 2003. Update on pathogenesis of cystic fibrosis lung disease. *Curr. Opin. Pulm. Med.* **9:**486–491

Drumm, M.L., Wilkinson, D.J., Smit, L.S., Worrell, R.T., Strong, T.V., Frizzell, R.A., Dawson, D.C, Collins, F.S. 1991. Chloride conductance expressed by delta F508 and other CFTRs in *Xenopus* oocytes. *Science* **254:**1797–1799

Gadsby, D., Nairn, A.C. 1999. Control of CFTR channel gating by phosphorylation and nucleotide hydrolysis. *Physiol. Rev.* **79:**77–107

Greger, R., Schreiber, R., Mall, M., Wissner, A., Hopf, A., Briel, M., Bleich, M., Warth, R., Kunzelmann, K. 2001. Cystic fibrosis and CFTR. *Pfluegers Arch.* **443:**3–7

Harris, A.L. 2001. Emerging issues of connexin channels: Biophysics fills the gap. *Q. Rev. Biophys.* **34:**325–472

Huang, S., Dudez, T., Scerri, I., Thomas, M.A., Giepmans, B.N., Suter, S., Chanson, M. 2003. Defective activation of c-Src in

cystic fibrosis airway epithelial cells results in loss of tumor necrosis factor-alpha-induced gap junction regulation. *J. Biol. Chem.* **278:**8326–8332

Kausalya, P.J., Reichert, M., Hunziker, W. 2001. Connexin45 directly binds to ZO-1 and localizes to the tight junction region in epithelial MDCK cells. *FEBS Lett.* **505:**92–96

Ko, S.B., Shcheynikov, N., Choi, J.Y., Luo, X., Ishibashi, K., Thomas, P.J., Kim, J.Y., Kim, K.H., Lee, M.G., Naruse, S., Muallem, S. 2002. A molecular mechanism for aberrant CFTR-dependent HCO_3^- transport in cystic fibrosis. *EMBO J.* **21:**5662–5672

Kotsias, B.A., Peracchia, C. 2005. Functional interaction between CFTR and Cx45 gap junction channels expressed in oocytes. *J. Membr. Biol.* **203:**143–150

Kunzelmann, K. 1999. The cystic fibrosis transmembrane conductance regulator and its function in epithelial transport. *Rev. Physiol. Biochem. Pharmacol.* **137:**1–70

Kunzelmann, K. 2001. CFTR: Interacting with everything? *News Physiol. Sci.* **16:**167–170

Lazrak, A., Peracchia, C. 1993. Gap junction gating sensitivity to physiological internal calcium regardless of pH in Novikoff hepatoma cells. *Biophys. J.* **65:**2002–2012

Nagel, G., Barbry, P., Chabot, H., Brochiero, E., Hartung, K., Grygorczyk, R. 2005. CFTR fails to inhibit the epithelial sodium channel ENaC expressed in *Xenopus laevis* oocytes. *J. Physiol.* **564:**671–682

Park, O.M., Ko, S.B., Choi, J.Y., Muallem, G., Thomas, P.J., Pushkin, A., Lee, M.S., Kim, J.Y., Lee, M.G., Muallem, S., Kurtz, I. 2002. The cystic fibrosis transmembrane conductance regulator interacts with and regulates the activity of the HCO_3^- salvage transporter human Na^+-HCO_3^- cotransport isoform 3. *J. Biol. Chem.* **277:**50503–50509

Peracchia, C. 1990a. Increase in gap junction resistance with acidification in crayfish septate axons is closely related to changes in intracellular calcium but not hydrogen ion concentration. *J. Membr. Biol.* **113:**75–92

Peracchia, C. 1990b. Effects of caffeine and ryanodine on low pHi-induced changes in gap junction conductance and calcium concentration in crayfish septate axons. *J. Membr. Biol.* **117:**79–89

Peracchia C. 2004. Chemical gating of gap junction channels. Roles of calcium, pH and calmodulin. *Biochim. Biophys. Acta (Biomembranes) 1662:61–80*

Peracchia, C., Wang, X., Li, L., Peracchia, L.L. 1996. Inhibition of calmodulin expression prevents low-pH-induced gap junction uncoupling in *Xenopus* oocytes. *Pfluegers Arch.* **431:**379–387

Peracchia, C., Wang, X.G., Peracchia, L.L. 1999. Is the chemical gate of connexins voltage sensitive? Behavior of Cx32 wild-type and mutant channels. *Am. J. Physiol.* **276:**C1361–C1373

Peracchia, C., Wang, X.G., Peracchia, L.L. 2000. Slow gating of gap junction channels and calmodulin. *J. Membr. Biol.* **78:**55–70

Reczek, D., Berryman, M., Bretscher, A. 1997. Identification of EBP50: A PDZ-containing phosphoprotein that associates with members of the ezrin-radixin-moesin family. *J. Cell. Biol.* **139:**169–179

Schwiebert, E.M., Benos, D.J., Egan, M.E., Stutts, M.J., Guggino, W.B. 1999. CFTR is a conductance regulator as well as a chloride channel. *Physiol. Rev.* **79:**145–166

Sheppard, D.N., Welsh, M.J. 1999. Structure and function of the CFTR chloride channel. *Physiol. Rev.* **79:**23–45

Spray, D.C., Harris, A.L., Bennett, M.V.L. 1981. Equilibrium properties of a voltage-dependent junctional conductance. *J. Gen. Physiol.* **77:**77–93

Stergiopoulos, K., Alvarado, J.L., Mastroianni, M., Ek-Vitorin, J.F., Taffet, S.M., Delmar, M. 1999. Hetero-domain interactions as a mechanism for the regulation of connexin channels. *Circ. Res.* **84:**1144–1155

Wang, S., Li, M. 2001. Molecular studies of CFTR interacting proteins. *Pfluegers Arch.* **443:**62–64

Wang, S., Yue, H., Derim R.B., Guggino, W.B. UM. 2000. Accessory protein facilitated CFTR-CFTR interaction, a molecular mechanism to potentiate the chloride channel activity. *Cell* **103:**169–179