Mechanism of G551D-CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) Potentiation by a High Affinity ATP Analog

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Cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel gated by ATP binding and hydrolysis at its nucleotide binding domains (NBD). The NBDs dimerize in a head-to-tail configuration, forming two ATP binding pockets (ABP) with the ATP molecules buried at the dimer interface. Previous studies have indicated that ABP2, formed by the Walker A and B motifs of NBD2 and the signature sequence of NBD1, is the site critical for the ATP-dependent opening of CFTR. The G551D mutation in ABP2, the third most common cystic fibrosis-associated mutation, abolishes ATP-dependent gating, resulting in an open probability that is ~100-fold lower than that of wild-type channels. Interestingly, we found that the ATP analog N\(^{\beta}\)-(2-phenylethyl)-ATP (P-ATP) increases G551D currents mainly by increasing the open time of the channel. This effect is reduced when P-ATP is applied together with ATP, suggesting a competition between ATP and P-ATP for a common binding site. Introducing mutations that lower the nucleotide binding affinity at ABP2 did not alter significantly the effects of P-ATP on G551D-CFTR, whereas an equivalent mutation at ABP1 (consisting of the Walker A and B motifs of NBD1 and the signature sequence of NBD2) dramatically decreased the potency of P-ATP, indicating that ABP1 is the site where P-ATP binds to increase the activity of G551D-CFTR. These results substantiate the idea that nucleotide binding at ABP1 stabilizes the open channel conformation. Our observation that P-ATP enhances the G551D activity by binding at ABP1 implicates that ABP1 can potentially be a target for drugs to bind and increase the channel activity.

The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel that belongs to the ATP binding cassette super family (1). Mutations in the gene coding of CFTR cause cystic fibrosis (CF), a genetic disease characterized by defective transport of chloride ions across several epithelial tissues (1–3).

CFTR has two nucleotide binding domains (NBD) that control the channel gating by binding and hydrolysis of ATP. Recent studies revealed that the NBDs of CFTR may dimerize as observed in other ATP binding cassette proteins (4, 5). Upon dimerization of the two NBDs of CFTR in a head-to-tail configuration, two ATP binding pockets (ABP1 and ABP2) are formed with the ATP molecules sandwiched at the interface. Zhou et al. (6) studied the role of each ABP in the gating of CFTR by mutating amino acids that interact with the adenine ring of ATP, Trp-401 in ABP1 and Tyr-1219 in ABP2, and showed that each ABP plays a different role in CFTR gating. The results led to the conclusion that ABP2 is the site critical for the ATP-dependent opening of the CFTR channel (6, 7), whereas ATP binding to ABP1 may contribute to the stability of the open channel conformation (6).

The importance of the signature sequence in CFTR gating is attested by the fact that mutations such as G551D and G1349D in this region of the protein are associated with CF. G551D, located in the signature sequence of NBD1, is one of the most common CF-associated mutations (the Cystic Fibrosis Mutation Database). G551D-CFTR channels exhibit much lower open probability than wild-type (WT) channels (7–9), and consequently, the patients carrying this mutation present a severe phenotype (10, 11). G551D has been used as a model in pharmacological characterizations of CFTR (9, 12–15), but the fundamental mechanism for the functional defect has been unclear until recently. We found that the G551D mutation completely eliminates the ability of ATP to increase the opening rate of the channel (7), consistent with the idea that ATP binding to ABP2 is critical for the ATP-dependent opening of CFTR channels (6). The observed low activity of G551D-CFTR likely represents the rare ATP-independent openings seen with the WT-CFTR channels in the absence of ATP (7, 16).

Although G551D-CFTR does not respond to ATP, interestingly Cai et al. (9) showed that the ATP analog 2'-deoxy-ATP increases the P\(_o\) of G551D-CFTR. It remains unclear which nucleotide binding sites this ATP analog acts on to potentiate G551D-CFTR. We have previously (17) showed that the high affinity ATP analog P-ATP prolonged the open time of WT-CFTR channels by binding to ABP1. Since the G551D mutation, located in ABP2, may not affect the function of ABP1, we tested the hypothesis that P-ATP potentiates G551D-CFTR by binding to ABP1. We found that P-ATP increases G551D cur-
rents primarily by increasing the open time of the channel. By introducing mutations that lower the nucleotide binding affinity at each NBD, we were able to conclude that this effect of P-ATP on G551D-CFTR is through binding of the nucleotide to ABP1. These observations suggest that ABP1 can serve as a molecular target for the development of CFTR potentiators.

EXPERIMENTAL PROCEDURES

Site-directed Mutagenesis—The constructs containing single mutations (G551D, W401G, and Y1219G) have been described previously (6, 7). Additional point mutations were introduced into G551D-CFTR by using a QuikChange XL kit (Stratagene, La Jolla, CA) according to the manufacturer’s protocol. The mutations were confirmed by sequencing (DNA core, University of Missouri-Columbia).

Transient Expression—Chinese hamster ovary cells were transiently transfected with WT or mutant CFTR as described previously (7). The transfected cells were used 2–6 days after transfection.

Electrophysiological Recordings and Data Analysis—All data were recorded at room temperature using an EPC9 amplifier (HEKA, Lambrecht/Pfalz, Germany). The membrane potential was held at −50 mV for all experiments. Data were filtered at 100 Hz with an eight-pole Bessel filter (Warner Instrument, Hammed, CT) and digitized at a sampling rate of 500 Hz. Downward deflections in the current trace indicate channel opening. The pipette solution contained (in mM): 140 N-methyl-D-glucamine chloride (NMDG-Cl), 2 MgCl2, 5 CaCl2, and HEPES (pH 7.4 with NMDG). The bath solution contained (in mM): 145 NaCl, 5 KCl, 2 MgCl2, 1 CaCl2, 5 glucose, 5 HEPES, and 20 mm sucrose (pH 7.4 with NaOH). After a seal was obtained, the patch was excised into a perfusion solution containing (in mM): 150 NMDG-Cl, 10 EGTA, 10 HEPES, 8 Tris, and 2 MgCl2 (pH 7.4 with NMDG).

The steady-state mean currents were calculated with Igor (WaveMetrics, Lake Oswego, OR). Recordings with up to four channel open steps were further filtered at 50 Hz and used for single channel kinetic analysis. Mean open times were calculated as described previously (7) using a program developed by Dr. Csanady (18). Since the activity of G551D is extremely low, the number of channels in the membrane patch cannot be ascertained. Although the analysis program allows us to determine the mean open time of the channel (\(\tau_o\)) accurately, the closed time constant (\(\tau_c\)), which is very sensitive to the number of channels present in the patch, is grossly underestimated. To better estimate the mean closed time we employed a strategy based on our previous results (7). We first obtained \(\tau_c\) in the control condition as described above. Since the \(P_o\) of G551D-CFTR is \(0.004\) \(\approx \frac{1}{120}\) of the \(P_o\) for WT-CFTR, \(\tau_c\) can be calculated based on the equation, \(\tau_c = (\tau_o / P_o) - \tau_o\). To calculate \(\tau_c\) in the presence of P-ATP, we used the same method except that the \(P_o\) in the presence of P-ATP is the control \(P_o\) multiplied by the -fold increase of the mean current by P-ATP.

Reagents—Mg-ATP was purchased from Sigma-Aldrich. cAMP-dependent protein kinase was purchased from Promega (Madison, WI). P-ATP (N6-(2-phenylethyl)-ATP) was purchased from Biolog Life Science Institute (Bremen, Germany).

RESULTS

Effect of P-ATP on G551D-CFTR—Fig. 1A shows a continuous current trace from an excised inside-out patch of a Chinese hamster ovary cell expressing G551D-CFTR. The channels were activated with 1 mM ATP + 25 units/ml cAMP-dependent

FIGURE 1. Reversible activation of G551D-CFTR by P-ATP. A, P-ATP, a high affinity ATP analog, increases G551D-CFTR activity. B, structure of P-ATP. The ATP analog has an extra ring at the N6 position. C, P-ATP dose-response relationship for G551D-CFTR channels. Currents at different [P-ATP] were normalized to the current level in the absence of P-ATP. The solid line represents a fit to the data using the Hill equation, \(K_{1/2} = 6.3 \pm 2.5 \mu M\). Data are presented as means ± S.E., n = 4–15 for each data point.
ATP Analog Potentiates G551D-CFTR

**FIGURE 2. P-ATP dependent gating of G551D-CFTR in excised patches.** A, single-channel current traces for G551D-CFTR in the presence and absence of 10 μM P-ATP. B, summary of the mean open time and the mean closed time for G551D-CFTR in the presence and absence of P-ATP (n = 11). The mean open time increases almost 5-fold in the presence of 10 μM P-ATP, whereas the mean closed time slightly decreases. C, summary of the -fold increase for the mean current, mean open times, and mean opening rate (n = 7–11). Data are presented as means ± S.E.

Protein kinase (not shown). After removal of ATP and cAMP-dependent protein kinase, the channel activity remained unchanged as described previously (7). The addition of 10 μM P-ATP increased the mean current by 6.2 ± 0.7-fold (n = 11). Similar results were obtained for G551D-CFTR expressed in human epithelial cells CFPAC-1 (see supplemental materials).

To further quantify the effect of P-ATP on G551D currents, we measured the channel activity at different [P-ATP] and calculated the ratio between the mean current in the presence of P-ATP and the mean control current (washout). The P-ATP dose-response relationship (Fig. 1C) shows that G551D-CFTR currents can be enhanced as much as 7-fold at 50 μM P-ATP with a K\text{\textsubscript{\text{o}}} of −6 μM.

In patches with sporadic openings, we could observe in detail the effect of P-ATP on the channels. Fig. 2A shows representative current traces. The −50-s control trace shows 15 openings, with a mean open time of −350 ms. In the presence of 10 μM P-ATP, most of the openings are several seconds long, but the number of opening events (18 openings) is not very different from the control. This effect of P-ATP on the channel open time is reversible because after P-ATP is removed, the duration of the opening events returns to hundreds of milliseconds. Results from single channel kinetic analysis (see “Experimental Procedures”) are summarized in Fig. 2B. The mean open time increases in the presence of P-ATP, whereas the closed time constant does not change significantly. Fig. 2C shows the -fold increase in the mean current, the mean open time (τ\text{\textsubscript{o}}), and the opening rate (1/τ\text{\textsubscript{o}}). The results indicate that the increase of the G551D channel activity by P-ATP is achieved mainly by an increase in the open time of the channel. G551D channels have extremely long closed times, τ\text{\textsubscript{c}} ≪ τ\text{\textsubscript{o}}; consequently, the channel activity is directly proportional to the open time (i.e., P\text{\textsubscript{\text{o}}} = τ\text{\textsubscript{o}}/(τ\text{\textsubscript{c}} + τ\text{\textsubscript{o}}) ≡ τ\text{\textsubscript{o}}/τ\text{\textsubscript{c}}). That the -fold increase of the mean current is similar to the -fold increase in the mean open time also indicates that the number of channels (n) likely does not increase with P-ATP. Based on these results, we can safely conclude that the increase in G551D-CFTR activity in the presence of P-ATP is mainly due to an increase in τ\text{\textsubscript{o}}.

**ATP and P-ATP Compete for the Same Binding Sites**—We next tested whether the effect of P-ATP on G551D-CFTR is through binding to the same site(s) ATP may bind. Since ATP has little effect on G551D, one expects that the effect of P-ATP will be diminished by the presence of ATP if they share a common binding site. Fig. 3 compares the effect of 10 μM P-ATP in the presence and absence of 2.75 mM ATP. In the presence of ATP, the effect of P-ATP is dramatically reduced, but removal of ATP rapidly restores the potentiation of P-ATP on G551D-CFTR, suggesting that these two nucleotides can readily exchange at a binding site. The expanded sections of the trace show the effect of P-ATP on the channels. In the presence of ATP and P-ATP, we can rarely observe a long-lasting opening, but when ATP is removed, and only P-ATP is present, abundant long-lasting openings are observed.

**Where Does P-ATP Bind to Increase the G551D-CFTR Current?**—To determine to which ABP P-ATP binds to exert its effect on G551D-CFTR channels, we introduced mutations that lower the nucleotide binding affinity at each ABP. As demonstrated previously, W401G and Y1219G are two mutations that can serve this purpose. Trp-401 was shown interacting directly with the adenosine ring of ATP via a stacking mechanism in the crystal structure of NBD1 from human CFTR (Ref. 19, Protein Data Bank (PDB) code 1XMI). Tyr-1219 was identified as the counterpart of Trp-401 in NBD2 through homology modeling and sequence analysis (6). It was found that, under the WT-CFTR background, the Y1219G mutation, but not the W401G mutation, causes a rightward shift of the ATP is dose-response curve (6). A similar result was observed for P-ATP (see supplemental materials).

Although the Y1219G mutation decreases the apparent affinity of ATP and P-ATP in WT background, introducing this mutation into the G551D background has little influence on the...
effect of P-ATP. Fig. 4A shows a representative trace of G551D/Y1219G-CFTR channels in the absence and presence of 10 μM P-ATP. 10 μM P-ATP increased the activity of this double mutant by 5.0 ± 0.6-fold (n = 7), and as in the case of G551D channels, this increase in activity is mainly due to an increase in the open time of the channel (Fig. 4B). Fig. 5 shows the normalized P-ATP dose-response relationship. The G551D/Y1219G-CFTR P-ATP dose response is very similar to the G551D-CFTR dose response, suggesting that lowering the binding affinity at the ABP2 site does not alter the effect of P-ATP.

In contrast, introducing W401G in the G551D background significantly reduced the effect of P-ATP. The activity of W401G/G551D-CFTR channels in the presence of 10 μM P-ATP is smaller than the activity of G551D channels under the same conditions. The mean current -fold increase is 1.9 ± 0.2 for W401G/G551D-CFTR when compared with 6.2 ± 0.7 for G551D-CFTR. However, the increase in the mean current is correlated with the increase in the open time. The activity of the double mutant can be further enhanced by increasing the [P-ATP] (Fig. 6). The rightward shift of the P-ATP dose-response relationship suggests that mutating Trp-401 to glycine at ABP1 lowers the P-ATP binding affinity (Fig. 6). We thus conclude that P-ATP binds to ABP1 to increase the open time of G551D channels and consequently enhance the activity of this mutant.

**DISCUSSION**

The current results show that P-ATP, a high affinity ATP analog, binds to ABP1 to enhance G551D-CFTR, and it does so mainly by increasing the open time of the channel. This conclusion is important for two main reasons. Mechanistically, it establishes that nucleotide binding at ABP1 can stabilize the open state even for G551D-CFTR, a mutant irresponsive to ATP; pharmacologically, it suggests that ABP1 may be a potential molecular target for drugs that could potentiate G551D-CFTR.

The gating of CFTR channels is controlled by ATP binding and hydrolysis at the two NBDs, which dimerize upon ATP binding, leading to the opening of the channel. ATP hydrolysis at ABP2 leads to the destabilization of the dimer and closing of the channel. Phosphorylated CFTR channels can open in the absence of ATP but with a very low P_o (7, 20). Our previous studies suggest that the two ABPs play asymmetrical roles in CFTR gating. ABP2 was identified as the site that catalyzes the ATP-dependent opening of the CFTR channel (6, 7), whereas nucleotide binding to ABP1 contributes mainly to the stabilization of the dimer, resulting in a longer open time (6, 16, 17, 20, 21).

Previously, we showed that ATP fails to increase the opening rate of G551D-CFTR (7), and this observation led us to conclude that G551D-CFTR presents only ATP independent openings. Similar observations were made by Wang et al. (22). These results are consistent with the ideas that ABP2 is the site that couples ATP binding to the channel opening and that the G551D mutation specifically abolishes the function of ABP2. Thus, other nucleotides such ADP or AMP-PNP, which presumably act on ABP2 (7, 16), were not effective on G551D-CFTR (7). The data presented in the current report indicate
that for G551D-CFTR, the function of ABP1 may not be jeopardized since P-ATP can bind to this site to stabilize the open channel conformation. The significance of this conclusion is that the G551D construct presents a great opportunity to investigate specifically the interaction of nucleotides with ABP1, a yet challenging issue for several reasons. First, under the WT construct, the ATP dose-response relationship mostly reflects the affinity of ATP to ABP2; a functional assay that quantitatively assesses ATP binding affinity to ABP1 is still lacking (6, 21). Second, there are some discrepancies about the exact molecular interactions between ATP and ABP1 in the crystallographic literature (Ref. 19, PDB code 1XMI; Ref. 23, PDB code 1ROX). Third, since ligand binding and subsequent conformational changes are coupled for any allosteric proteins (24), biochemical studies with ligands that are ineffective in inducing conformational changes may not provide a whole picture of ligand–receptor interactions.

It is interesting to note that the effects of P-ATP echo what has been reported by Cai et al. (9), that 2′-deoxy-ATP increased the activity of G551D channels. They also showed an increase of the mean open time of the channels by 2′-deoxy-ATP. The main difference is that the closed time is decreased by 61% in the presence of 2′-deoxy-ATP (9), whereas the current report showed minimal effect of P-ATP on the closed time constant. However, it should be pointed out that it is extremely difficult to accurately estimate \( \tau_c \) because of the low \( P_o \) of G551D. Cai et al. (9) obtained the closed time of the channels, assuming that the total number of channels in the patch was the same as the maximal number of channel opening steps observed. Given the extremely low \( P_o \) of G551D, this practice inevitably results in an underestimation of the closed time. Regardless of the difference between results in Cai et al. (9) and the current report, it is interesting that 2′-deoxy-ATP and P-ATP are chemically distinct and thus likely interact with ABPs in a different manner. If it turns out that 2′-deoxy-ATP also binds to ABP1 to potentiate G551D-CFTR, it may be possible to design even more effective nucleotides for this mutant.

If we assume that the ATP-independent openings of G551D involve the dimerization of the NBDs, an outstanding question remains to be answered. When G551D channels open, is ABP2 occupied or empty? Based on the crystal structure of the NBD1 of CFTR (Ref. 19, PDB code 1XMI; Ref. 23, PDB code 1ROX), ATP binds to the Walker A and B motifs but not to the signature sequence. Since the G551D mutation is located in the signature sequence of NBD1 (ABP2), one could assume that the nucleotide binding at ABP2 is not affected by this mutation, but instead, the post-binding events may be impaired. We speculate that perhaps the electrostatic repulsion between the negatively charged ATP, or P-ATP, molecule and the negatively charged aspartate play a role in hindering the dimerization process. Then, the dimer may only form after the nucleotide dissociates from this binding site. In this case, the open state represents a dimer with only ABP1 occupied. Alternatively, if an NBD dimer can form in the presence of ATP (or P-ATP) bound at ABP2, we need to explain why ATP (or P-ATP) fails to increase the opening rate of G551D channels. In this case, perhaps the Gly to Asp mutation affects the post-dimerization conformational changes that allow the channel gate to open.

Since we do not know whether the G551D open state involves the dimerization of the NBDs, we need to consider the possibility that the openings of G551D may be unrelated to the dimerization of the NBDs. Recently, Wang et al. (22) showed that the activity of mutant CFTR channels whose NBD2 has been completely deleted can open and close in the absence of ATP, bypassing ATP binding and dimerization of the NBDs. Thus, we cannot rule out the possibility that the openings of G551D are decoupled from the NBD dimerization. However, since binding of P-ATP to ABP1 decreases the closing rate of the G551D channels, we have to conclude that even under this scenario, the transmembrane domains are not totally decoupled from the NBDs in G551D-CFTR. More studies are needed to further understand the mechanisms by which ATP-independent gating occurs.

The goal of understanding how CFTR works is to guide in the development of drugs for therapeutical interventions in cystic fibrosis. G551D is a disease-associated mutation that traffics normally to the membrane (7, 25) but with defective gating. In this particular case, understanding the gating mechanism of the channel is extremely important to finding a potentiator that restores the channel function. Many compounds have been shown to increase G551D-CFTR currents (9, 12, 13, 15, 26), but none of them seems to be potent enough to completely restore the \( P_o \) of the channel to WT levels. Since we lack a detailed

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**FIGURE 6. P-ATP effect on W401G/G551D-CFTR.** A, single-channel traces of W401G/G551D-CFTR in the presence or absence of P-ATP. B, increasing [P-ATP] above 10 \( \mu M \) further increases the activity of the double mutant (\( n = 3–7 \)).
understanding of how these compounds work or where they bind, it is very difficult to design a drug that may accomplish the task of rescuing the defective channel. Working in that direction, recently Moran et al. (14) predicted several potential binding sites at the dimer interface for genistein, one of the most extensively studied CFTR potentiators. Later, analysis of the mutated binding sites seemed to support this prediction (27). Our observation that P-ATP potentiates G551D-CFTR by binding to ABP1 suggests that ABP1 may be a target for drugs to bind and enhance the mutant channel activity. It is important to point out that for ABP1 to serve as a potential drug target, it is critical that ATP is not occluded in the binding site, as suggested previously by some biochemical studies (28, 29). Our data (Fig. 3) clearly indicate that ATP and P-ATP readily exchange at ABP1, challenging the notion of a prolonged nucleotide occlusion at this site. It should be interesting to examine biochemically whether the G551D mutation abolishes nucleotide occlusion at this site. It should be interesting to examine biochemically whether the G551D mutation abolishes nucleotide occlusion at this site. Nevertheless, we believe that these new findings will likely serve as a structural framework for the future development of potentiators for CFTR mutants such as G551D.

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REFERENCES

1. Riordan, J. R., Rommens, J. M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M. L., Ianuzzo, M. C., Collins, F. S., and Tsui, L.-C. (1989) Science 245, 1066–1073
2. Welsh, M. J., and Smith, A. E. (1993) Cell 73, 1251–1254
3. Quinton, P. M. (1990) J. Gen. Physiol. 128, 2709–2717
4. Vergani, P., Lockless, S. W., Nairn, A. C., and Gadsby, D. C. (2005) Nature 433, 876–880
5. Mense, M., Vergani, P., White, D. M., Alberg, G., Nairn, A. C., and Gadsby, D. C. (2006) EMBO J. 25, 4728–4739
6. Zhou, Z., Wang, X., Liu, H.-Y., Zou, X., Li, M., and Hwang, J. H. (2006) J. Gen. Physiol. 128, 413–422
7. Bompadre, S. G., Sohma, Y., Li, M., and Hwang, T.-C. (2007) J. Gen. Physiol. 129, 285–298
8. Drumm, M. L., Wilkinson, D. J., Smit, L. S., Worrell, R. T., Strong, T. V., Frizzell, R. A., Dawson, D. C., and Collins, F. S. (1991) Science 254, 1797–1799
9. Cai, Z., Taddei, A., and Sheppard, D. N. (2006) J. Biol. Chem. 281, 1970–1977
10. Cutting, G. R., Basch, L. M., Rosenzweig, B., Wu, Z., Zielenski, J., Tsui, L.-C., Antonarakis, S. E., and Kazazian, H. H. (1990) Nature 346, 366–369
11. Kerem, B.-S., Zielenski, J., Markiewicz, D., Bozon, D., Gazit, E., Yahav, I., Kennedy, D., Riordan, J. R., Collins, F. S., Rommens, J. M., and Tsui, L.-C. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 8447–8451
12. Illek, B., Zhang, L., Lewis, N. C., Moss, R. B., Dong, J. Y., and Fischer, H. (1999) Am. J. Physiol. 277, C833–C839
13. Galietta, L. J., Springsteel, M. F., Eda, M., Niedzinski, E. J., By, K., Haddadin, M. J., Kurth, M. J., Nantz, M. H., and Verkman, A. S. (2001) J. Biol. Chem. 276, 19723–19728
14. Moran, O., Galietta, L. J., and Zegarra-Moran, O. (2005) CMLS Cell. Mol. Life Sci. 62, 446–460
15. Pedemonte, N., Sonawane, N. D., Taddei, A., Hu, J., Zegarra-Moran, O., Suen, Y. F., Robins, L. I., Dicus, C. W., Willenbring, D., Nantz, M. H., Kurth, M. J., Galietta, L. J., and Verkman, A. S. (2005) Mol. Pharmacol. 67, 1797–1807
16. Bompadre, S. G., Ai, T., Cho, J. H., Wang, X., Sohma, Y., Li, M., and Hwang, T.-C. (2005) J. Gen. Physiol. 125, 361–375
17. Zhou, Z., Wang, X., Li, M., Sohma, Y., Zou, X., and Hwang, T.-C. (2005) J. Physiol. (Lond.) 569, 447–457
18. Csanady, L. (2000) Biophys. J. 78, 785–799
19. Lewis, H. A., Zhao, X., Wang, C., Sauder, J. M., Rooney, I., Noland, B. W., Lorimer, D., Kearins, M. C., Conners, K., Condon, B., Maloney, P. C., Guggino, W. B., Hunt, J. F., and Emtage, S. (2005) J. Biol. Chem. 280, 1346–1353
20. Bompadre, S. G., Cho, J. H., Wang, X., Zou, X., Sohma, Y., Li, M., and Hwang, T.-C. (2005) J. Gen. Physiol. 125, 377–394
21. Powe, A. C., Nakkash, L. A., Li, M., and Hwang, T.-C. (2002) J. Physiol. (Lond.) 539, 333–346
22. Wang, W., Bernard, K., Li, G., and Kirk, K. L. (2007) J. Biol. Chem. 282, 4533–4544
23. Lewis, H. A., Buchanan, S. G., Burley, S. K., Conners, K., Dickey, M., Dwork, M., Fowler, R., Gao, X., Griggino, W. B., Hendrickson, W. A., Hunt, J. F., Kearins, M. C., Lorimer, D., Maloney, P. C., Post, K. W., Rajashekhar, K. R., Rutter, M. E., Sauder, J. M., Shriver, S., Thibodeau, P. H., Thomas, P. J., Zhang, M., Zhao, X., and Emtage, S. (2005) EMBO J. 23, 282–293
24. Colquhoun, D. (1998) Br. J. Pharmacol. 125, 924–947
25. Gregory, R. J., Rich, D. P., Csanady, L., Lorimer, D., Kearins, M. C., Conron, A. G., Post, K. W., Rajashekhar, K. R., Rutter, M. E., Sauder, J. M., Shriver, S., Thibodeau, P. H., Thomas, P. J., Zhang, M., Zhao, X., and Emtage, S. (2005) EMBO J. 23, 282–293
26. Ai, T., Bompadre, S. G., Wang, X., Hu, S., Li, M., and Hwang, T.-C. (2004) Mol. Pharmacol. 65, 1415–1426
27. Zegarra-Moran, O., Monteverde, M., Galietta, L. J., and Moran, O. (2007) J. Biol. Chem. 282, 9098–9104
28. Basso, C., Vergani, P., Nairn, A. C., and Gadsby, D. C. (2003) J. Gen. Physiol. 122, 333–348
29. Aleksandrov, L., Aleksandrov, A. A., Chang, X. B., and Riordan, J. R. (2002) J. Biol. Chem. 277, 15419–15425