A dipeptidyl aminopeptidase-like protein remodels gating charge dynamics in Kv4.2 channels

Kevin Dougherty
Thomas Jefferson University

Manuel Covarrubias
Thomas Jefferson University

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Dipeptidyl aminopeptidase–like proteins (DPLPs) interact with Kv4 channels and thereby induce a profound remodeling of activation and inactivation gating. DPLPs are constituent components of the neuronal Kv4 channel complex, and recent observations have suggested the critical functional role of the single transmembrane segment of these proteins (Zagha, E., A. Ozaita, S.Y. Chang, M.S. Nadal, U. Lin, M.J. Saganich, T. McCormack, K.O. Akinsanya, S.Y. Qi, and B. Rudy, 2005. J. Biol. Chem. 280:18853–18861). However, the underlying mechanism of action is unknown. We hypothesized that a unique interaction between the Kv4.2 channel and a DPLP found in brain (DPFX-S) may remodel the channel’s voltage-sensing domain. To test this hypothesis, we implemented a robust experimental system to measure Kv4.2 gating currents and study gating charge dynamics in the absence and presence of DPFX-S. The results demonstrated that coexpression of Kv4.2 and DPFX-S causes a −26 mV parallel shift in the gating charge-voltage (Q-V) relationship. This shift is associated with faster outward movements of the gating charge over a broad range of relevant membrane potentials and accelerated gating charge return upon repolarization. In sharp contrast, DPFX-S had no effect on gating charge movements of the Shaker B Kv channel. We propose that DPFX-S destabilizes resting and intermediate states in the voltage-dependent activation pathway, which promotes the outward gating charge movement. The remodeling of gating charge dynamics may involve specific protein–protein interactions of the DPFX-S’s transmembrane segment with the voltage-sensing and pore domains of the Kv4.2 channel. This mechanism may determine the characteristic fast operation of neuronal Kv4 channels in the subthreshold range of membrane potentials.

INTRODUCTION

Auxiliary β subunits of Kv channels influence gating through diverse mechanisms, which in the best known instances involve direct interactions with intracellular domains of the channel (Jerng et al., 2004a; Heinemann and Hoshi, 2006). Interactions with transmembrane core domains (pore domain [PD] and voltage-sensing domain [VSD]) are also plausible and may help to explain the promiscuous effects of certain Kv channel β subunits (e.g., MiRP) on Kv channel permeation and gating (Hanlon and Wallace, 2002; Jerng et al., 2004a; Cai et al., 2006). The dipeptidyl aminopeptidase–like proteins (DPLPs), a recently discovered family of β subunits of neuronal Kv4 channels, may also use the latter type of interactions to influence function. DPLPs are analogous to the CD26 surface antigen, but lack enzymatic activity, and consist of a variable intracellular N-terminal domain followed by a single membrane-spanning segment and a large extracellular C-terminal domain (Kin et al., 2001; Nadal et al., 2003; Strop et al., 2004). DPLPs shift the voltage-dependent properties of Kv4 channels and accelerate their inactivation gating kinetics (Nadal et al., 2003; Jerng et al., 2004b; Ren et al., 2005; Zagha et al., 2005). Relative to the Kv4 α subunits expressed alone, Kv4–DPLP complexes exhibit a substantially leftward-shifted conductance-voltage relationship, which may result from remodeling different aspects of activation and inactivation gating (Ayer and Sigworth, 1997; Yifrach and MacKinnon, 2002). More concretely, however, a possible explanation for the leftward-shifted voltage dependence emerged from recent studies that identified putative interactions between the sole transmembrane segment of the DPLPs and discrete components of the Kv4 VSD (Ren et al., 2005; Zagha et al., 2005). Therefore, we hypothesized that the DPLP transmembrane segment may remodel the gating charge displacements that control Kv4 channel activation gating through interactions with the S4 voltage sensor. Such interactions may reshape the energetic landscape of the channel’s voltage sensor during gating.

To test the hypothesis and gain more direct insights into the mechanisms of DPLP action, it is necessary to measure the Kv4 gating current (Ig). Under voltage-clamp conditions and in the absence of ion conduction, the gating charge displacements in the Kv channel
protein generate the nonlinear ON and OFF capacitative transients of the $I_g$, which correspond to the apparent outward and inward movements of the VSDs, respectively (Bezanilla, 2000, 2005). To block ion conduction and thereby unveil the Kv4.2 $I_g$ for the first time, we engineered a charybdotoxin (CTX) binding site in the Kv4.2 PD (Kim et al., 2004). Then, to examine the remodeling of the $I_g$, the CTX-sensitive Kv4.2 channel was expressed heterologously in the absence and presence of DPPX-S, a DPLP expressed in the brain (Nadal et al., 2003). In a specific manner, DPPX-S induced dramatic changes in the voltage dependence and kinetics of the $I_g$, which result from promoting the movement of the Kv4.2 voltage sensor. These observations strongly suggest the presence of unique functional interactions between DPPX-S and the Kv4.2 VSD. To the best of our knowledge, no other Kv channel subfamily exhibits this type of functional remodeling by a specific auxiliary β subunit.

**MATERIALS AND METHODS**

**Molecular Biology and Reagents**

Kv4.2 cDNA (a gift from M. Sheng, Massachusetts Institute of Technology, Cambridge, MA) is maintained in pRC-cytomegalovirus (CMV; Invitrogen), whereas the DPPX-S cDNA (a gift from B. Rudy, New York University School of Medicine, New York, NY) is maintained in pSG5 (Stratagene). Shaker β Kv channels bearing the mutations W434F and T449V (a gift from R. Horn, Thomas Jefferson University, Philadelphia, PA) is maintained in the GWI-CMV vector (British Biotechnology). The Kv4.2CTX mutant was created with a site-directed mutagenesis kit (QuickChange; Stratagene). Shaker β Kv channels bearing the mutations W434F and T449V (a gift from R. Horn, Thomas Jefferson University, Philadelphia, PA) is maintained in the GWI-CMV vector (British Biotechnology). The Kv4.2CTX mutant was created with a site-directed mutagenesis kit (QuickChange; Stratagene) and confirmed by automated sequencing at the Nucleic Acid Facility of the Kimmel Cancer Center (Thomas Jefferson University, Philadelphia, PA). The transfection of tsA201 cells (a gift from R. Horn) was accomplished by the calcium-phosphate method (O’Leary and Horn, 1994), and a plasmid containing the CD8 gene was cotransfected to allow the identification of individual transfected cells by labeling them with beads bearing anti-CD8 antibody (Dynal). Recombinant CTX (98% pure) was purchased from Sigma-Aldrich.

**Electrophysiology**

Ionic currents were measured in the tight-seal whole-cell configuration of the patch-clamp method with the following pipette (intracellular) solution: 120 mM KF, 1 mM CaCl₂, 2 mM MgCl₂, 11 mM EGTA, and 10 mM HEPES, pH 7.2, adjusted with KOH; and the following external bath solution: 150 mM NaCl, 2 mM KCl, 1.5 mM CaCl₂, 1 mM MgCl₂, and 10 mM HEPES, pH 7.4, adjusted with NaOH. Series resistances (2–5 MΩ) were compensated to yield a total voltage error of ≤3 mV. Peak chord conductance ($G_p$) and its voltage dependence were calculated and analyzed as reported previously (Beck et al., 2002). $I_g$ was measured with the following pipette (intracellular) solution: 105 mM CsF, 35 mM NaCl, 10 mM EGTA, and 10 mM HEPES, pH 7.4, adjusted with CsOH; and the following external solution: 135 mM NMG-Cl, 10 mM HEPES, 1 mM MgCl₂, and 1 mM CaCl₂, pH 7.4, adjusted with HCl. The $I_g$ from Shaker Kv channels (W434F) was recorded with the Cs-containing pipette solution and the Na-containing bath solution described. A P/4 leak subtraction protocol consisting of four subpulses from a subsweep holding potential of −153 mV was used to subtract passive components of the total current and isolate the $I_g$. All $I_g$ measurements were filtered at 5 kHz and sampled at 25 kHz. Analyses and graphical displays were produced with pClamp (version 9.0; Axon Instruments, Inc.) and Origin (version 7.0; OriginLab) software. The calculated liquid junction potential was subtracted off-line. All measurements were taken at room temperature (22–25°C).

To examine the effect of CTX on Kv4.2 wild type and Kv4.2CTX, the corresponding mRNAs were injected into *Xenopus* oocytes alone or together with mRNA encoding DPPX-S. Using the two-electrode voltage-clamp technique reported previously (Beck et al., 2002), the Kv4.2 whole-oocyte currents were elicited by 400-ms voltage steps from −100 mV to +50 mV. A standard frog Ringer’s saline in the bath was supplemented with 100 μg/ml BSA to avoid nonspecific binding of CTX to the recording chamber. CTX solutions were perfused manually by means of a disposable syringe, and immediately after each experiment, the recording chamber was bathed for at least 10 min in a concentrated 0.5 M NaCl solution to remove all residual CTX (Anderson et al., 1988).

**Data Analysis and Model Simulations**

$Q_{on}$ and $Q_{off}$ values were obtained by integrating the area under the current trace for the length of the entire depolarization. Normalized $Q_{V}$ relationships were described by assuming a Boltzmann function:

$$Q(V) = 1/(1 + \exp((V - V_{1/2})/k)),$$

and the $G_p$-$V$ relationships were described by assuming a Boltzmann function raised to the fourth power; $k$ represents the slope factor, and $V_{1/2}$ represents the midpoint voltage of the relationships. $I_g$ relaxations were described by assuming an exponential function or the sum of exponential terms:

$$I_g(t) = \sum_i A_i \exp(-t/\tau_i),$$

where $\tau_i$ represents the time constant, and $A_i$ represents the corresponding amplitude. The voltage-dependence of the time constants was determined according to the following equation:

$$\tau(V) = \tau_0 \exp(-z e_0 V/k_B T),$$

where $\tau_0$ represents the time constant at 0 mV; $z$ represents the apparent charge, $e_0$ represents the electronic charge, and $k_B T$ has its usual thermodynamic meaning. All data are expressed as mean ± SE, and the one-way ANOVA test was used to evaluate differences. Model simulations were conducted in the IChSim simulator developed by J. De Santiago-Castillo (www.ichsim.ionchannels.org). Rate constants were assumed to depend exponentially on membrane potential, and for comparative purposes, all simulated data were analyzed as described for the experimental observations.

**Online Supplemental Material**

Fig. S1 reports the results of an experiment that investigated the possible impact of DPPX-S on Shaker B gating charge dynamics. Online supplemental material is available at http://www.jgp.org/cgi/content/full/jgp.2006009668/DC1.

**RESULTS**

DPPX-S Remodels Gating of the CTX-sensitive Kv4.2 Channel

Generally, the $I_g$ is several orders of magnitude smaller than the corresponding ionic current. Thus, to examine
the effects of DPPX-S on Kv4.2 gating charge dynamics, the ionic current must be eliminated. Kv4 channels are typically insensitive to external blockers of most Kv channels (i.e., TEA and pore toxins). Therefore, according to a previous study (Kim et al., 2004), we engineered a Kv4.2 mutant with three pore substitutions (K353G, A359D, and K379V) that are sufficient to confer high sensitivity to CTX, a potent K\(^+\) channel pore toxin (Fig. 1, A–C). The voltage-dependent and kinetic properties of the ionic currents mediated by the Kv4.2 wild type and the Kv4.2 triple mutant (Kv4.2 CTX) were generally similar, as shown previously (Fig. 1, D–F; and Table I; Kim et al., 2004). An exception was the peak conductance-voltage relationship (Gp-V relationship) of the Kv4.2 CTX channel, which appeared moderately shifted to the left (Table I). This shift was not investigated further. More importantly, however, DPPX-S remodeled the Kv4.2 wild-type and Kv4.2 CTX ionic currents similarly and as reported by others (Nadal et al., 2003; Jerng et al., 2004b, 2005; Zagha et al., 2005). The hallmark changes include faster macroscopic inactivation at depolarized membrane potentials, a leftward-shifted Gp-V relationship, and faster recovery from inactivation at hyperpolarized membrane potentials (Fig. 1, D–F; and Table I). These results justified the use of the Kv4.2 CTX channel to investigate the remodeling of gating charge dynamics by DPPX-S.

**Figure 1.** CTX sensitivity of Kv4.2 wild-type and Kv4.2 CTX channels expressed in *Xenopus* oocytes. (A and B) Outward whole-oocyte K\(^+\) currents mediated by Kv4.2 CTX and Kv4.2 CTX:DPPX-S channels before (control saline in the bath) and after exposure to 1 nM CTX in control saline (see Materials and methods). Currents were elicited by 400-ms step depolarizations from \(-100\) mV to \(+50\) mV. (C) Dose-response relationships for Kv4.2 WT, Kv4.2 CTX, and Kv4.2 CTX:DPPX-S channels. Both Kv4.2 CTX and Kv4.2 CTX:DPPX-S channels are sensitive to CTX in the subnanomolar or nanomolar range. The solid line superimposed on the symbols is the best-fit Hill equation of this form: \(y = (A_2 + (A_1 - A_2))/(1 + (x/K_0)^h)\). \(A_1\) and \(A_2\) represent the maximum and minimum, \(K_0\) represents the apparent dissociation constant, and \(h\) represents the Hill coefficient. The best-fit parameters were as follows: Kv4.2 CTX, \(K_0 = 0.83 \text{ nM}\) and \(h = 1.04;\) and Kv4.2 CTX:DPPX-S, \(K_0 = 1.48 \text{ nM}\) and \(h = 1.27\) (\(n \geq 3\) experiments). DPPX-S does not significantly alter the CTX sensitivity of Kv4.2 CTX channels (\(P > 0.05\) by one-way ANOVA). Data are expressed as mean \(\pm\) SE. (D) Whole-cell (tsA201; see Materials and methods) Kv4.2 CTX currents evoked by a 400-ms step depolarization to \(+32\) mV from a holding potential of \(-108\) mV. Note that the macroscopic inactivation of Kv4.2 CTX channels is accelerated by DPPX-S. The solid lines are best-fit fourth-order Boltzmann functions with the following parameters: \(V_{1/2}\) (Kv4.2 CTX) = \(-13.2\) mV, \(k\) (Kv4.2 CTX) = \(23.2\) mV; and \(V_{1/2}\) (Kv4.2 CTX:DPPX-S) = \(-43.6\) mV, \(k\) (Kv4.2 CTX:DPPX) = \(23.6\) mV. The dashed line indicates the zero-conductance level. (F) Recovery from inactivation at \(-110\) mV. The solid lines are the best-fit exponential functions with the following parameters: \(\tau\) (Kv4.2 CTX) = \(186\) ms; and \(\tau\) (Kv4.2 CTX:DPPX-S) = \(116\) ms. The changes induced by DPPX-S are similar to those reported by others for Kv4.2 wild type (Nadal et al., 2003; Jerng et al., 2004b). Data in E and F are expressed as mean \(\pm\) SE.

**Figure 2.** \(I_\text{f}\) from Kv4.2 CTX and Kv4.2 CTX:DPPX-S channels. (A) \(I_\text{f}\) from Kv4.2 CTX channels expressed in tsA201 cells in the presence of 100 nM CTX and 105 mM intracellular CsF. From a holding potential of \(-153\) mV, currents were elicited by a series of 12-ms voltage steps between \(-143\) and \(+57\) mV in 20-mV increments. (B) \(I_\text{f}\) from Kv4.2 CTX:DPPX-S channels recorded under conditions identical to those used in A. Note the acceleration of the \(I_\text{f}\)-ON and \(I_\text{f}\)-OFF kinetics in the presence of DPPX-S.
Kv4.2<sub>WT</sub>:DPPX

Kv4.2<sub>WT</sub>

of Kv4.2 CTX and DPPX-S resulted in faster I<sub>g-ON</sub> and

Shaker B W434F

titude of this shift (quantitatively, the relaxation of the I<sub>g-ON</sub> was approxi-

mated as an exponential decay (Fig. 5 A). In the absence and presence of DPPX-S, this analysis provided an overall evaluation of the forward steps in the voltage-dependent activation process. In both conditions, the best-fit exponentials yielded voltage-dependent time constants that
decrease with membrane depolarization. Clearly, however, DPPX-S reduced these time constants uniformly at membrane potentials greater than or equal to −50 mV (Fig. 5 B). The time constant–voltage relationships were
described by assuming exponential voltage dependencies in the absence and presence of DPPX-S (Fig. 5 B). At 0 mV, the time constant (τ<sub>α</sub>) is fivefold faster in the presence of DPPX-S than in its absence, but the effective valence (α) was not affected by DPPX-S (∼1 66). The I<sub>g-OFF</sub> relaxations at −153 mV were better described by

assuming the sum of two exponential terms (Fig. 5, C and D). The fractional amplitudes of the exponential terms are not affected much by DPPX-S (Fig. 5 D). In contrast, DPPX-S decreases both the fast and slow I<sub>g-OFF</sub> time constants by 3- and 3.7-fold, respectively (Fig. 5 D). Overall, the analysis of the Kv4.2<sub>CTX</sub> I<sub>g</sub> demonstrates a general acceleration of gating charge dynamics by DPPX-S.

DPPX-S Promotes Voltage-dependent Activation of Kv4.2 Channels

The effects of DPPX-S on Kv4.2 gating charge dynamics could result from selective changes in the voltage-dependent transitions of the activation pathway. To test this hypothesis qualitatively, we simulated the I<sub>g</sub> by

assuming a general form of the Zagotta-Hoshi-Aldrich (ZHA) kinetic model without the final G<sub>f</sub> state (Zagotta et al., 1994). Currently, there is no specific kinetic model that explains all major features of Kv4.2 channel gating unambiguously. In the ZHA model, each subunit of the Kv channel tetramer acts independently and undergoes two sequential voltage-dependent transitions that correspond to the major movements of the voltage sensor. Assuming that DPPX-S induces a fivefold increase in the forward rates constants of both transitions (α × 5 and γ × 5) was sufficient to simulate the main

| Kv4.2<sub>CTX</sub>:DPPX | V<sub>1/2</sub> (mV) | k (mV) | V<sub>1/2</sub> (mV) | Effective charge ε<sub>0</sub> | Slope |
|-------------------|--------------|-------|-----------------|-----------------|------|
| Shaker B W434F    | -46.3 ± 2.3<sup>a</sup> (4) | 2.77 ± 0.2 (4) | -52.2 ± 0.4<sup>c</sup> (4) | 3.1 ± 0.2 (4) |
| Shaker B W434F + DPPX-S | -46.3 ± 2.3<sup>a</sup> (4) | 2.77 ± 0.2 (4) | -52.2 ± 0.4<sup>c</sup> (4) | 3.1 ± 0.2 (4) |

<sup>a</sup> The leftward shift induced by DPPX-S (ΔV<sub>1/2</sub> = −29 mV) is highly significant at P < 0.01.

<sup>b</sup> The leftward shift induced by DPPX-S (ΔV<sub>1/2</sub> = −28 mV) is highly significant at P < 0.01.

<sup>c</sup> The Gp-V midpoint voltages (at Gp/Gmax = 0.5) of the Kv4.2<sub>CTX</sub> mutant in the absence and presence of DPPX-S were considerably different from those of the Kv4.2<sub>WT</sub>. This difference was not examined further.

<sup>d</sup> The leftward shift induced by DPPX-S (ΔV<sub>1/2</sub> = −26 mV) is highly significant at P < 0.0001.

<sup>e</sup> The leftward shift induced by DPPX-S (ΔV<sub>1/2</sub> = −6 mV) appeared modestly significant at P = 0.045. Differences between other parameters (k and effective charge Z) were not statistically significant for all pairs in the absence and presence of DPPX-S. The number of independent determinations for all tabulated measurements is indicated in parentheses.
observed changes closely (Fig. 6), which are (a) dramatically accelerated I_g-ON; (b) uniformly reduced time constants of the I_g-ON relaxation over the examined range of membrane potentials (approximately fivefold smaller at 0 mV); (c) a leftward-shifted Q_ON-V relationship; and (d) a leftward-shifted G-V relationship. As observed, both relationships were shifted by $-25$ mV. Although there is a good general agreement between the simulated and observed data, a limitation of the simulations is that the ZHA model does not include coupled inactivation transitions. As a result, the simulated steady-state activation curves (Fig. 6 D) differed from the observed data (Fig. 1 E), because the Gp-V relationship is influenced by inactivation and late opening transitions (Ayer and Sigworth, 1997; Yifrach and MacKinnon, 2002), which are not fully characterized in Kv4 channels.

The $I_g$ from the Shaker B Kv Channel Is Not Affected by DPPX-S

DPPX-S is a membrane protein with a single transmembrane segment that could interact promiscuously with the VSD in the transmembrane core of other Kv channels and thereby alter their biophysical properties. To test this possibility, we investigated the effect of DPPX-S on the $I_g$ of the Shaker B channel. The $I_g$ from nonconducting Shaker B channels (W434F mutant) and Shaker B channels exposed to CTX or in the absence of permeant ions exhibits similar properties (Perozo et al., 1993; Schoppa and Sigworth, 1998). Therefore, we expressed the Shaker B W434F mutant alone or coexpressed it with DPPX-S in tsA201 cells and studied the $I_g$. This $I_g$ is typically $>1$ nA at positive membrane potentials and exhibits the characteristic biophysical properties that fully agree with previous studies (Fig. S1, available at http://www.jgp.org/cgi/content/full/jgp.200609668/DC1). In sharp contrast with the results obtained with the Kv4.2CTX channel, however, DPPX-S had no significant effects on the kinetics of the $I_g$ or the voltage dependence of the gating charge (Fig. S1 and Table I). At the

![Figure 3](http://www.jgp.org/cgi/content/full/jgp.200609668/DC1)

![Figure 4](http://www.jgp.org/cgi/content/full/jgp.200609668/DC1)
protein level, this result is consistent with the absence of a physical interaction between Kv1.4, a Shaker-type channel, and DPPY (Ren et al., 2005), but it does not support another recent study, which reported effects of DPPY on Kv1.4 ionic current kinetics (Li et al., 2006). Thus, the dramatic remodeling of Kv4.2CTX gating charge dynamics by DPPX-S is relatively specific.

**DISCUSSION**

In vivo, most Kv channels exist as part of macromolecular complexes that include a variety of specific β subunits (Hanlon and Wallace, 2002). Although there is considerable information on the mechanisms responsible for the biophysical effects of β subunits that interact with intracellular channel domains (T1 or the C-terminal region; Jerng et al., 2004a; Callsen et al., 2005; Heinemann and Hoshi, 2006), much less is known about those that may interact with the transmembrane domains of the channel’s core (PD and VSD; Hanlon and Wallace, 2002; Jerng et al., 2004a; Cai et al., 2006). The Kv channel VSD has been the subject of intense research and controversy, and major breakthroughs have been made toward solving the mechanisms of voltage-dependent activation (Ahern and Horn, 2004, 2005; Chanda et al., 2005; Long et al., 2005b; Posson et al., 2005; Tombola et al., 2006). Nevertheless, the possible remodeling of the Kv channel VSD by specific membrane-spanning β subunits that affect voltage-dependent gating remained largely unexplored. Therefore, we investigated the interactions between DPPX-S and Kv4.2 channels by examining gating charge dynamics. The main results led us to suggest that DPPX-S promotes the outward movement of the Kv4.2 gating charge in a specific manner.

The Impact of DPPX-S on Kv4.2 Gating Charge Dynamics: Mechanistic Implications

A recent study suggested that residues in the S1 and S2 transmembrane segments of Kv4.3 channels are critical determinants of DPLP action (Ren et al., 2005). In addition, this and other studies have suggested that the functional remodeling of Kv4 channels by DPLPs depends on transmembrane interactions involving the single membrane-spanning segment of DPLPs (Ren et al., 2005; Zagha et al., 2005). Thus, given the results of this study, it is tantalizing to propose that the transmembrane segment of DPPX-S is influencing the relative stability of various conformations of the VSD through changes in the membrane environment and packing of the channel’s core. DPPX-S could thereby facilitate the shift of the VSD conformations from reluctant to permissive.
Kinetic modeling (Fig. 6) showed that acceleration of the outward gating charge movement induced by DPPX-S may result from destabilization of the resting and intermediate states of the Kv4.2 subunits, which promotes the permissive conformation of the VSD. These results are consistent with reduced energy barriers of the voltage-dependent transitions in the presence of DPPX-S; therefore, this auxiliary subunit may exert a catalytic effect on voltage-dependent gating of Kv4.2 channels.

By promoting voltage-dependent activation, DPPX-S may also accelerate coupled inactivation indirectly, which could account for the observed acceleration of the development of inactivation (Fig. 1). The extent of additional direct effects of DPPX-S on Kv4 inactivation gating is not yet clear. The acceleration of the \( I_{A}^{OFF} \) by DPPX-S (Fig. 5, C and D) may involve interactions between inactivation and gating charge movement. A concrete possibility is that recovery from inactivation limits the observed rate of return or remobilization of the gating charge (Roux et al., 1998). Thus, given that DPPX-S accelerates the recovery from inactivation (Fig. 1 E), the return of the gating charge is consequently faster.

A physical interaction between DPPX-S and the Kv4.2 VSD is plausible in the light of the recently published crystal structure of a mammalian Kv channel (Long et al., 2005a). Four VSDs surround the pore structure in the center of the tetramer and are substantially exposed to possible interactions with neighboring membrane proteins. Those interactions may involve specific contacts between the transmembrane segment of DPPX-S, the VSD, and the PD. Further biochemical and structural studies are necessary to understand the stoichiometry and architecture of such a Kv channel complex, the identity of the unique interactions, and the physical basis of the functional remodeling. The presence of the DPPX-S transmembrane segments as part of the Kv4 channel complex may reconfigure the architecture of the VSD in an important way.

Neurophysiological Implications of Remodeling Kv4.2 Gating Charge Dynamics by DPPX-S

Various studies suggest strongly that DPLPs are integral parts of the native Kv4 channel complex that underlies the somatodendritic A-type \( K^{+} \) current (\( I_{SA} \)) in the...
mammalian brain (Nadal et al., 2003; Jerng et al., 2004a, 2005). Critical functions of IS_A include the regulation of repetitive firing frequency, dampening of backpropagating action potentials, and compartmentalization of dendritic excitability (Jerng et al., 2004a). These functional roles depend on the ability of IS_A to operate in the subthreshold range of membrane potentials. By remodeling gating charge dynamics, DPPX-S could be the key determining factor of IS_A operation in the subthreshold range of membrane potentials. KChIPs are also integral components of native Kv4 channels (Jerng et al., 2004a); however, these cytosolic proteins interact with the N-terminal region and the intracellular T1 domain directly (Zhou et al., 2004; Callsen et al., 2005) and are not likely to interact with the VSD or the PD. The extent to which KChIPs may remodel gating charge dynamics through indirect allosteric interactions has not been investigated.

Conclusion

This study reports the profound tune-up of the Kv4.2 VSD by a specific β subunit (DPPX-S). Based on these observations and current knowledge, this study strongly supports the concept of specific interactions between the single membrane-spanning segment of DPPX-S and the channel’s VSD in the transmembrane core. The typical operating membrane potential range of the neuronal Kv4 channel may depend on this exceptional interaction.

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