Figures and figure supplements

Voltage-dependent dynamics of the BK channel cytosolic gating ring are coupled to the membrane-embedded voltage sensor

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Figure 1. Voltage dependence of gating ring rearrangements is associated to activation of the RCK1 binding site. G-V (left panels) and E-V curves (right panels) obtained simultaneously at several Ca\textsuperscript{2+} concentrations from (a) the
Figure 1 continued

BK667CY construct, (b) mutation of the RCK1 high-affinity site (D362A/D367A), (c) mutation of the Ca\(^{2+}\) bowl (SDSA), or (d) both (D362A/D367A 5D5A). Note that the voltage dependence of the E signal is only abolished after mutating the RCK1 high-affinity binding site (b) or both (d). Data corresponding to each Ca\(^{2+}\) concentration are color-coded as indicated in the legend at the bottom. Solid curves in the G-V graphs represent Boltzmann fits. For reference, grey shadows in (a–d) left panels represent the full range of G-V curves corresponding to non-mutated BK667CY channels from 0 \(\mu\)M Ca\(^{2+}\) to 95 \(\mu\)M Ca\(^{2+}\) (indicated with colored dashed lines). Data points and error bars represent average ± SEM (n = 3–14, N = 2–8). Part of the data in (a, b and c) are taken from (Miranda et al., 2013) and (Miranda et al., 2016).

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Figure 2. Modification of the voltage dependence of gate opening does not affect the gating ring voltage-dependent conformational changes. (a) Topology of the BKα subunit where the voltage sensing domain (VSD), Ca\(^{2+}\) sensing domain (gating ring, GR) and pore domain (PD) are indicated by colored dashed lines boxes (see main text for a full description). (b) The three BK functional modules (VSD, PD, GR), schematically represented as colored boxes, interact allosterically. (c) Diagram representing the main effect of the F315A mutation, which is the uncoupling of the VSD to the PD. (d) G-V (left panel) and E-V curves (right panel) obtained simultaneously at several Ca\(^{2+}\) concentrations after mutation of the F315 site to alanine (BK667CY\(^{F315A}\)). It should be noted that the extent of the shifts induced by the mutation are smaller than previously reported (Carrasquel-Ursulaez et al., 2015), which could arise from the different experimental conditions and/or our fluorescent construct. (e) The interaction with the γ1 subunit favors the VSD-PD coupling mechanism (f) G-V (left) and E-V curves (right) of BK667CY α subunits co-expressed with γ1 subunits. In all panels, data corresponding to each Ca\(^{2+}\) concentration are color-coded as indicated in the bottom legend. Colored dashed lines represent the G-V and E-V curves corresponding to BK667CYα channels (Miranda et al., 2013; Miranda et al., 2016). The solid curves in the G-V graphs represent Boltzmann fits. The full range of G-V curves from 0 μM Ca\(^{2+}\) to 95 μM Ca\(^{2+}\) from BK667CY is represented as a grey shadow in left panels (d and f), for reference. Data points and error bars represent average ± SEM (n = 3–8; N = 2–3).

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Figure 3. Co-expression with β subunits. (a) β1 subunits have been shown to directly regulate VSD function, shifting $V_{\text{th}}$ to more negative values (b). Left panel, G-V curves obtained at several Ca$^{2+}$ concentrations after co-expression of BK667CY with the β1 subunit, which induces a leftward shift in the E-V curves obtained simultaneously (right). (c) β3bNβ1 chimeras produce similar effects to β1 on VSD function, since they retain the N-terminal region of β1 (Castillo et al., 2015). (d) G-V (left) and E-V curves (right) of BK667CY α subunits co-expressed with the β3bNβ1 chimera. Data corresponding to each Ca$^{2+}$ concentration are color-coded as indicated in the legend at the bottom. Colored dashed lines represent the G-V and E-V curves corresponding to BK667CYα channels (Miranda et al., 2013, Miranda et al., 2016). The solid curves in the G-V graphs represent Boltzmann fits. The full range of G-V curves from 0 μM Ca$^{2+}$ to 95 μM Ca$^{2+}$ from BK667CY is represented as a grey shadow in left panels (b and d), for reference. Data points and error bars represent average ± SEM (n = 3–10; N = 2–4).

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Co-expression with β3b subunits. (a) Representative currents obtained after applying depolarizing pulses to inside-out patches expressing BK667CYα (left) or BK667CYα + β3b channels, in the presence of 12 μM Ca²⁺. (b) Left panel, G-V curves obtained at several Ca²⁺ concentrations after co-expression of BK667CY with β3b subunits, inducing no appreciable changes in the E-V curves obtained simultaneously (right). Note that when β3b is co-expressed with the BK667CY construct, the kinetics of inactivation are different than those observed with wild-type BK channels (Xia et al., 2000). At first glance, it appears as if the off rate of inactivation is largely increased. Note also that there is a substantial current reduction at the tails as blockade increased with very positive potentials, which is not observed with wild-type BK channels. The simplest interpretation is that the insertion of the fluorescent protein interferes with the kinetics of blockade mediated by the β3b NH2-terminal region (Lingle et al., 2001). Understanding this discrepancy will require further study.

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Figure 4. Mutation of charged residues of BK VSD. VSD activation was altered by mutation of charged residues in the VSD that modify its voltage of half activation, $V_{1/2}$.

(a) The R210E mutation induces a negative shift of $V_{1/2}$.

(b) G-V (left panel) and E-V curves (right panel) obtained simultaneously from constructs BK667CY containing the R210E mutation at several Ca$^{2+}$ concentrations.

(c) The E219R mutation produces a negative shift of $V_{1/2}$.

(d) G-V (left panel) and E-V curves (right panel) obtained simultaneously from constructs BK667CY containing the E219R mutation at several Ca$^{2+}$ concentrations.

(e) The R213E mutation induces a large positive shift of $V_{1/2}$ values.

(f) G-V (left panel) and E-V curves (right panel) obtained simultaneously from constructs BK667CY containing the R213E mutation at several Ca$^{2+}$ concentrations.

Data corresponding to each Ca$^{2+}$ concentration are color-coded as indicated in the bottom legend. Colored dashed lines represent the G-V and E-V curves corresponding to non-mutated BK667CYa channels (Miranda et al., 2013; Miranda et al., 2016). The solid curves in the G-V graphs represent Boltzmann fits. The full range of G-V curves from 0 μM Ca$^{2+}$ to 95 μM Ca$^{2+}$ from BK667CY is represented as a grey shadow in left panels (b), (d) and (f), for reference. Data points and error bars represent average ± SEM (n = 4–10; N = 3–4).

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Figure 5. Voltage dependence of gating ring rearrangements after specific activation of RCK1 high-affinity binding site by Cd²⁺. (a) Effect of the VSD E219R mutation on the selective activation of RCK1 by Cd²⁺. (b) G-V (left panels) and E-V curves (right panels) obtained simultaneously at several Ca²⁺ concentrations from constructs BK667CYE219R. (c) VSD R201Q mutation induces a positive shift of Vₚₐₐ. (d) G-V (left panels) and E-V curves (right panels) obtained simultaneously at several Cd²⁺ concentrations from constructs BK667CYR201Q. (e) Effect of the F315A mutation on the selective activation of RCK1 by Cd²⁺. (f) G-V (left panels) and E-V curves (right panels) obtained simultaneously at several Cd²⁺ concentrations from constructs BK667CYF315A. Data corresponding to each Cd²⁺ concentration are color-coded as indicated in the legend at the bottom. Colored dashed lines represent the G-V and E-V curves corresponding to BK667CYα channels (Miranda et al., 2013; Miranda et al., 2016). The solid curves in the G-V graphs represent Boltzmann fits. The full range of G-V curves from 0 µM Cd²⁺ to 100 µM Cd²⁺ corresponding to non-mutated BK667CY is represented as a grey shadow in left panels (b), (d), and (f), for reference. Data points and error bars represent average ± SEM (n = 3–4; N = 2).

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Figure 6. Voltage dependence of gating ring movements triggered by $\text{Ba}^{2+}$. (a) The RCK2 site is selectively activated by $\text{Ba}^{2+}$, which additionally induces pore block. (b) FRET efficiency ($E$) data obtained at several $\text{Ba}^{2+}$ concentrations from BK667CY constructs (Miranda et al., 2016). (c) Effect of the VSD R210E mutation after selective activation of the RCK2 binding site by $\text{Ba}^{2+}$. (d) $E$-$V$ curves obtained at several $\text{Ba}^{2+}$ concentrations from Figure 6 continued on next page.

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Figure 6 continued

BK667CY\textsuperscript{R210E} constructs. (e) Effect of the VSD R213E mutation after selective activation of the RCK2 binding site by Ba\textsuperscript{2+}. (f) E-V curves obtained at several Ba\textsuperscript{2+} concentrations from BK667CY\textsuperscript{R213E} constructs. (g) Effect of the F315A mutation after selective activation of the RCK2 binding site by Ba\textsuperscript{2+}. (h) E-V curves obtained at several Ba\textsuperscript{2+} concentrations from BK667CY\textsuperscript{F315A} constructs. Data corresponding to each Ba\textsuperscript{2+} concentration are color-coded according to the legend at the bottom. For reference, the curve corresponding to 100 µM Ba\textsuperscript{2+} from the BK667CY construct shown in (b) is also shown as a colored dashed line in panels (b, d, f and h). Data points and error bars represent average ± SEM (n = 4–6; N = 2–3). DOI: https://doi.org/10.7554/eLife.40664.008
Figure 6—figure supplement 1. Additional experiments to characterize voltage dependence of gating ring movements triggered by Ba\(^{2+}\). FRET efficiency (E) data obtained at several Ba\(^{2+}\) concentrations from: (a) BK667CY constructs including D362A/D367A mutations to knockout the RCK1 domain.
Figure 6—figure supplement 1 continued

binding site (BK667CYD362A D367A). (b) BK667CYD362A D367A with additional VSD R210E mutation (BK667CYD362A D367A R210E). (c) BK667CYD362A D367A with additional VSD E219R mutation (BK667CYD362A D367A E219R). For reference, the curve corresponding to 100 μM Ba²⁺ from the BK667CY construct shown in Figure 6a is also shown as a grey line in panels (a, b, and c). Ba²⁺ concentrations are indicated in legend of panel a. (d) E-V curves obtained simultaneously at several Ca²⁺ concentrations (indicated in the legend) from constructs BK667CYD362A D367A after addition of 100 μM bbTBA. Data points and error bars in all panels represent average ± SEM (n = 3). Data in panel (a) were partly obtained from (Miranda et al., 2016). DOI: https://doi.org/10.7554/eLife.40664.009