Disruption of the RIM1 effects on VDCC inactivation by the β₄-GK domain and BADN. (a) Structure of β₄-GK in comparison with the WT β₄b. β₄-GK corresponds to 183-407 of rat β₄b. (b) Pulldown assay of β₄-GK constructs with RIM1 1079-1463. GST fusion protein demonstrates that β₄-GK does not interact with RIM1 1079-1463. cDNA of β₄-GK is subcloned in the expression plasmid pCMV-tag3. GST fusion proteins immobilized on glutathione-Sepharose beads are incubated with cell lysate obtained from myc-β₄-GK-expressing HEK293 cells. Bound proteins are analyzed by WB using anti-myc antibody. (c) Inactivation of Ca₂,2,2 currents in BHK cells expressing α₂δ and β₄-GK. For comparison of inactivation time courses before and after expression of RIM1 constructs, the peak amplitudes are normalized for Ba²⁺ currents elicited by 2-s pulses to 10 mV from a Vₖ of -100 mV. 10 mM Ba²⁺ is used as a charge carrier. (d) Inactivation curves of Ca₂,2,2 currents in BHK cells expressing α₂δ and β₄-GK. (e) Dominant-negative effect of BADN for RIM1 effects on inactivation properties of VDCCs. Inactivation kinetics of Ca₂,2,1 currents in BHK cells expressing α₂δ and β₁a. (f) Inactivation curves of Ca₂,2,1 in BHK cells expressing α₂δ and β₁a. The differences at > −80 mV between vector and RIM1, or those between RIM1 plus BADN and RIM1 are significant (P < 0.05).