Supporting Information

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SI Materials and Methods

Characterization of Functional Coupling Between Channel-Forming Cbv1 and WT or Mutated Rβ1-Subunits. The presence of voltage- and calcium-gated potassium channels (BK) β1 subunits functionally coupled to BK channel-forming (cbv1) α-subunits was confirmed by the introduction of macroscopic current slow activation kinetics compared with the kinetics of currents mediated by homomeric BK α-channels (1, 2), which was previously described for cbv1 subunits (3). After excision of inside-out macropatches from the oocytes, a depolarizing 200-ms-long voltage step from 0 to +80 mV was applied. Activation data were fitted with a single exponential function using a Chebyshev approximation to obtain the activation time constant (τ_{act}). This procedure was performed using a built-in option in Clampfit 9.2 (Molecular Devices).

Electrophysiological Recording and Following Analysis of cbv1 + Rβ1T165,L157,L158 Macroscopic Currents. Oocytes were prepared for patch-clamping as described (4), with the inside-out configuration being used to record macroscopic ionic current. Bath and electrode solutions contained 130 mM Kgluconate, 5 mM EGTA, 1.6 mM HEDTA, 2.28 mM $MgCl_2$ ($[Mg^{2+}]_{free}$ = 1 mM), 15 mM Hepes, 5.22 mM $CaCl_2$ ($[Ca^{2+}]_{free}$ = 10 μM), pH 7.35. Free Ca^{2+} and Mg^{2+} were calculated using Max Chelator (C. Patton, Stanford University, Palo Alto, CA) and validated experimentally with Ca^{2+}-sensitive/reference electrodes (Corning) (5).

Patch pipettes were pulled from glass capillaries (Drummond) (1). The procedure gave tip resistances of 5–7 MΩ when filled with electrode solution. An Ag/AgCl electrode was used as ground electrode. Experiments were carried out at room temperature (21 °C). BK currents were acquired using an EPC8 (HEKA) amplifier and digitized at 1 kHz using a Digidata 1320A A/D converter and pCLAMP8 software (Molecular Devices). Macroscopic currents were evoked from a holding potential of −80 mV by 200-ms-long, 10-mV depolarizing steps from −150 to +150 mV. Currents were low pass-filtered at 1 kHz with an eight-pole Bessel filter 902LPF (Frequency Devices) and sampled at 5 kHz. Current amplitude was averaged within 100–150 ms after the start of the depolarizing step.

Macroscopic conductance (G)–V plots were fitted to a Boltzmann function of the type $G(V) = G_{max}/1 + \exp[(-V + V_{1/2})/k]$. Boltzmann fitting routines were run using the Levenberg–Marquardt algorithm to perform nonlinear least squares fits.

Data Fitting and Plotting. Data fitting and plotting were performed using Clampfit 9.2 (Molecular Devices) and Origin 7.0 (Originlab).

1. Orio P, Rojas P, Ferreira G, Latorre R (2002) New disguises for an old channel: MaxiK channel beta-subunits. *News Physiol Sci* 17:156–161.
2. Brenner R, Jegla TJ, Wickenden A, Liu Y, Aldrich RW (2000) Cloning and functional characterization of novel large conductance calcium-activated potassium channel beta subunits, hKCNMB3 and hKCNMB4. *J Biol Chem* 275:6453–6461.
3. Bukiya AN, Vaithianathan T, Toro L, Dopico AM (2009) Channel beta2-4 subunits fail to substitute for beta1 in sensitizing BK channels to lithocholate. *Biochem Biophys Res Commun* 390:995–1000.
4. Dopico AM, Anantharam V, Treistman SN (1998) Ethanol increases the activity of $Ca^{(++)}$-dependent K^+ (mslo) channels: Functional interaction with cytosolic Ca^{++}. *J Pharmacol Exp Ther* 284:258–268.
5. Dopico AM (2003) Ethanol sensitivity of BK_{Ca} channels from arterial smooth muscle does not require the presence of the beta 1-subunit. *Am J Physiol Cell Physiol* 284: C1468–C1480.

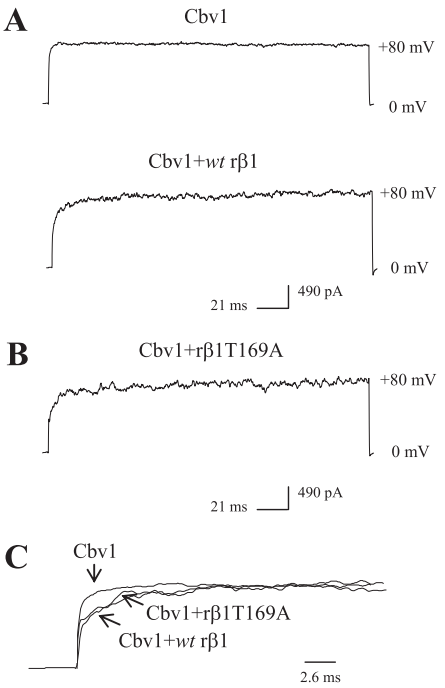

Fig. S1. Macroscopic currents mediated by association of BK channel-forming cbv1 subunits with either WT (*A Lower*) or mutant rβ1- (*B*) subunits exhibit significantly slower activation kinetics compared with the subunits of homomeric cbv1-mediated current (*A Upper*). (*C*) Expanded time scale traces from macroscopic current records shown in *A* and *B* underscore the different activation rate of cbv1 vs. cbv1 + WT rβ1 and cbv1 + rβ1T169A constructs. Ionic currents were evoked from inside-out macropatches as described in *SI Materials and Methods*. Current activation following a step from 0 to +80 mV was best fitted to a single exponential function to obtain the activation time constant (τ_{act}).

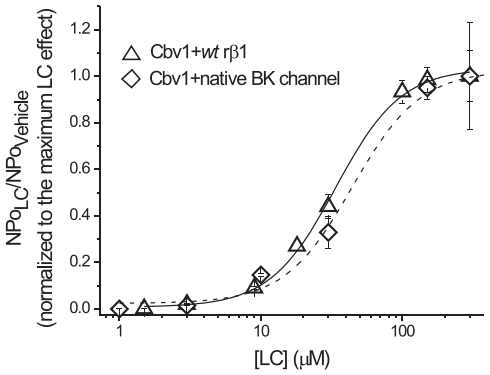

Fig. S2. Concentration-response curves (CRCs) for lithocholate (LC) -induced potentiation of channel activity show similar characteristics when evaluated with recombinant cbv1 + WT rβ1-channels expressed in *Xenopus* oocytes and native cerebrovascular BK channels: EC_{50} = 40.4 and 44.3 μM, respectively, E_{max} ~300 μM, and apparent Hill number (defined as the slope of logit-log plot of LC action on channel steady-state activity) is ~1.32 in both cases.

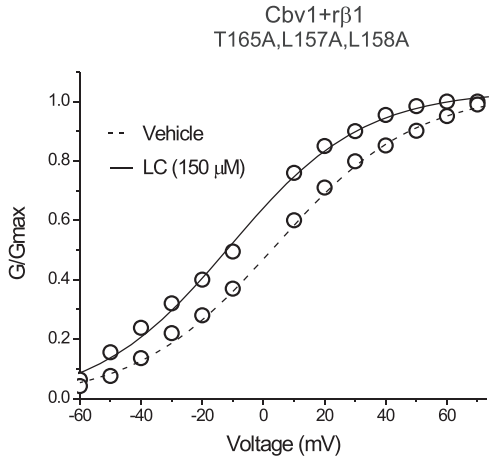

Fig. S3. G/G_{max}–V curves obtained from cbv1 + rβ1T165,L157,L158-mediated macroscopic currents show a parallel leftward shift in response to 150 μM LC. This fact indicates that, despite mutations in the proposed LC interaction site in the outer region of BK β1 TM2 domain, LC effect remains independent of membrane voltage, a finding that is characteristic of LC action on recombinant cbv1 + WT β1-constructs and native BK channels (1).

1. Bukiya AN, Liu J, Toro L, Dopico AM (2007) Beta1 (KCNMB1) subunits mediate lithocholate activation of large-conductance Ca2+-activated K+ channels and dilation in small, resistance-size arteries. *Mol Pharmacol* 72:359–369.

Table S1. Macroscopic current activation time constants (τ_{act}) from the different cbv1 + rβ1 constructs are significantly larger than those constants from homomeric cbv1 channels whether the heteromeric construct is LC-sensitive (cbv1 + WT rβ1) or -resistant (cbv1 + mutant rβ1s)

BK channel construct	τ_{act} (ms)
cbv1	1.05 ± 0.28
cbv1 + WT rβ1	3.57 ± 0.23*
cbv1 + rβ1T165A,T169A	4.01 ± 1.49†
cbv1 + rβ1T169A	6.50 ± 2.33*
cbv1 + rβ1T169S	3.15 ± 0.56†
cbv1 + rβ1L172A,L173A	7.48 ± 2.31*

These data indicate that both WT and mutated rβ1s are functionally coupled to cbv1 channels.

*$P < 0.01$; significantly different from cbv1 (one-way ANOVA followed by Bonferroni test).

†$P < 0.05$.