Bukiya et al., http://www.jgp.org/cgi/content/full/jgp.201010519/DC1

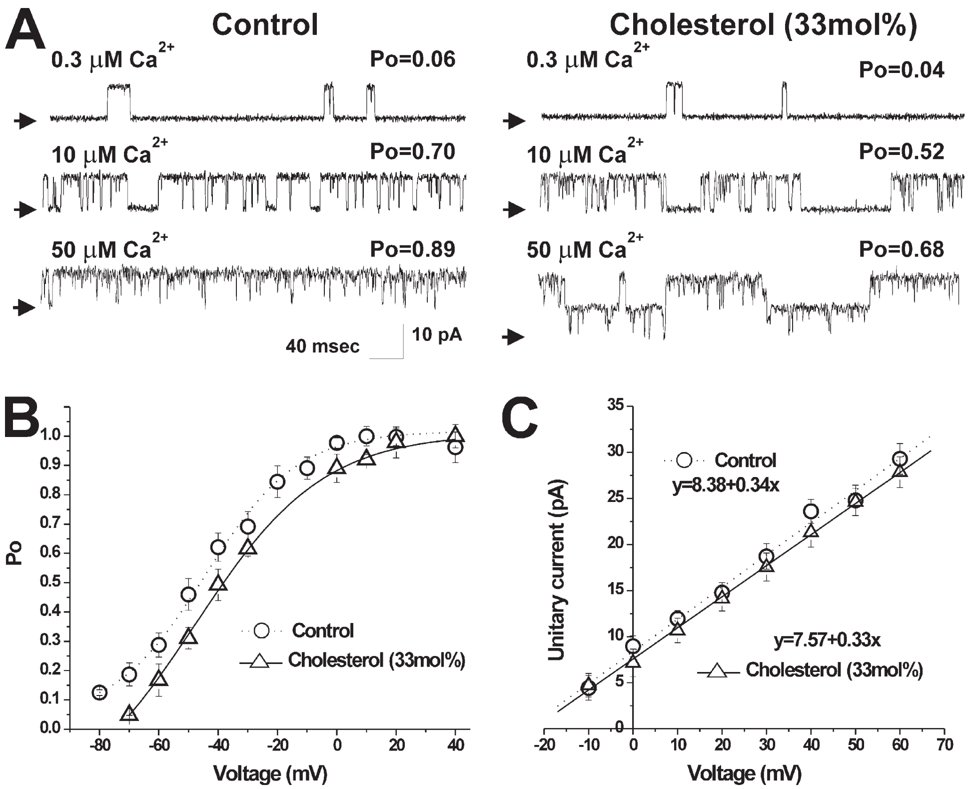

Figure S1. Basic properties of BK channels in POPE/POPS 3:1 (wt/wt) bilayers. (A) Original records of cbv1 channel activity obtained at 0.3, 10, and 50 µM Ca^{2+} at the cytosolic side of the control sterol-free POPE/POPS 3:1 (wt/wt) and 33 mol% cholesterol-containing lipid bilayer. Records show a progressive increase in Po as $[Ca^{2+}]_i$ is increased. Channel openings are shown as upward deflections; arrows indicate the baseline. The membrane potential for each recording was set to 0 mV. (B) A voltage (V)–Po plot obtained after the incorporation of cbv1 protein into control (*n* = 5) versus cholesterol-containing (*n* = 7) bilayers underscores that cholesterol presence does not alter the voltage dependence of cbv1 channel gating. (C) Cbv1 channel unitary current amplitude (i)–voltage (V) relationships from records obtained in 300/30 mM K^+ render unitary (slope) conductances of ≈340 and ≈330 pS for control (*n* = 5) and cholesterol-containing (*n* = 7) bilayers; these values are characteristic of BK channels. For B and C, data were obtained at $[Ca^{2+}]_i$ = 10 µM. Curve fitting was performed using Origin7 (OriginLab).

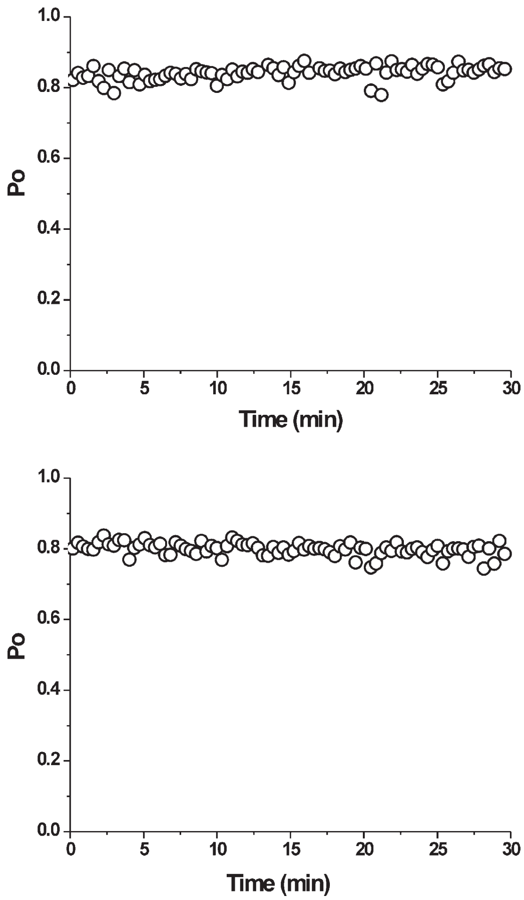

Figure S2. BK channel activity (Po) lacks significant variation over 30 min of gap-free recording. The two plots depict cbv1 channel behavior from two independent, sterol-free bilayer preparations. Each data point corresponds to the average cbv1 Po of 30-s segments, which were computed for a total of 30 min in gap-free mode from control bilayers. V_m = 0 mV and $[Ca^{2+}]_i$ = 10 µM.

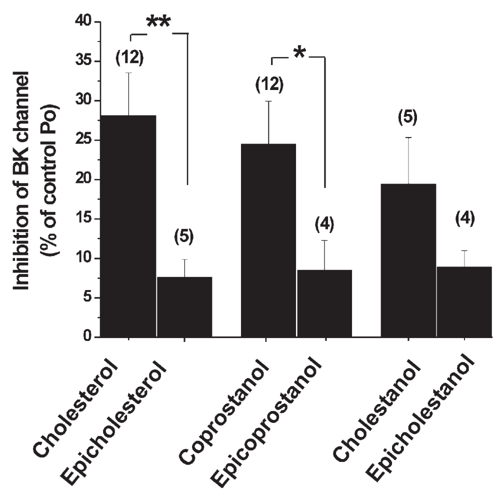

Figure S3. Direct comparisons of BK channel inhibition evoked by each pair of respective monohydroxysterol isomers. The three β monohydroxysterols (i.e., cholesterol, coprostanol, and cholestanol) inhibit the BK channel, whereas their α isomers (i.e., epicholesterol, epicoprostanol, and epicholestanol) fail to do so. *, significantly different from control ($P < 0.05$); **, significantly different from control ($P < 0.01$); *n*, number of bilayers.

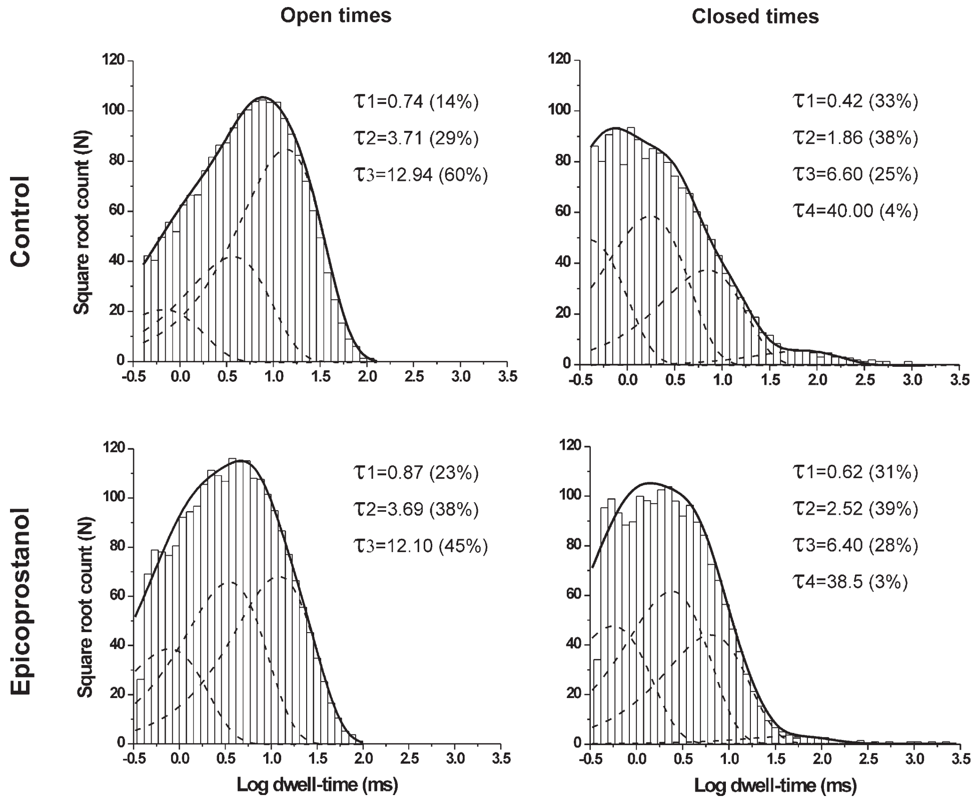

Figure S4. Noninhibitory steroid epicoprostanol slightly modifies open- and closed-time distributions of BK (cbv1) channels. Open- (left) and closed- (right) time distributions of cbv1 channels incorporated into control (sterol-free) and epicoprostanol-containing bilayers. Dwell-time distributions were obtained from 30 min of gap-free recording of cbv1 activity in each bilayer type. Each graph shows the channel time constants (τ; in milliseconds) obtained from the individual components of the fit, with the relative contribution of each component given in parentheses as a percentage of the total fit. Dashed lines indicate the individual components of the fit, and solid lines represent the composite fit. Po values are 0.7 and 0.66 for control and epicoprostanol-containing bilayers, respectively.

Table S1

Cbv1 channel time constants from POPE/POPS bilayers in the absence (control) and presence of inhibitory sterols

Times	Control *n* = 6	Cholesterol *n* = 7	Coprostanol *n* = 4	Cholestanol *n* = 5
Closed times				
τ1 (ms)	0.31 ± 0.06	0.37 ± 0.08	0.43 ± 0.08	0.45 ± 0.06
Proportion (%)	37.00 ± 1.33	36.00 ± 2.81	32.00 ± 2.36	37.40 ± 1.75
τ2 (ms)	1.37 ± 0.24	1.86 ± 0.28	2.34 ± 0.48	2.28 ± 0.18
Proportion (%)	31.66 ± 2.10	31.71 ± 4.12	31.50 ± 1.47	34.20 ± 1.80
τ3 (ms)	5.16 ± 0.82	7.56 ± 0.84	8.24 ± 1.15	8.19 ± 0.78
Proportion (%)	26.17 ± 2.12	24.00 ± 1.79	30.83 ± 3.13	23.00 ± 1.09
τ4 (ms)	30.98 ± 5.2	52.00 ± 9.17	43.09 ± 8.36	37.25 ± 1.65
Proportion (%)	5.17 ± 0.54	8.29 ± 2.24	5.67 ± 0.79	5.40 ± 0.75
Open times				
τ1 (ms)	0.72 ± 0.08	0.54 ± 0.09	0.64 ± 0.08	0.75 ± 0.13
Proportion (%)	16.20 ± 2.00	20.72 ± 2.60	20.60 ± 2.10	21.00 ± 3.10
τ2 (ms)	3.79 ± 0.34	2.75 ± 0.34	2.89 ± 0.64	3.74 ± 0.48
Proportion (%)	28.47 ± 0.60	32.86 ± 2.39	35.40 ± 4.27	32.00 ± 2.05
τ3 (ms)	13.03 ± 0.96	9.38 ± 0.63	11.34 ± 2.46	11.57 ± 0.81
Proportion (%)	55.33 ± 2.59	46.42 ± 3.77	44.00 ± 3.64	47.00 ± 3.49

Individual time constants (τ) were obtained from the individual exponential components after fitting open- and closed-time distributions with exponential probability functions. The contribution of each time constant to the total fit (i.e., Proportion) is expressed as a percentage.