Supporting Information

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SI Experimental Procedures

Electrophysiology. Expressed SK currents recorded in the excised outside-out patch configurations were evoked in symmetrical high (~160 mM) K^+ conditions, using an internal solution that contained 1 μM free Ca^{2+}. Due to low expression rates, whole-cell recordings were made of rSK1* channel subunits expressed in tsA201 cells. Pipettes were fabricated from KG-33 glass (Friedrich & Dimmock) and filled with an internal solution of composition: KAsp (97 mM) and KCl (20 mM) or KCl (117 mM), Hepes (10 mM), EGTA (10 mM), Na_2ATP (1.5 mM), $CaCl_2$ (9.65 mM; calculated free $[Ca^{2+}]_i$, 1 μM), and $MgCl_2$ (2.34 mM; calculated free $[Mg^{2+}]_i$, 1 mM), pH 7.4, with ~40 mM KOH. Cells were bathed in a control external solution that consisted of KAsp (97 mM) and KCl (20 mM) or KCl (127 mM), Hepes (10 mM), EGTA (10 mM), $CaCl_2$ (6.19 mM; calculated free $[Ca^{2+}]_i$, 60 nM), and $MgCl_2$ (1.44 mM; calculated free $[Mg^{2+}]_i$, 1 mM), pH 7.4, with ~40 mM KOH. For concentration response curves, solutions were rapidly exchanged using an RSC200 rapid switcher (Biologic). Expressed SK currents were evoked by a 1-s voltage ramp from −100 to +100 mV.

For concentration-inhibition relationships, data points representing current block were fit with a variable slope Hill equation in the form

$$I/I_{\text{cont}} = A_{\text{min}} + \left(\frac{A_{\text{max}} - A_{\text{min}}}{1 + 10^{(\text{Log IC}_{50} - X)\times n_{\text{h}}}}\right), \qquad \textbf{[S1]}$$

where I_{cont} is the amplitude of current at −60 mV in the absence of drug, I is the amplitude of current observed at a given concentration of blocker [(X), expressed in logarithmic units], A_{min} is $I_{\text{min}}/I_{\text{cont}}$, A_{max} is $I_{\text{max}}/I_{\text{cont}}$, IC_{50} is the concentration of blocker that blocks 50% of the sensitive current, and n_{h} is the Hill coefficient.

Where data were best fitted by the sum of two Hill equations, the equation

$$I/I_{\text{cont}} = A_{\text{min}} + \left(\frac{A_{\text{frac}} - A_{\text{min}}}{1 + 10^{(\text{Log IC}_{50,\text{a}} - X)\times n_{\text{h,a}}}}\right) + \left(\frac{A_{\text{max}} - A_{\text{frac}}}{1 + 10^{(\text{Log IC}_{50,\text{b}} - X)\times n_{\text{h,b}}}}\right) \qquad \textbf{[S2]}$$

was used, where A_{frac} is the amplitude of the current at the maximum of the high-sensitivity component (I_{frac})/I_{cont}, $\text{IC}_{50,\text{a}}$ is the IC_{50} of the high-sensitivity component, $n_{\text{h,a}}$ is the Hill coefficient of the high-sensitivity component, $\text{IC}_{50,\text{b}}$ is the IC_{50} of the low-sensitivity component, and $n_{\text{h,b}}$ is the Hill coefficient of the low-sensitivity component.

Radioligand Binding. Membranes were prepared for binding experiments as described previously (1). Saturation binding of ^{125}I-apamin (Perkin-Elmer Life Sciences) was carried out as described previously (1). Data were fit with a Hill equation of the form

$$\text{Bound/Total bound} = \frac{[\text{apamin}]^{n_{\text{h}}}}{([\text{apamin}] + K_{\text{D}})^{n_{\text{h}}}} \qquad \textbf{[S3]}$$

with K_{D} being the dissociation constant of the peptide and n_{h} the Hill coefficient. For all experiments, a $1/Y$ (where Y = bound/total bound) weighting procedure was used, which gave more weight to the smaller values of radioactivity (i.e., those that are close to the K_{D}).

1. Lamy C, et al. (2010) Allosteric block of $K_{Ca}2$ channels by apamin. *J Biol Chem* 285: 27067–27077.