Bergson P, Lipkind GM, Lee SP, Duban ME, Hanck DA.Verapamil Block of T-type Calcium Channels. *Molecular Pharmacology.*

Supplemental Methods: Chemical Synthesis

Unless otherwise noted, all reactions were carried out using reagents of >98% purity from Sigma-Aldrich-Fluka, Inc. Methylation of tertiary amines to provide the *p*-toluene sulfonate salts was achieved by treatment with methyl *p*-toluene sulfonate (e.g. Aubry, 2006). This reagent was used as a fresh supply with careful exclusion of moisture. Agent ***C1*** was prepared directly from N,N-diethylmethylamine and agents ***1q*** and ***2q*** were prepared, respectively, from the free bases of (±)-verapamil•HCl and devapamil•HCl (D888•HCl, A-444312.1, a kind gift of Abbott Laboratories, Abbott Park, IL). Free bases were produced by adjusting ≈30 mg/mL aq. solutions of the hydrochlorides to pH ≈12 with 5% (w/v) NaOH, extracting thrice with *tert*-butyl methyl ether, drying the organic phase with $MgSO_4$, evaporating and exhaustive further drying *in vacuo*, followed by immediate synthetic use. Methylation was achieved using 1.0 equiv. of methyl *p*-toluene sulfonate at 0.2M concentration with 3.0-7.5 hr. reflux in anhydrous acetonitrile (Fluka 006950) with magnetic stirring under a 4Å molecular sieve-dried nitrogen atmosphere in 150°C oven-dried glassware. Scales were 2-5 mmol except for ***2q***, which was at 0.11 mmol. Reaction progress was followed by analytical thin layer chromatography on 0.25 mm SiO_2 plates (EM Reagent 60-F), eluting with 4:1 dichloromethane-methanol, and visualizing by standard oxidations or by quench of UV plate fluorescence. Reactions proceeded to completion or nearly so as judged by disappearance of methyl *p*-toluene sulfonate. Products provided by drying *in vacuo* were hygroscopic clear-to-white foamy solids, were largely pure after reaction completion (^{1}H pulsed, Fourier-transform nuclear magnetic resonance spectroscopy at 300 MHz, yields 86-104%), but were dried rigorously *in vacuo* and isolated in analytically pure form by medium pressure LC (0.7 MPa, Biotage SP1, 40–63 μM KP-SIL SiO_2, linear gradients of methanol in dichloromethane). Subsequent spectroscopic data (^{1}H and ^{13}C NMR, electrospray ionization-mass spectrometry, high resolution mass spectrometry) were consistent with the structures reported and literature data available. Purified products were stored at –20 °C in the dark with rigorous exclusion of moisture (dry N_2) until use. Spectroscopic methods were reapplied 8 mos. into the study to confirm shelf life of compounds under laboratory storage conditions. Reagent ***C1*** was tested alone in cells as a control, to ensure that effects seen were not due to residual tosylate in the prepared compounds. Recordings in the presence of this compound were indistinguishable from control.

Aubry C, Wilson, AJ, Jenkins, PR, Mahale, S, Chaudhuri, B, Maréchal, JD, Sutcliffe, MJ. (2006) Design, synthesis and biological activity of new CDK4-specific inhibitors, based on fascaplysin. *Org Biomol Chem* **4**(5):787-801.

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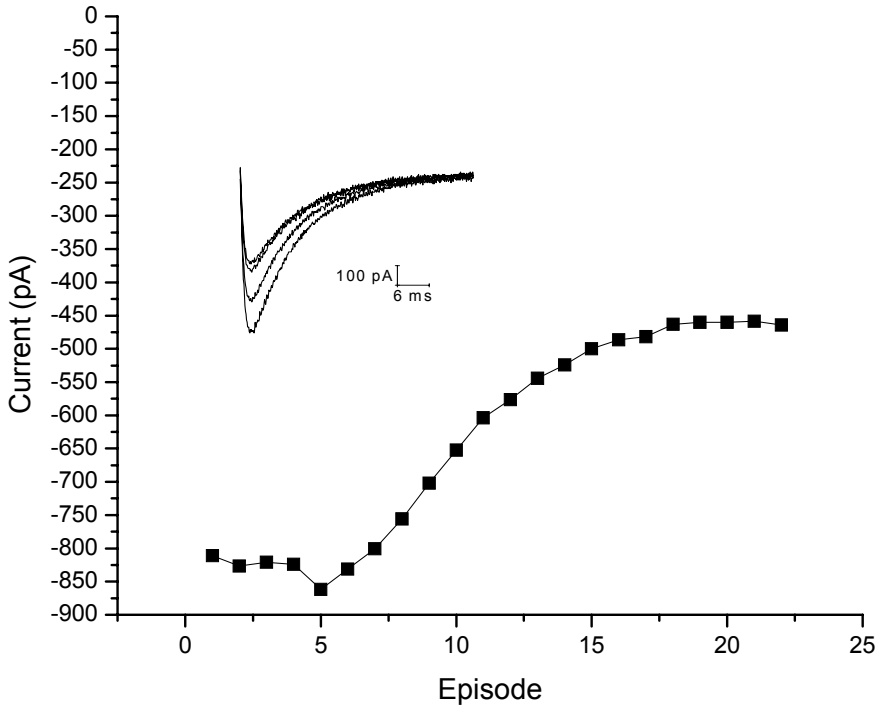

Supplemental Figure 1: Drug wash on. PAAs were applied in the bath using a gravity flow perfusion system. After control recordings were made, a 0.2 Hz train of depolarizations from the holding potential of -110 mV to a depolarized potential of -10 mV. The solution was changed from control to a solution containing, in this example, 20 μM verapamil at sweep 5. The train was run until the peaks reached a plateau, typically after 10 to 15 sweeps. Inset: sample traces from sweeps 1, 10, 15 and 20.

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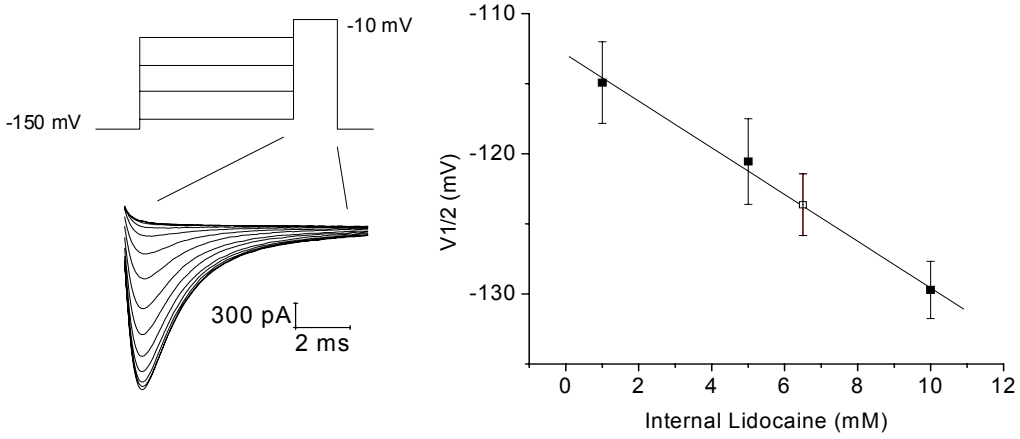
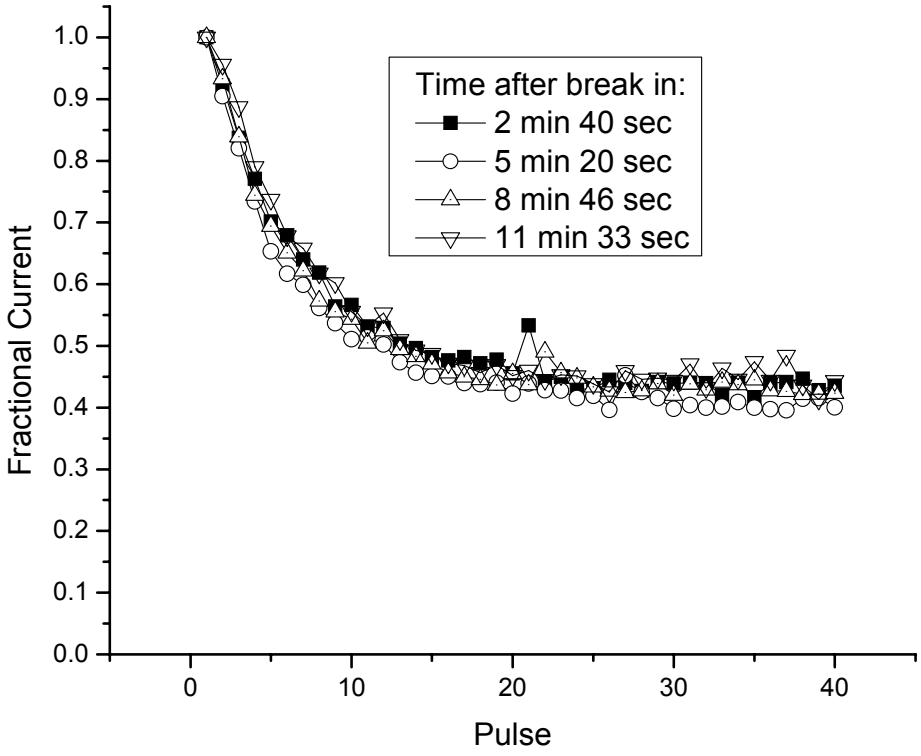
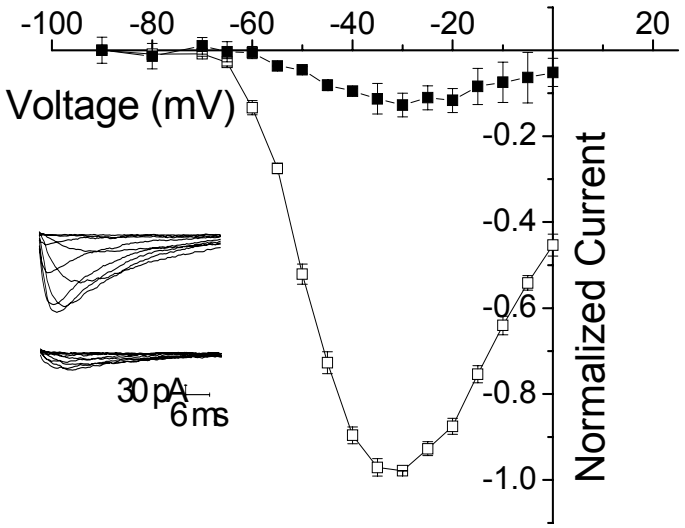


Supplemental Figure 2: Dose dependence of intracellularly applied lidocaine in $Na_V1.5$. One hallmark of lidocaine inhibition of I_{Na} is that it produces a dose dependent shift in steady state voltage dependent channel availability. To evaluate the efficacy of intracellularly applied lidocaine, HEK 293 cells stably expressing $Na_V1.5$ were exposed to various concentrations of lidocaine in the pipette. A. Representative current generated using a steady-state voltage- dependent sodium channel availability protocol. Peak currents at each voltage were plotted and fit using the Boltzman equation to determine $V_{1/2}$. B: mean $V_{1/2} \pm$ SEM at three doses of intracellular lidocaine (black squares, n=3-6) is compared to mean $V_{1/2}$ in the presence of 1 mM extracellular lidocaine (red square, n=11). It would require approximately 6.5 mM intracellular lidocaine to produce a similar shift in $V_{1/2}$. Cells were cultured as described in the main methods. Recording methods were also similar. The bath solution contained, in mM: 20 NaCl, 120 CsCl, 2 $CaCl_2$, 10 HEPES, pH 7.4. The pipette solution contained, in mM: 100 CsF, 45 CsCl, 10 EGTA, 10 HEPES, pH 7.4. Lidocaine (Sigma-Aldrich, St. Louis, MO) was dissolved in bath solution.



Supplemental Figure 3: Stability of effects with drug in the pipette. The amount of block elicited by a given concentration of PAA did not change with time after break in. In this example, successive trains of depolarizations to -30 mV were run in the presence of 200 μM D575. Peak currents were normalized to the last pulse in the train, and control values were subtracted. No increase in block is observed over the life of the cell.



Supplemental Figure 4: MTSET blocks L1831C channels. Mean current voltage relationship ± SEM produced in the absence (open squares) and presence (filled squares) of 2 mM MTSET in L1831C channels, with 2 mM Ca^{2+} in the bath. Currents were normalized to peak current at –30 mV in the absence of MTSET. N=3. Inset: sample traces recorded in the absence (top) and presence (bottom) of 2 mM MTSET.

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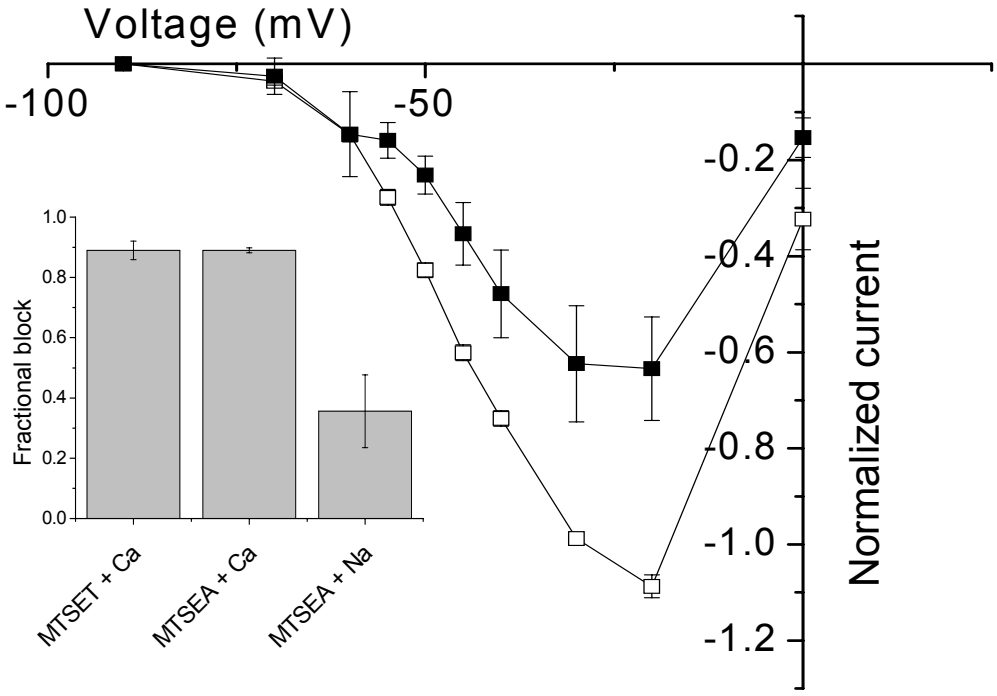

Supplemental Figure 5: Block by MTS reagents is sensitive to the species of permeant ion. Mean current voltage relationship ± SEM produced in the absence (open squares) and presence (filled squares) of 200 µM MTSEA in Q1831C channels, in the absence of Ca^{2+} in the bath. Currents were normalized to peak current at –30 mV in the absence of MTSEA. N=4. Inset: Fractional block at -30 mV is equivalent between MTSET and MTSEA in the presence of Ca^{2+}; current carried by Na^{+} is relatively spared. N=3-4 for each condition. Bath solution in the absence of Ca^{2+} contained, in mM: 140 NaCl, 10 HEPES, 1 EGTA and was titrated to a pH of 7.4 with NaOH.

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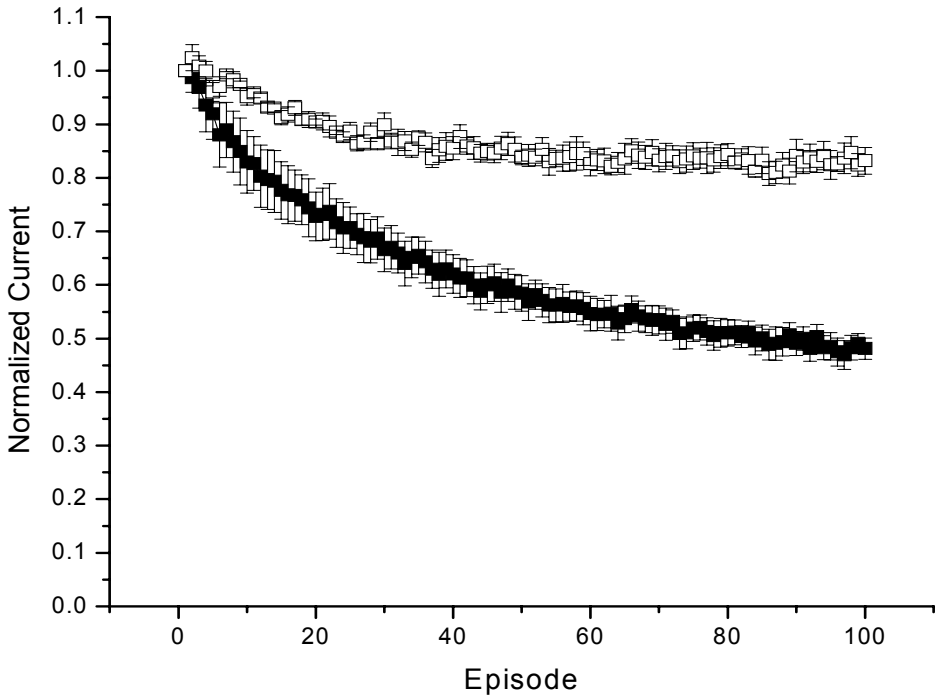

Supplemental Figure 6: 20 µM D575 elicits more block in cells expressing L1825W. Mean peak currents ± SEM, recorded with 20 µM D575 in the pipette, in wild type (open squares, n=3) and L1825W channels (filled squares, n=6). Currents were normalized to the first pulse of the train and mean control values were subtracted.