

“Cerebrovascular dilation via selective targeting of the cholane steroid-recognition site in the BK channel beta1 subunit by a novel nonsteroidal agent”, Bukiya A, McMillan J, Fedinec A, Patil S, Miller D, Leffler C, Parrill A, Dopico A. Molecular Pharmacology.

Supplemental Methods

Arterial wall $[Ca^{2+}]_i$ measurements. Adult male Sprague-Dawley rats were decapitated using a guillotine. Middle cerebral arteries were isolated on ice under microscope (Nikon SMZ645; Nikon, Tokyo, Japan) and cut into 1 to 2 mm-long segments. Segments were incubated in physiological saline solution (composition in main text) containing 3 μ M fura-2AM for 45 min. Endothelium was removed by passing an air bubble into the vessel lumen for 90 seconds prior to vessel cannulation. A segment was cannulated at each end, bathed by physiological saline (PSS, composition in main text) containing 1 μ M paxilline to block BK channels, and kept in the dark for 15 min. Fura-2 was alternatively excited at 340 or 380 nm by using a PC-driven hyper-switch (Ionoptix, Milton, MA) for ratiometric detection of free $[Ca^{2+}]$ levels within each arterial segment.