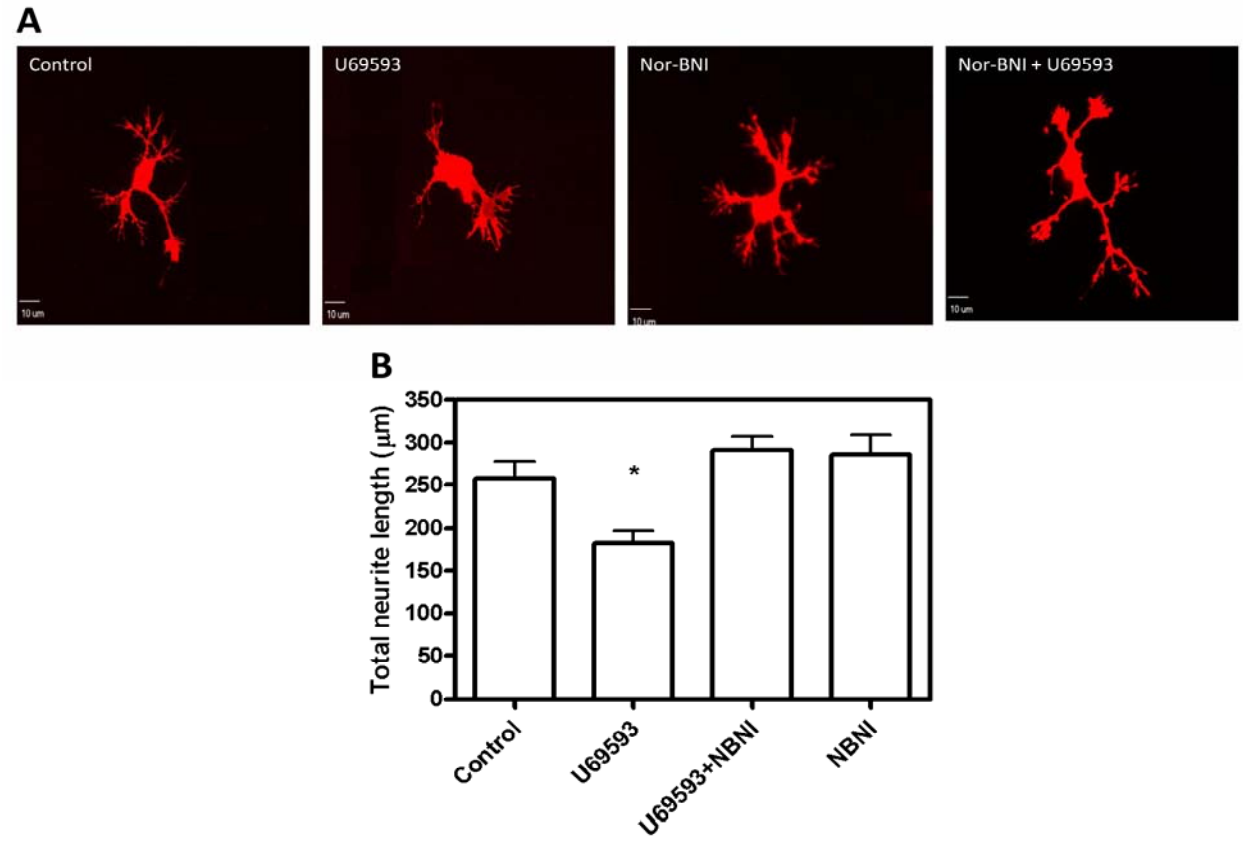
Kappa-opioid receptor inhibition of calcium oscillations in spinal cord neurons

Lakshmi Kelamangalath, Shashank M. Dravid, Joju George, Jane V. Aldrich, Thomas F. Murray
Department of Pharmacology, Creighton University, Omaha, NE 68178 (LK., SMD., JG., TFM).
Department of Medicinal Chemistry, The University of Kansas, Lawrence, Kansas 66045-7582 (JVA).

Molecular Pharmacology

Supplemental Figure-1



Supplemental Figure-1 legend

Activation of kappa opioid receptors affects neurite outgrowth in spinal neurons:

Inasmuch as the synchronous spontaneous calcium oscillations are primarily a developmental phenomenon that reflects the formation of functional synapses, we examined the functional consequences of kappa opioid receptor mediated suppression of calcium oscillations. Three hours after plating, spinal cord neurons in culture were exposed to various treatments (control vehicle, 300 nM U69593, 300 nM nor-BNI or U69593 + nor-BNI) for 17 hours followed by assessment of the effect on total neurite outgrowth (supplementary figure 1). After the termination of treatment, media was removed and the neurons were pre-fixed in 1.5% (w/v) paraformaldehyde (PFA) in PBS for 15 min. Following a brief wash with PBS, tungsten particles (1.1 µm diameter) coated with the lipophilic carbocyanine dye DiI were delivered diolistically into the neurons at 100 psi using a Helios Gene Gun system (Bio-Rad, Hercules, CA) fitted with a polycarbonate 20µm pore filter (Small Parts, Miramar, FL). DiI was allowed to diffuse along neuronal dendrites and axons in PBS containing 0.01% (w/v) thimerosal for 48 h at 4^{o}C, and then labeled neurons were post-fixed in 4% PFA for 10 min. The fixed neurons were again washed in PBS and coverslips were mounted onto subbed slides in aqueous medium. Z-stacked images were acquired using the Olympus IX 71 spinning disk confocal microscope attached to Hamamatsu ORCA-ER digital camera. DiI was excited using a mercury arc discharge light source and the images were captured with a 60X oil-immersion objective using Slide Book 4.2 software. Each neuron was scanned at 0.2 µm intervals along the z-axis for a depth of 5µm (25 planes). For quantitative analysis, a three-dimensional perspective was rendered by the Surpass module/Filament module of Imaris software (Bitplane Scientific Solutions, Saint Paul, MN). Analyis of the neurite outgrowth data by one way ANOVA showed a significant effect of

treatment ($p<.001$). Exposure of spinal neurons to the kappa opioid receptor agonist, U69593, produced a significant inhibition of neurite outgrowth as compared to control (Control, 257.8 ± 19.9 µm; U69593, 182.6 ± 15, $p< 0.05$, Bonferroni's multiple comparison test). To confirm the involvement of kappa opioid receptors in the response to U69593, spinal neurons were co-incubated with nor-BNI and the effect on neurite outgrowth assessed. Treatment with the kappa receptor antagonist nor-BNI was eliminated the inhibition of neurite outgrowth produced by U69593 (nor-BNI, 285 ± 23.8 µm; U69593+ nor-BNI, 290.7 ± 16.6 µm). These results demonstrate the involvement of kappa opioid receptors in the regulation of neurite outgrowth in embryonic spinal cord neurons.