Presynaptic Inhibition of Gamma-Aminobutyric Acid Release in the Bed Nucleus of the Stria Terminalis by Kappa Opioid Receptor Signaling

Supplemental Information

Brain Slice Preparation

Mice were decapitated under isoflurane anesthesia and their brains were rapidly removed and placed in ice-cold sucrose artificial cerebrospinal fluid (ACSF): (in mM) 194 sucrose, 20 NaCl, 4.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.2 NaH_2PO_4, 10.0 glucose, and 26.0 $NaHCO_3$ saturated with 95% O_2/5% CO_2. Coronal slices containing dorsolateral bed nucleus of the stria terminalis (BNST) (Bregma 0.26–0.02 mm), identified using the internal capsule, anterior commissure, and stria terminalis as landmarks, were prepared using a Leica VT1200 vibratome (Wetzlar, Germany). Slices were stored in a holding chamber for at least 45 minutes before being transferred to a submerged recording chamber perfused with heated, oxygenated ACSF at a rate of approximately 2 ml/min. Slices were allowed to equilibrate for 30 min before electrophysiological recordings. Dorsolateral BNST neurons were directly visualized with infrared video microscopy (Olympus, Center Valley, PA). Recording electrodes (3–5 MΩ) were pulled with a Flaming-Brown Micropipette Puller (Sutter Instruments, Novato, CA) using thin-walled borosilicate glass capillaries.

Stereotaxic Surgery

Mice were anesthetized with ketamine/xylazine and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). Microinjection needles (33 gauge) connected to a 2 µl Hamilton syringe were bilaterally inserted directly above the central nucleus of the amygdala (CeA) (coordinates from Bregma: -1.6 AP, ±3.4 ML, -4.9 DV). Following virus injection to the CeA,

GABAergic neurons were transduced with virus coding for double floxed ChR2(H134R)-EYFP under control of the EF1α promoter over ten minutes followed by 10 minutes to allow diffusion of viral particles away from the injection site. Mice were allowed to recover for 4-6 weeks to achieve maximal infection and protein expression in the BNST prior to electrophysiological experiments (1). The CeA injection site was evaluated in each experiment via fluorescence microscopy.

Construct and AAV Preparation

DNA plasmids coding double floxed-ChR2-EYFP were obtained from the laboratory of Karl Deisseroth. Plasmid DNA were grown and collected using a standard plasmid maxiprep kit (Qiagen, Hilden, Germany). Following plasmid purification and restriction digest, and sequencing to assure DNA fidelity, purified AAV was produced, using calcium phosphate precipitation methods by the UNC Vector Core facilities (AAV serotype 2; University of North Carolina at Chapel Hill). Viral titers used were > 10^{12} g.c. (genome copies) /mL.

Internal Pipette Solution: KCl vs CsCl

In order to maintain consistency, the internal solutions used for all electrophysiology data were potassium-based, instead of the cesium-based internal that is known to provide better signal-to-noise ratio. The following data demonstrate that experiments conducted with the cesium-based internal solution reflect kappa opioid receptor effects in inhibitory postsynaptic currents in similar manners as the potassium-based internal solution.

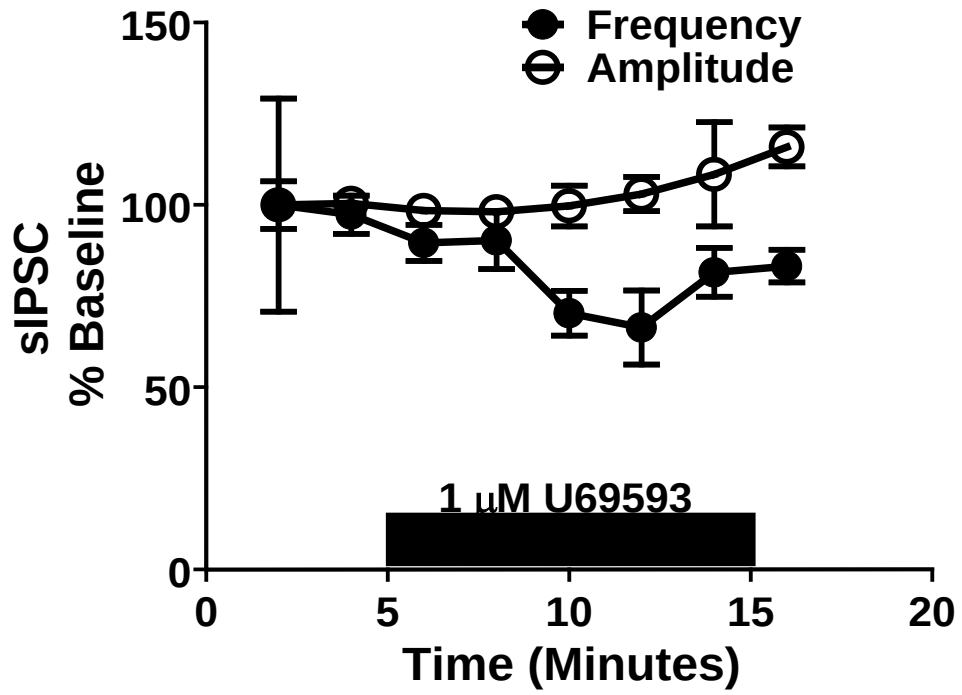

Figure S1. sIPSC recorded with cesium-based internal solution. Cesium-based internal solution produced similar U69593-induced attenuation in sIPSC as potassium-based internal solution. sIPSC, spontaneous inhibitory postsynaptic currents.

Drugs

(+)-(5α,7α,8β)-N-Methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide (U69593, 1 uM), a synthetic agonist highly selective for the kappa opioid receptor (2), was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in 0.1 M HCl; Dynorphin A (300 nM) and Norbinaltorphimine (Nor-BNI, 100 nM) were from Tocris (Ellisville, MO), and were both dissolved in distilled water. Nor-BNI is an opioid receptor antagonist highly selective for the kappa opioid receptor (3); Baclofen (10 μM) was from Ascent Scientific (Princeton, NJ) and dissolved in distilled water with 1 molar equivalent of NaOH; 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX, 10 uM) and 4-[4-(4-

Fluorophenyl)-2-[4-(methylsulfinyl)phenyl]-1H-imidazol-5-yl]pyridine (SB203580, 20 μM) were from Ascent and dissolved in distilled water; Picrotoxin (5 uM), 1,4-Diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene (U0126, 20 μM), and alpha-[Amino[(4-aminophenyl)thio]methylene]-2-(trifluoromethyl)benzeneacetonitrile (SL327, 10 μM) were from Ascent and dissolved in DMSO.

Supplemental References

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2. La Regina A, Petrillo P, Sbacchi M, Tavani A (1988): Interaction of U-69,593 with mu-, alpha- and kappa-opioid binding sites and its analgesic and intestinal effects in rats. *Life Sci* 42293-301.
3. Portoghese PS, Lin CE, Farouz-Grant F, Takemori AE (1994): Structure-activity relationship of N17'-substituted norbinaltorphimine congeners. Role of the N17' basic group in the interaction with a putative address subsite on the kappa opioid receptor. *J Med Chem* 37:1495-1500.