Online Supplement

Solutions and materials

All chemicals were purchase from Sigma (St. Louis, MO) and Invitrogen (Eugen, OR) unless otherwise stated. Tyrode solution for measuring intracellular calcium transient as follows: (in mol/L) 135 NaCl, 4.5 KCl, 11 Glucose, 1 $MgCl_2$, 2 $CaCl_2$ and 20 HEPES (pH 7.4). Tyrode solution for norepinephrine release experiments contained (in mol/L) 120 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 25 $NaHCO_3$, 2 $CaCl_2$, 1.2 KH_2PO_4 and 11 glucose and was aerated with 95% O_2 +5% CO_2 (pH 7.4). Fura-2/AM was acquired from Molecular Probes (Eugene, OR). L-15 blocking medium: 96.8% L-15 medium supplemented with 0.6% D-(+)-Glucose solution, 2mM L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, 10% Fetal bovine serum. Plating medium: 90% L-15 medium supplemented with 24mM $NaHCO_3$, 38mM Glucose, 50 units/ml penicillin, 50 µg/ml streptomycin, 50 ng/ml Nerve growth factor, 10% Fetal bovine serum.

Western blotting

The primary antibodies with optimized dilutions used included: anti-CAPON (abcam 1:1000), anti-nNOS (Invitrogen 1:500). The results were normalised to β-actin that served as loading control (anti-β actin: abcam, 1:2500). Brain lysate was used as a positive control throughout the experiments.

Measurement of intracellular calcium concentration

Intracellular free calcium concentration $[Ca^{2+}]_i$ of individual cultured stellate neurons was determined using Fura-2 acetoxymethyl ester (Fura-2/AM) fluorescence ratio imaging as previously described[19]. Loaded neurons were imaged every 2 seconds with CoolSnap digital CCD camera connected to an OptoLED fluorescence imaging system (Cairn Research Ltd) housed on an inverted microscope (Nikon Eclipse Ti-U) equipped with a 40×/1.30, oil-immersion objective. The cover slip containing the neurons was placed in a temperature-controlled (36±0.5 ^{o}C), gravity fed, perfusion chamber (volume: 100 µl). The depolarization-induced $[Ca^{2+}]_i$ transient was acquired by 30 seconds exposure to 50 mmol/L KCl (with equimolar reduction in NaCl) in the Tyrode solution. Fura-2AM was excited alternately at 355nm and 380 nm and fluorescence emissions acquired at 510 nm. Fluorescence excitation ratios were transformed into $[Ca^{2+}]_i$ concentrations using the equation derived by Grynkiewicz et al. below.

$([Ca^{2+}]_i = K_d \times (S_{f2}/S_{b2}) \times (R - R_{min})/(R_{max} - R))$.

Measurement of tissue NOS activity

The activity of NOS was assessed by measuring the conversion of [^{14}C]L-Arginine to [^{14}C] L-Citrulline as described previously[23, 24]. Briefly, separation of the products of L-arginine metabolism was obtained by HPLC (Jasco Ltd.) and on-line radiochemical detection using a Beta-Ram scintillation counter (Lablogic Systems Ltd). Data were collected and analyzed using Azur software (Datalys, France). A total of 24 SHR were used and divided into 4 groups of 6 animals: Empty virus, empty virus with AAAN, Ad.CAPON and Ad.CAPON with AAAN. Left and right stellate ganglia from 2 animals were isolated, pooled and homogenized using ice-cold Krebs' HEPES Buffer containing nor-NOHA (5 µmol/L, to inhibit arginase activity) because

ganglia from one animal does not give enough protein. Following centrifugation (13,000 rpm for 10 mins at 4°C), NOS cofactors (10 μmol/L FAD, 10 μmol/L FMN, 1 mmol/L NADPH, 10 μmol/L BH4) were added in the presence or absence of the specific nNOS inhibitor, AAAN (10 μmol/L). 0.75 μL of radiolabelled [^{14}C] L-arginine (Hartmann Analytic GmbH) was then added and the samples were incubated for 4 hours (37oC). Samples were then de-proteinated by the addition of trichloroacetic acid (10%). Samples were centrifuged and transferred to vials for analysis by HPLC. Standards of ^{14}C-labelled L- arginine (1 μmol/L), L-citrulline (0.1 μmol/L), and L-ornithine (0.2 μmol/L) were used to determine retention time. Chromatograms were then analysed using Azur software and NOS activity expressed as the percent conversion of arginine to citrulline.