Supplementary Data

Amyloid-β-Induced Amyloid-β Secretion: A Possible Feed-Forward Mechanism in Alzheimer’s Disease

Ian T. Marsden, Laurie S. Minamide and James R. Bamburg∗
Department of Biochemistry and Molecular Biology and Molecular, Cellular and Integrative Neuroscience Program, Colorado State University, Fort Collins, CO, USA

Accepted 2 January 2011

RODENT TOTAL Aβ ELISA
RAβ_{1-40} and RAβ_{1-42} pellets were solubilized in 10 μL of DMSO and diluted further with a phosphate buffered saline containing Tween and BSA (PBSTB; Covance). Wells to be used on the assay plate were washed 1× with PBSTB and standards or samples (100 μL per well) were applied and plates incubated overnight at 4°C. Wells were washed 5× with 300 μL PBSTB. SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Scientific) was added at 300 μL to each well. Chemiluminescence was quantified by photon counting between 5 and 10 min after substrate addition using a Perkin-Elmer Victor V multi-mode microplate reader operating at room temperature with no filter. The optimal concentration of capture antibody that allowed for maximal binding of rodent Aβ to the plate was determined to be 5 μg/mL. Standard curves using 1.6 μg/mL detection antibody and synthetic RAβ_{1-40} and RAβ_{1-42} between 0 and 1,200 pg/mL were identical for both forms of Aβ and were linear from 10 to 600 pg/mL (Supplementary Fig. 2).

ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES
Ethanol washed coverslips (12 × 22 mm) were treated with 2% 3-aminopropyltriethoxy-silane in ace-

*Correspondence to: James R. Bamburg, Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO 80523, USA. Tel.: +1 970 491 6096; Fax: +1 970 491 0494; E-mail: jbamburg@lamar.colostate.edu.

ISSN 1387-2877/11/$27.50 © 2011 – IOS Press and the authors. All rights reserved.
tone (10 sec dip), rinsed in water, air dried and UV sterilized. Chick plasma (2 × 4 μL aliquots) was spread into two 5 mm diameter circles near one end of the coverslip, two slices were placed side by side on the plasma and the slices were each covered with 4 μL of fresh plasma/thrombin mixture (4 μL chick plasma: Cocalico Biologicals Inc., Reamstown, PA) and 4 μL thrombin (150 NIH units/mL; MP Biomedicals, Inc.) in Gey’s BSS/glucose (per 100 mL: 97 mL Gey’s BSS (Sigma), 2 mL 25% glucose, 1 mL Pen/Strep). After the plasma clotted, the coverslip was inserted into a flat sided tube (Nuncloth Delta Tubes, Nalge Nunc, Rochester, NY) and 600 μL slice culture medium added. Tubes were placed at a 5º angle in a roller incubator (10 revolutions per hour) at 35°C. The original slice medium (per 205 mL: 50 mL horse serum, 50 mL Hanks BSS, 4 mL 25% glucose, 100 mL minimum essential medium containing glutamax (250 μL/100 mL), HEPES (4.76 g/L) and Pen/Strep (1 mL)) was replaced on day 2 with Neurobasal A medium containing (per 50 mL): 48 mL Neurobasal A, 180 μL 25% glucose, 625 μL GlutaMAX 1, 1 mL B27 supplements and 250 μL Pen/Strep. The Neurobasal A medium was replaced every 2–3 days. Slices were allowed to recover for at least 1 week after dissection before treatment.