ACTIONS OF GLUTAMATE RECEPTOR AGONISTS ON INTRACELLULAR FREE CALCIUM [Ca]$_i$ IN CULTURED TURTLE CEREBELLAR NEURONS.

A.W. Krause, I.C. Haralabatos, and T.J. Sejnowski. Department of Biophysics, The Johns Hopkins University, Baltimore, MD 21218

Glutamate (GLU) receptor activation, particularly the N-methyl-D-aspartate (NMDA) subtype, is thought to affect long-term neuronal plasticity via modulation of ([Ca]$_i$). We have investigated this modulation using the [Ca]$_i$-sensitive fluorescent dye fura-2, in cerebellar cultures from the turtle.

Turtle cerebella were dissected from 2-6 week embryos, minced, then dissociated by trituration. Cells were incubated at 33° C in 3% CO_2 at pH 7.4-7.6 in a modified Basal Eagle's Medium supplemented with 10% fetal bovine serum on coverslips coated with polylysine and/or polyornithine. Cells at 2-25 days in culture were stained 1-2 hours with 5 µM fura-2AM, postincubated without dye at least 30 min and then observed on an inverted microscope at 22-24° C with continuous perfusion of turtle Ringer saturated with 95% O_2/5% CO_2. Koehler epifluorescence excitation (with 10 nm bandpasses) at 380 nm and either 345 or 350 nm was used with aperture-limited detection of fluorescence by a photon-counting PMT for maximum sensitivity.

Granule and Purkinje neurons and glia were studied as identified by morphology and parallel examination of cell-specific antibody staining. GLU, NMDA, kainate, and aspartate were applied from pressure micropipettes or by bath perfusion. In these neurons, GLU and NMDA frequently induced long-term [Ca]$_i$ elevation (>30 min) from resting levels (60-120 nM) to from 250 nM to >1 µM. Kainate and aspartate generally elicited fewer and more transient (10 sec - 5 min) [Ca]$_i$ increases. Surprisingly, 2-amino-5-phosphonovalerate (APV) also increased [Ca]$_i$, even in turtle Ringer without calcium (including 5 mM of the [Ca]$_i$ chelator EGTA), as did GLU and NMDA. Thus, these compounds can cause calcium release from intracellular stores. Glia rarely responded to these compounds and [Ca]$_i$ changes were usually slight (<50 nM). Oscillations in neuronal [Ca]$_i$ were sometimes observed with or without drug application.

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