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BRAIN RESEARCH

Short Communication

Glutamate receptor-mediated inhibition of L-glutamate efflux from cerebral cortex in vitro

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ABSTRACT

We tested whether glutamate receptor ligands affect oxygen–glucose deprivation-evoked L-glutamate efflux from adult rat cerebrocortical prisms. The uncompetitive NMDA antagonist AR-R15896AR inhibited efflux (IC_{50} 34 μM, 87% maximal inhibition). AMPA/kainate receptor blockade (NBQX, 100 μM) or Group II metabotropic glutamate receptor activation (DCG-IV, 10 μM) inhibited efflux (41%, 67% respectively) but Group I mGluR blockade (CPCCOEt/MPEP, 10 μM) was without effect. These data support a modulatory effect of glutamate receptors on L-glutamate efflux.

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Abbreviations:
AR-R15896AR, (S)-α-phenyl-2-pyridine-ethanamine dihydrochloride
CPCCOEt, 7-(hydroxyimino) cyclopropa[b]chromen-1a-carboxylate ethyl ester
DCG-IV, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine
HBS, HEPES-buffered saline
MPEP, 2-methyl-6-(phenylethynyl) pyridine
NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide
OGD, oxygen and glucose deprivation

Extra-cellular L-glutamate concentration is rapidly elevated in and around an ischemic brain region and drugs that suppress glutamate release or inhibit glutamate receptor activity have been examined for their utility in treating a number of brain disorders, including acute ischemic stroke, epilepsy and schizophrenia (Lipton, 1999; Small and Buchan, 1997; Lipton and Rosenberg, 1994; Moghaddam, 2003). We have previously reported on an in vitro model of acute damage to cerebral cortical tissue, using prisms of adult rat cortex, with simulated ischemia (oxygen and glucose deprivation, OGD) as the stimulus (Nelson et al., 2000, 2003). The endpoint assayed in this model is efflux of endogenous L-glutamate, known to be associated with cerebral ischemic damage from many previous studies in vitro and in vivo (Gaspary et al., 1994; Phillis et al., 1994; Lipton, 1999; Lipton and Rosenberg, 1994). There are several possible sources for efflux of L-glutamate in ischemic brain and our own findings suggest components due to exocytotic release, reversal of uptake by amino acid transporters and osmotically-activated anion channels (Nelson et al., 2000, 2003), with little contribution from cell lysis under the conditions used.

It seemed likely that activation of glutamate receptors might influence the efflux process, possibly via a feedback mechanism. NMDA-type glutamate receptors in particular have long been associated with acute brain injury (Lipton, 1999; Small and Buchan, 1997; Bano et al., 2005). In the present study therefore, we examined the effects of glutamate receptor subtypes on OGD-induced glutamate efflux, using selective pharmacological ligands.

NBQX, CPCCOEt, MPEP and DCG-IV were purchased from Tocris-Cookson Ltd., Bristol, UK. AR-R15896AR was supplied by AstraZeneca R&D Södertälje, Sweden.

Young adult, female, Wistar rats (200–250 g) were killed by cervical dislocation, brain rapidly removed and prisms of cerebral cortex (cross sectional area 350×350 μm) prepared by cutting in two orthogonal planes using a McIllwain tissue slicer (Nelson et al., 2000, 2003). Equal volumes of a suspension of prisms (2 ml, equivalent to about 200 mm^3 of tissue) were aliquoted into mesh baskets and incubated in control HBS or hypoxic/aglycemic HBS (OGD-HBS) at 37 °C. The OGD-HBS contained no added glucose and was bubbled with N_2 for at least 2 h prior to the experiment. The PO_2 values were between 5.4 and 5.7 kPa (measured with an I.L.16/40 blood gas analyser) for OGD-HBS and PO_2 of 22.6–23.3 kPa for the control HBS. In some experiments, glucose was omitted from HBS buffer ("aglycaemia" buffer), or HBS buffer was equilibrated with nitrogen ("hypoxic" buffer).

In time-course experiments, prisms were transferred to fresh HBS at 5 min intervals for 45 min. In uninterrupted incubation studies baskets remained in a single 2 ml vial during a 30 min incubation period. In previous experiments we observed only moderate cell destruction in this paradigm (as indicated by escape of lactate dehydrogenase from the tissue, Nelson et al., 2000). The appropriate gas (control: air, OGD: N_2) was blown over the liquid surface of the solutions throughout the experiment. Prisms were incubated under control and OGD conditions in the presence or absence of drugs.

AR-R15896AR was applied at a range of concentrations (1 μM–3 mM). NBQX (100 μM), DCG-IV (10 μM), CPCCOEt (10 μM) and MPEP (10 μM) were each applied at a single concentration, chosen for maximal effect with minimal non-selective action, based on previous pharmacological studies (Dev et al., 1996; Mutel et al., 1998; Uyama et al., 1997).

At the end of each experiment, supernatants (containing glutamate efflux) were retained and prisms were transferred to 1% Triton-X100 for 5 min to release all remaining glutamate. Supernatants and tissue lysates were spun at 13,400×*g* for 5 min at 4 °C and glutamate assayed fluorimetrically using the conversion of $NADP^+$ to NADPH by glutamate dehydrogenase.

In each experiment, L-glutamate efflux was expressed as a percentage of the total L-glutamate present in the tissue (effluxate + tissue lysate). In bar charts, pooled data show efflux normalized with respect to the mean OGD-evoked efflux in the absence of drugs. Inhibitory effects of drugs on efflux are expressed as fractional inhibition, i.e. the fraction of OGD-dependent efflux (efflux during OGD – control efflux) that was inhibited by drug treatment. All data are presented as mean, SD, n = at least 5, being the number of independent experiments pooled. For statistical analysis, one-way analysis of variance (ANOVA) with Dunnet's post-hoc test was used for uninterrupted incubation experiments, and 2-way ANOVA