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Effect of ischemia and intra-amygdaloid kainate injection on the density of NMDA binding sites in the hippocampal CA1 region

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The N-methyl-D-aspartate (NMDA) receptor channel complex, a subtype of glutamate receptors, plays an important role in neuronal plasticity Cotman and Iversen, 1987). The role of the NMDA receptor has been much studied, notably in the CA1 region of the hippocampus which contains a particularly high density of NMDA binding sites in the stratum radiatum i.e. the terminals field of the Schäffer collaterals (Monaghan and Cotman, 1985). The exact localisation of these binding sites (i.e. pre- or post-synaptic) has however not been fully determined. We now report the results of a quantitative autoradiographic study that involved the selective destruction of CA3 or of CA1. Our results suggest that most NMDA binding sites are located postsynaptically (on pyramidal neurons or interneurons) but not on the Schäffer collaterals.

Seventeen male wistar rats (200-230 g) were used in the present study. Eleven rats were used for one of the two following procedures.

(1) Selective destruction of CA1 pyramidal cells (n = 6) by means of bilateral hemispheric ischemia (Pulsinelli and Brierley, 1979). The vertebral arteries were electrocauterized under chloral hydrate anaesthesia (7%) and both carotid arteries were isolated. The next day, the unanaesthetized rats had the carotid arteries clamped for 30 min.

(2) Destruction of CA3 pyramidal cells (n = 5): kainate (a neurotoxin analogue of glutamate) was injected into the amygdala (1.2 μg in 0.3 μl of phosphate buffer, pH 7.4) (Ben-Ari, 1985). Six additional rats were used as controls.

The rats were killed 10 days after ischemia or kainate injection and the brain was removed and sliced (20 μm) in a cryostat. The visualization of NMDA binding sites was done by quantitative autoradiography (Monaghan and Cotman, 1985) according to the following procedure: after preincubation in Tris-acetate (50 mM, pH 7.2) to remove endogenous competitive ligands the slices were incubated in the same buffer containing 150 nM L-[^{3}H]glutamate (NEN, 50-55 Ci/mM); alternate sections were incubated in the same medium either in the presence of 150 μM cold glutamate to determine non-specific glutamate binding or in the presence of 150 μM NMDA. NMDA binding sites were evaluated by subtraction of glutamate binding in the presence of NMDA from the total binding. The extent and the specificity of the lesion were verified by means of cresyl-violet staining.

The ischemic treatment induced a highly significant reduction (60%, $P < 0.005$) of NMDA binding sites in the stratum radiatum of CA1 (fig. 1). Glutamate binding sites were reduced by 40% after ischemia; 80% of this loss was represented by NMDA binding sites. The mean values (fmol/mg tissue $\pm$ S.E.M.) for glutamate and NMDA binding sites were, respectively, 321.5 $\pm$ 15 and 179 $\pm$ 9 in the control rats (n = 6) and 189 $\pm$ 16 and 75.6 $\pm$ 6 in the ischemic rats (n = 5).

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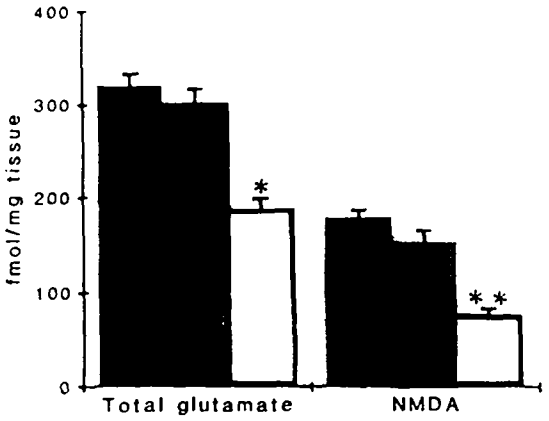

Fig. 1. Mean density (fmol/mg tissue ± S.E.M.) of specific total L-[^{3}H] glutamate and NMDA binding sites in CA1 of control (solid bars), kainate-treated (stippled bars) and ischemic (open bars) rat hippocampi. NMDA binding sites were evaluated as described in the text. Note the significant loss of both glutamate and NMDA in the hippocampus of ischemic rats. * and ** refer to $P < 0.05$ and $P < 0.005$, respectively (Student's t-test).

In contrast, the massive and almost complete destruction of CA3 pyramidal neurons and Schäffer collaterals by an intra-amygdaloid injection of kainate ($n = 6$), had no significant effect on the distribution and density of either glutamate or NMDA binding sites in the stratum radiatum of CA1 (303.4 ± 19 and 154 ± 17 fmol/mg tissue ± S.E.M., for glutamate and NMDA, respectively).

To conclude, the present results suggest that most NMDA binding sites in CA1 (60%) are located postsynaptically, on the dendrites of the pyramidal cells and not on the Schäffer collaterals. The sites which remained after ischemia (40%), could be located on CA1 interneurons which seemed relatively resistant to the ischemic procedure. Preliminary observations also suggested that the binding sites to TCP (a NMDA channel blocker) and to glycine (strychnine-insensitive NMDA allosteric sites) are also co-localized postsynaptically on CA1 neurons.

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