ACTIVATION OF AMPA AND KAINATE GLUTAMATE RECEPTORS IMPAIRS THE VIABILITY OF OLIGODENDROCYTES *IN VITRO*.

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Oligodendrocytes myelinate axons in the Central Nervous System. This cell type mainly develops shortly after the majority of neurons are generated and therefore, it is likely that neurons play an important role in controlling oligodendrocyte development. Thus, it is known that the proliferation and survival of oligodendrocytes depends on electrical activity in axons and in the reciprocal contacts they establish, respectively (for a recent review, see Barres and Raff, 1996). Axon to oligodendrocyte signalling underlies the generation of the precise number of oligodendrocytes necessary to myelinate the entire extent of a given population of axons. Unfortunately, little information is available about the molecules participating in this signalling process.

Here, we tested whether glutamate, the most abundant excitatory neurotransmitter in the brain, is involved in the shaping of the oligodendrocyte population, by studying the effects of glutamate agonists in oligodendrocyte cultures of the developing rat optic nerve.

Glutamate activates different receptor subtypes that can be grouped into two categories, ionotropic and metabotropic receptors. Ionotropic receptors contain integral cationic ion channels that are associated with the ligand binding site, and are classified into alfa-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainate and N-methyl-D-aspartate (NMDA) subtypes, according to their preferred agonist (Hollmann and Heinemann, 1994).

In addition to their presence in neurons, non-NMDA glutamate receptors have also been found in glial cells (for a recent review, see Steinhäuser and Gallo,1996).

In the current study, we first investigated by immunocytochemistry the types of ionotropic glutamate receptors expressed by oligodendrocytes *in vitro*. Cultures were derived from P7 rat optic nerves and grown in chemically-defined medium (Barres et al., 1992). After 5 days, these cultures were highly enriched in oligodendrocytes (>95%) as assessed by employing antibodies to galactocerebroside C, and used for immunolabelling following a previously described procedure (Puchalski et al., 1994). Briefly, cells were fixed in 4% paraformaldehyde and permeabilized in PBS containing 1% Triton X-100. After blocking with 5% donor serum, primary antibodies to ionotropic glutamate receptor subunits were applied and the immunodetection procedure was carried out using biotinylated secondary antibodies followed by incubations with ABC complex and with the peroxidase substrate.

The use of specific subunit antibodies indicated that the glutamate receptors expressed in the oligodendrocyte cultures were made of AMPA subunits (GluR2-4), kainate subunits (GluR5-7, KA2) and the NMDA subunit NMDAR1 (Fig. 1).

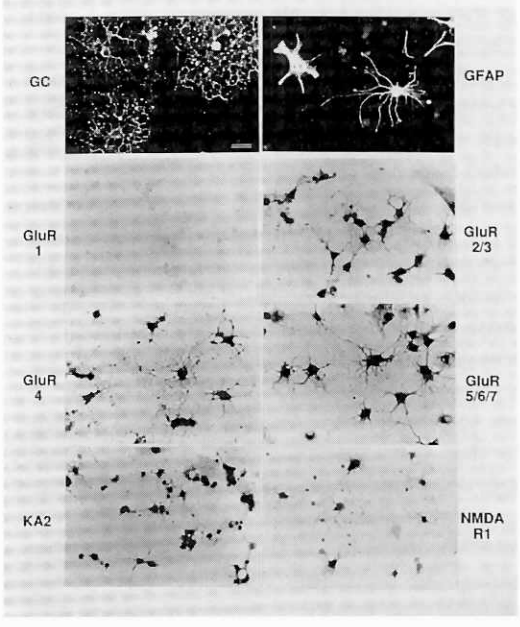

Figure 1.- Expression of ionotropic glutamate receptor subunits in differentiated oligodendrocytes. Cultures were at 5 days in vitro and derived from rat optic nerve. The upper immunofluorescence photograph to the left shows the expression of galactocerebroside C in oligodendrocytes. A few GFAP+ cells were also observed in these cultures (<5%; top right). All subunits expressed are present in oligodendrocyte cell bodies, while subunits GluR4-7 and NMDAR1 are more abundant in the cell processes. Bar=25 μm.

Because functional NMDA receptors are heteromeric in nature (Hollmann and Heinemann, 1994), we infer that the main ionotropic glutamate receptors expressed in oligodendrocytes are those belonging to the AMPA and kainate subfamilies.

Accordingly, we assayed the effects of their main agonists (AMPA and kainate) and antagonists (CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione) on the viability of mature oligodendrocytes *in vitro*.

Cell viability and cell death was measured by counting cells which were labelled with fluorescein diacetate (FDA) and propidium iodide (PI), respectively. Application of AMPA 100 μM resulted in a slight reduction in the number of viable cells which was evident only after 24 hours and that was antagonised by CNQX (Fig. 2). In contrast, kainate, both at 10 μM and 100 μM, damaged up to about 30% of oligodendrocytes present in these cultures (Fig. 2). This cytotoxic effect was prevented by using the non-NMDA antagonist CNQX (30 μM).

The kainate concentration required to elicit the maximal effect strongly suggests that the cytotoxic action is mediated by kainate receptors (see Lerma et al., 1993).

The cytotoxic levels were not affected by cell density (data not shown), indicating that cytotoxicity is directly mediated by the receptor and not by substances released upon receptor activation.

Intriguingly, glutamate 1mM, the endogenous agonist of glutamate receptors, did not affect oligodendroglial cell viability, an event which might be due to the possible instability of this compound in the culture media as well as to its removal by excitatory amino acid transporters known to be present in oligodendrocytes.

Taken together, the results reported above indicate that the ionotropic glutamate receptors present in oligodendrocytes negatively regulate the survival of these cells *in vitro*. Although we do not know whether this is the case *in vivo*, it is possible that glutamate released from Ranvier nodes following electrical activity in axons (Weinreich and Hammerschlag, 1975) may be involved in the shaping of the oligodendrocyte population in a similar fashion to that observed *in vitro*.

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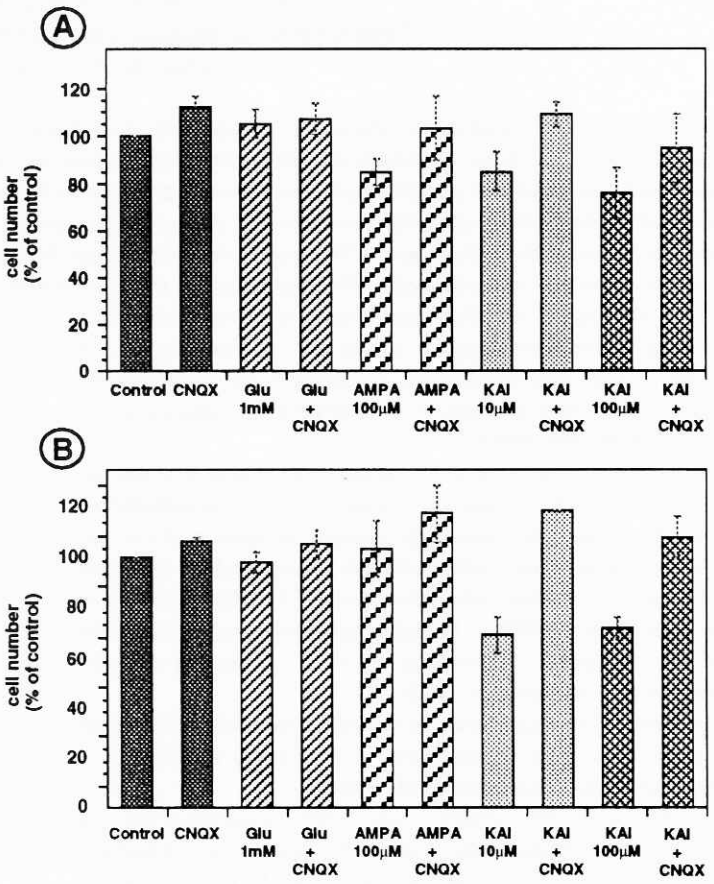

Figure 2.-- Effects of glutamate receptor agonists on the viability of oligodendrocytes *in vitro*. Optic nerve oligodendrocytes were cultured during 5 days on 12 mm diameter coverslips and then, agonists and/or antagonists were added to cultures. After exposure of 24 hr (A) or 72 hr (B), cells were labelled with FDA and PI and counted using a inverted microscope with a 20x objective. Ten fields/coverslip were counted in four independent experiments. Averages ± SEM (n≥30) are shown.

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