

Fine structural changes in the rat brain after local injections of gliotoxin, alpha-aminoadipic acid

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Summary. Toxic effects of locally injected gliotoxin, L-alpha-aminoadipic acid, were examined in the adult rat brain at the fine structural level. Astrocytes were the only cell type in the corpus striatum that showed clear structural degeneration 1 to 3 days after the treatment. With a 7 day survival period, two major structural changes were detectable, including tremendous invasion of astrocytes and their processes, and an appearance of a large number of microglia around injection sites. Both the stereospecificity and cellular specificity were firmly established on the toxic effects of this chemical.

Key words: Alpha-aminoadipic acid - Astroglia - Microglia - Degeneration - Striatum

Introduction

A specific gliotoxic effect of alpha-aminoadipic acid (alpha-AA), a six-carbon chemical analogue of glutamate, has been shown both in vitro and in vivo. The in vitro treatment of dissociated postnatal mouse cerebellum with alpha-AA resulted in a rapid nuclear and cytoplasmic swelling of astroglia, which were clearly identified by indirect immunofluorescence labeling with anti-human glial fibrillary acidic protein antiserum (Huck et al., 1984a). In addition, it was shown that ¹⁴C labeled alpha-AA was

taken up and accumulated selectively by cultured astrocytes, with presumptive neurons being free of the radiolabel (Huck et al., 1984b). Glial lesions were also observed in vivo in the arcuate nucleus of the hypothalamus and retina of early postnatal mice following subcutaneous administration of the chemical (Olney et al., 1971, 1980). In addition to the cellular specificity, comparison of D- with L-alpha-AA revealed a stereospecificity both in vitro (Huck et al., 1984a,b) and in vivo (Olney et al., 1980). L-alpha-AA was the only isomer effective on producing such specific glial degeneration.

Since the late 1970's, a considerable number of chemical analogues of glutamate and/or aspartate have been used as neurotoxin, both in the CNS and PNS (Coyle et al., 1981). There have been no reports, however, that involve the intracranial stereotaxic injection of the specific gliotoxin, alpha-AA and the analysis of its direct effects on the fine structure of different type of cells in the CNS.

Materials and methods

Experiments were performed on twelve male albino rats (Wistar, 280-320 g). Both L- and D-alpha-AA were dissolved in 0.05 M phosphate buffer (pH 7.4) prior to the operation. Under sodium pentobarbital anesthesia (65 mg/kg, i.p.), 50 to 250 nmol alpha-AA in 1 µl was stereotaxically injected into the striatum using 1-µl Hamilton microsyringe. Animals were sacrificed 1, 3 and 7 days after the injection, by transcardial perfusion with 300 ml of a fixative containing 4% paraformaldehyde, 0.5% glutaraldehyde and 0.54% glucose in 0.1 M phosphate buffer (pH 7.4). Following removal of the brain, the striatum was dissected out and serially sectioned on an Oxford Vibratome at 50 µm thickness. Alternate sections were Nissl stained and remaining ones were placed under a dissecting microscope. Checking those neighboring Nissl stained sections, rectangular areas of approximately 0.25

Glutotoxic effects on the brain fine structure

mm² lateral to the chemically damaged areas were dissected out as depicted in Fig. 1. Each dissected piece of the striatum was osmicated, dehydrated in graded alcohols, embedded in a mixture of Epon and Araldite, cut on an ultramicrotome, mounted onto grids, and then stained with lead citrate and uranyl acetate.

Results

At the light microscopic level, all the tissue compartments appeared to be destroyed in an area of approximately 500 μ m around the injection site 1 day after 125 nmol L-alpha-AA injection (Fig. 1a). The size of this chemically damaged area increased with the increased concentration of the toxic chemical. With a 3 day survival period, the tissue hole created by the chemical injection was mostly filled with a number of small cells which aggregated into the damaged area (Fig. 1b). 7 days following the injection, the damaged area around the injection site, was completely filled with large numbers of these small cells (Fig. 1c). We could not determine, however, the identity of these small cells nor classify them at the light microscopic level.

At the fine structural level, astrocytes were the only cell type that showed clear degeneration 1 day after L-alpha-AA injection. In fact, several membraneous inclusions could be detected in the damaged, vacuolated cytoplasm, while medium-sized neurons and microglia situated immediately adjacent to the damaged astrocyte, were quite normal in their morphology (Fig. 2a). A micrograph taken adjacent to the areas of Fig. 2a is shown in Fig. 2b, in which both large neuronal soma and oligodendrocyte can be seen are quite intact at the fine structural level. A more advanced stage of degeneration of astrocyte is shown in Fig. 2c, in which a number of vacuoles and membraneous inclusions were detected in the affected, swollen cytoplasm. In this degeneration stage, swelling and severe vacuolization with membraneous inclusions were also observed in the astrocytic processes surrounding intact endothelial cells (Fig. 2d). With the same or higher concentrations of the D-isomer of alpha-AA, however, there was no noticeable fine structural destruction of any cell type including astrocytes.

At 7 days after L-alpha-AA injection, there appeared a large number of active astrocytes and their processes containing distinct bundles of astroglial filaments (Fig. 3a). In addition to the changes of astrocytes, many microglia were also detected around injection sites with both the 3 and 7 day survival periods. These microglia were invariably surrounded by several layers of membraneous structures, which might be attributed to membranes of degenerating astroglia (Fig. 3b).

With a 3 day survival period, an intermediate picture could be obtained. Thus, there existed a whole sequence of degeneration and recovery processes of astrocytes. Included were totally disintegrated cells with many vacuoles and cell debris, cells with a few vacuoles and several bundles of glial filaments and cells with intact cytoplasm and tremendous numbers of filamentous glial bundles (not illustrated).

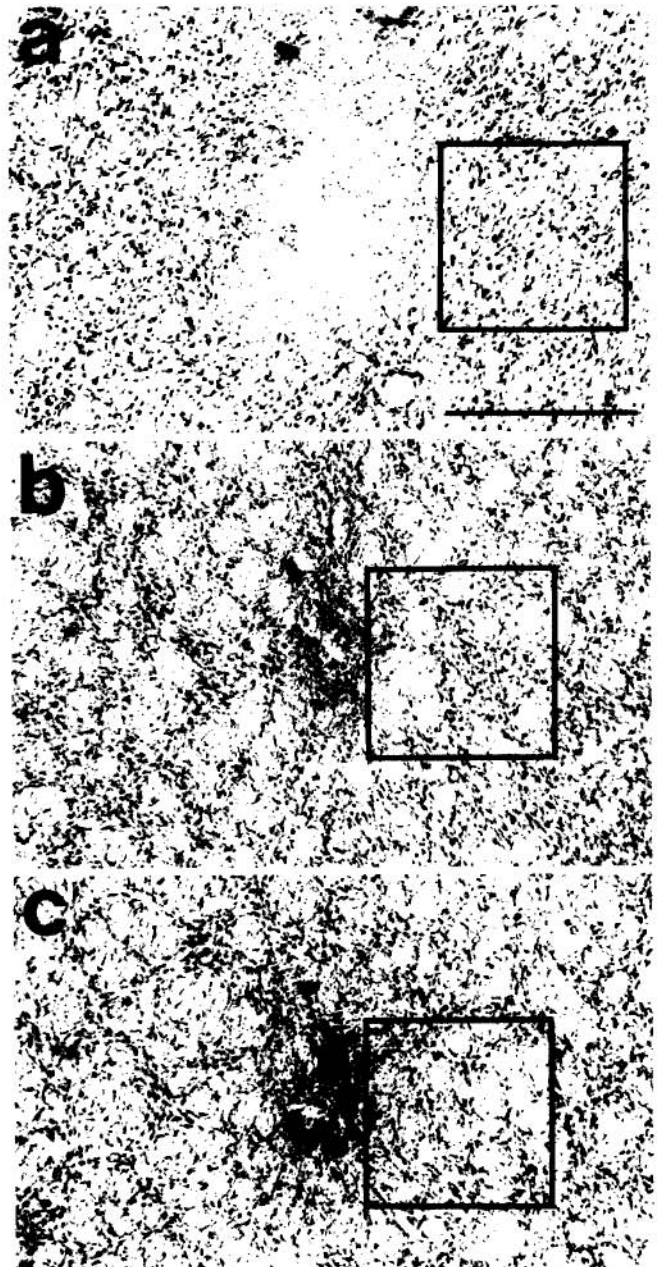


Fig. 1. Injection sites of 125 nmol L-alpha-AA in the rat striatum. Light micrographs were taken at 1 (a), 3 (b) and 7 (c) days after the stereotaxic injection of the toxic chemical. With the 3 and 7 day survival periods, a number of small cells aggregated into the chemically damaged areas. Corresponding rectangular areas were dissected out from neighboring unstained Vibratome sections and processed for electron microscopic analysis. Scale bar, 500 μ m.

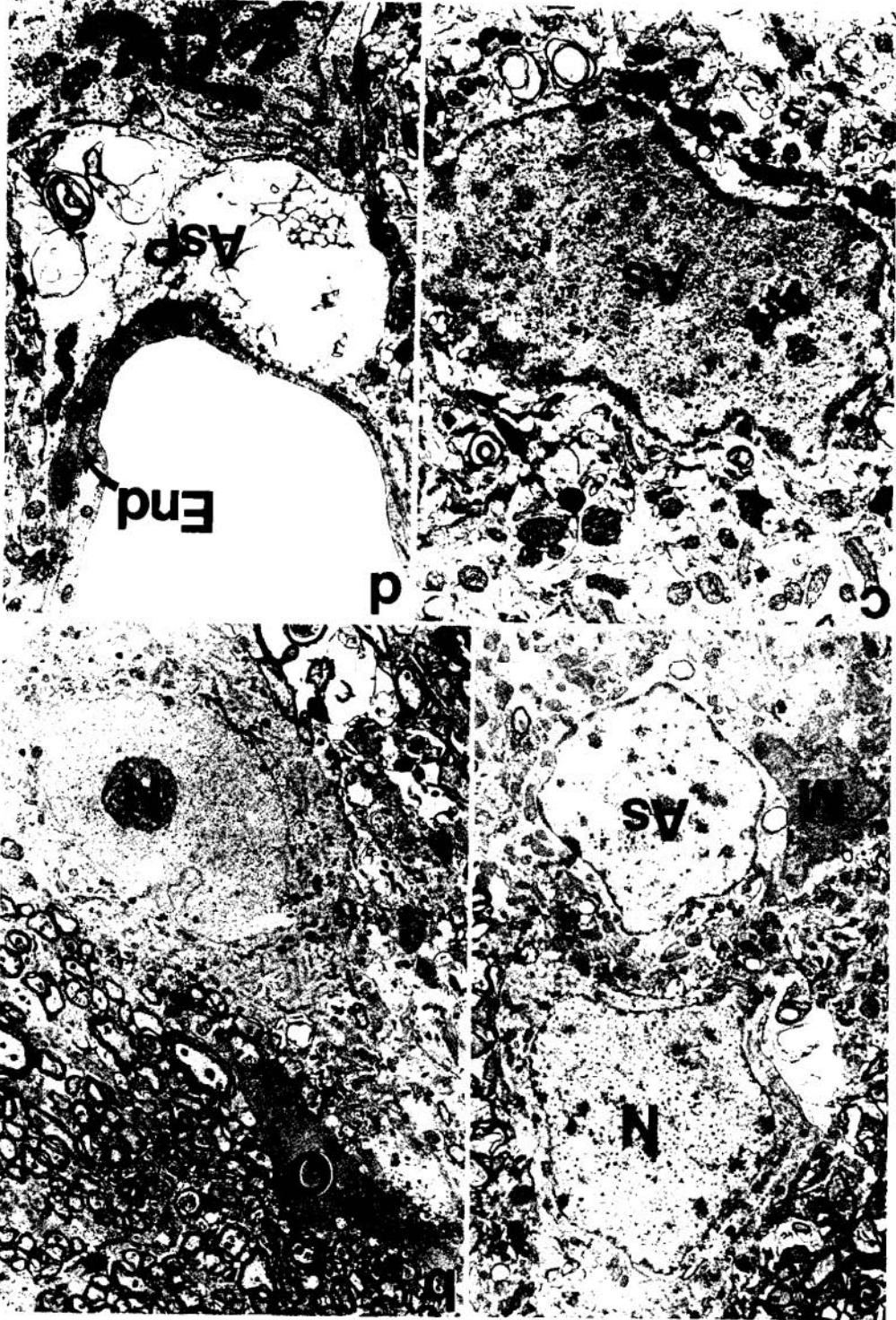


Fig. 2. Electron micrographs taken 1 day after the intrastriatal injection of 125 nmol L-alpha-Asp. a. Three distinct types of cells were detectable, of which only the astroglia (As) showed degenerating profiles with cytoplasmic vacuolization and membrane inclusions. Medium-sized neurons (N) and microglia (M), immediately adjacent to degenerating astroglia, were intact at the fine structural level. b. Micrograph taken from adjacent area to Fig. 2a. No degenerating profiles were detectable in both a large neuron (N) and an oligodendroglial cell (O). c. More advanced stage of degeneration of astroglial cytoplasm, with numerous vacuoles and membrane inclusions (As). d. Maximum state of vacuolization in the astrocytic process (Asp) surrounding an intact endothelial cell (End). Asp contained only cell debris and membrane inclusions in heavily vacuolized cytoplasm. Magnifications: a, x 4,700; b, x 3,800; c, x 8,000; d, x 11,600.

Gliotoxic effects on the brain fine structure

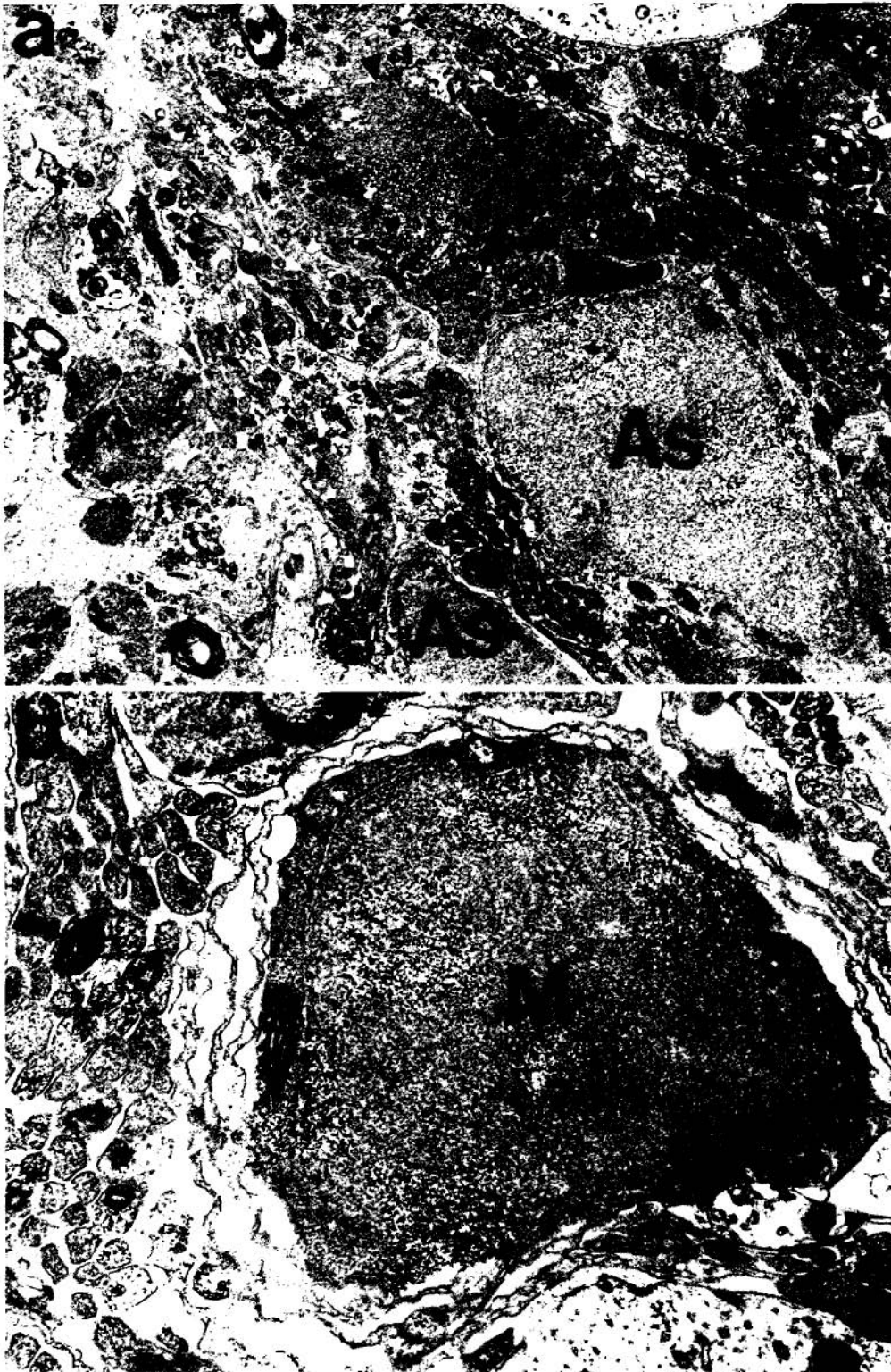


Fig. 3. 7 days after the intrastriatal injection of 125 nmol L-alpha-AA. a. Recovery of astrocytes (As) with many bundles of astroglial filaments (arrowheads). b. An example of microglia (M) which might have invaded areas occupied by degenerating astroglia. Those microglia were invariably surrounded by several layers of membrane structures which supposedly originated from membranes of lost astroglia. Magnifications, a, x 7,200; b, x 20,000

Discussion

The present study demonstrates that astrocytes were the only cell type which showed clear and intense degeneration at the fine structural level 1 to 3 days after L-alpha-AA injection into the rat striatum. This phenomenon was quite specific in the sense that only astrocytes were destroyed with a considerable amount of cytoplasmic vacuolization, leaving all the cell types other than astrocytes (neurons, oligodendroglia, microglia and endothelial cells) intact. This also implies that the astrocytic degeneration was not due to the simple physical damage induced by injection procedure, but was attributable to the specific chemical action. At 3 to 7 days after injection of the gliotoxin, there appeared a great number of active astrocytes, which is indicative of astroglial recovery from degeneration. In addition to the structural changes of astrocytes, many microglia were also seen to be surrounded by several layers of membranous structures, which might come from degenerating astroglial membranes. This presumably indicates that the microglia invaded and occupied the spaces left by the total disappearance of astrocytic cytoplasm.

The sequence of events which was observed in the present study (astroglial degeneration - microglial invasion - astroglial recovery) suggests that accumulating microglia around injection sites play a certain role in cleaning up degenerating astroglial debris, and that a large number of active astrocytes invade the lesion sites with the longer survival periods. It remains, however, to be determined whether these newly appearing active astrocytes are generated in response to various kinds of CNS damage including astroglial ablation (reactive astrocytes), or in the process of exhibiting reproduction of damaged astrocytes (recovering astrocytes). We have to await further precise experiments in order to answer this question. Additionally, the comparison of D- with L-isomer of the toxin suggests that only L-isomer is effective on specific astroglial degeneration.

In conclusion, alpha-AA is indeed a gliotoxin after its direct intracranial stereotaxic injection into the rat striatum, and its toxic effect is both cell specific and stereospecific. These results are in agreement with previous data from *in vitro* experiments (Huck et al., 1984a,b) or *in vivo* experiments employing subcutaneous administration of the toxin (Olney et al., 1971, 1980).

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