

NEURON-GLIA CROSS TALK IN RAT STRIATUM AFTER TRANSIENT FOREBRAIN ISCHEMIA

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1. INTRODUCTION

Striatum is highly vulnerable to transient forebrain ischemia induced by the 4 vessel occlusion (4VO) method (Brierley 1976, Pulsinelli et al. 1982, Zini et al. 1990a). Massive degeneration and loss of Nissl-stained neurons occur within 24 hr from an ischemia of long duration (30 min) (Pulsinelli et al. 1982). Neuronal loss is mainly restricted to the lateral part of caudate-putamen (Pulsinelli et al. 1982, Zini et al. 1990a). Cellular alterations include loss of medium-size spiny projection neurons (Pulsinelli et al. 1982, Francis and Pulsinelli 1982), largely corresponding to dopaminoceptive neurons (Benfenati et al. 1989, Zoli et al. 1989), and increase in reactive astrocytes (Pulsinelli et al. 1982, Grimaldi et al. 1990) and microglia (Gehrmann et al. 1982). On the other hand, large cholinergic (Francis and Pulsinelli 1982) and medium-size aspiny somatostatin (SS)/neuropeptide Y (NPY)-containing interneurons are resistant to the ischemic insult (Pulsinelli et al. 1982, Grimaldi et al. 1990). In a few instances, such as in the case of SS and NPY immunoreactivity (IR), the initial loss is followed by full recovery within 7 (SS) or 40 (NPY) days post-ischemia (Grimaldi et al. 1990). However, it is not known whether some kind of recovery is present for the bulk of medium-size spiny projections neurons after the first days post-ischemia.

This paper is divided into two parts: in the first, we report a study of the spatiotemporal relationships between neuron loss and recovery and astrocyte activation in the is-

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chemic striatum. In the second, we show some data on the characterization a possible molecular mechanisms linking neuronal lesion with astrocyte activation (i.e., the activation of the polyamine system) after striatal lesion.

2. SPATIOTEMPORAL RELATIONSHIPS BETWEEN NEURON LOSS AND RECOVERY AND ASTROCYTE ACTIVATION AFTER TRANSIENT FOREBRAIN ISCHEMIA

Three striatal cellular populations have been examined by means of immunocytochemistry coupled to computer-assisted image analysis:

- a. vulnerable spiny neurons (labelled for their content of DA and cyclic AMP regulated phosphoprotein mr32, DARPP-32, a phosphoprotein related to the transduction of D1 receptor (Hemmings *et al.* 1987)) which constitute more than 90% of the entire neuronal population.
- b. resistant aspiny neurons (labelled for their content of SS and NPY) which constitute around 1% of the entire neuronal population. They also contain high levels of nitric oxide synthase (NOS) (Vincent and Kimura 1992, Kawaguchi *et al.* 1995) and are known to be spared in a number of models of brain injury (Beal *et al.* 1986) and human brain pathology (Ferrante *et al.* 1986).
- c. reactive astrocytes, labelled for their increased content of glial fibrillary acidic protein (GFAP), an intermediate filament protein specific for differentiated astrocytes (Eng *et al.* 1985, Eng and De Armond 1982). Its increase is a marker of astroglial reaction to various types of brain injury (Grimaldi *et al.* 1990, Eng and De Armond 1982, Ludwin 1985, Bignami *et al.* 1980, Mathewson and Berry 1985, Brock and O'Callaghan 1987).

The 4VO method of Pulsinelli and Brierley (1982) was used to induce transient forebrain ischemia, as previously described (Zini *et al.* 1990a). Only those animals immediately losing their righting reflex, being unresponsive for 20 to 30 min after bilateral carotid occlusion, with an isoelectric electroencephalographic activity within 2–3 min after carotid artery occlusion and without recovery throughout the ischemic period, were studied. All the procedures used were in accordance with institutional (italian Ministero della Sanità) guidelines for animal care. Several times (4 hr, 1, 7 and 40 days, 8 months) after 30 min of forebrain ischemia, induced by 4VO were investigated.

Immunocytochemistry was performed as previously described (Agnati *et al.* 1988), using the avidin-biotin technique. Six sections/animal for each antiserum were taken at various coronal levels of the pre-commissural striatum (regularly spaced from bregma 1.7 to 0.2 mm, according to Paxinos and Watson 1982). The following primary antisera were used: mouse monoclonal antibody against DARPP-32 (16), rabbit polyclonal antiserum against GFAP (Dako, Glostrup, Denmark, lot no. 015), rabbit polyclonal antiserum against SS (Johansson *et al.* 1984), rabbit polyclonal antiserum against NPY (Peninsula, Merseyside, U.K., lot no. 006802–3) which have been previously characterized. The antisera were used in a dilution of 1:2000 for DARPP-32, 1:300 for GFAP, 1:1500 for NPY and SS.

Morphometric and microdensitometric analyses of the histological preparations were performed by means of an automatic image analyzer (IBAS I-II, Zeiss Kontron, Munich, FRG) (Zoli *et al.* 1990). Both a global microdensitometric analysis of DARPP-32 IR and manual count of DARPP-32 ir cells in 40x microscope fields (153x117 μm) were per-