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ISQUEMIA CEREBRAL TRANSITORIA EN EL JERBU COM A MODEL DE MALALTIA ISQUEMICA CEREBRAL: ESTUDI DE NEURONES INHIBIDORES CORTICALS DE CIRCUIT LOCAL I DE MECANISMES DE MORT CEL·LULAR.

Memòria per a optar al Grau de Doctor, presentada per Avelina Tortosa i Moreno.

Capítol 6

INCREASED B-AMYLOID PRECURSOR PROTEIN EXPRESSION IN ASTROCYTES IN THE GERBILS HIPPOCAMPUS FOLLOWING ISCHEMIA. ASSOCIATION WITH PROLIFERATION OF ASTROCYTES

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III.6 INCREASED B-AMYLOID PRECURSOR PROTEIN EXPRESSION IN ASTROCYTES IN THE GERBILS HIPPOCAMPUS FOLLOWING ISCHEMIA. ASSOCIATION WITH PROLIFERATION OF ASTROCYTES

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III.6.1 ABSTRACT

Increases in APP proteins, which contain the ßA4 senile plaque protein present in patients with Alzheimer disease (AD), have been shown to occur in models of neuronal damage and neurotoxic cell injury. This observation lead us to examine the espression of these proteins after transient ischemic episodes in the gerbils. Animals sacrified 2 to 28 days after ischemia , and APP detected by immunocytochemistry at the light microscopic level with an antibody raised against the C-terminal region of these proteins. The gliotic reaction was also examined using glial fibrillary acid protein (GFAP) immunoreactivity. Two days after ischemia neuronal cell death was observed in the hippocampal CA1 region accompained by astrocyte hypertrophy. These hypertrophic astrocytes were found to be GFAP positive but stained weakly for APP. Seven days after ischemia both astrocyte hypertrophia and hyperplasia, with identified mitotic figures, were observed. These hyperplasic astrocytes were intensely stained by the APP antibody. These stained APP positive astrocytes were observed up to 28 days after ischemia. These results show that neuronal cell death produced by transient ischemia is followed by an increased APP expression which appears to be associated with the hyperplasic astrocytes but not with the initial hypertrophy of this cell population. These results when taken together with those obtained in other models of neuronal damage or death, clearly suggest that APP expression follows neuronal death and is associated with astrocyte proliferation.

Key words: Brain ischemia, ßamyloid precursor protein, Astrocyte, Alzheimer's disease, gerbil brain, hippocampus, glial fibrillary acidic protein.

III.6.2 INTRODUCTION

ß-Amyloid precursor proteins (APP) form a family of polypeptides all encoded by the same gene localized on chromosome 21. Abnormal expression or proteolysis of APP has been proposed as being responsible for ß-amyloid deposition and senile plaque formation in the brains of patients with Alzheimer-type dementia (AD) (Selkoe, 1991; Joachim and Selkoe, 1992). The origin of the ß-amyloid plaque has not yet been resolved since potentially all cells in the central nervous system, including neurons, astrocytes, microglia, oligodendroglia anf endothelial cells, express and contain the APP mRNAs and their products and may be the primary source of ß-amyloid (Bahmanyar et al., 1987; Tanzi et al., 1988; Card et al., 1988; Higgins et al., 1988; Kawarabayashi et al., 1991a; Manning et al., 1988; Mita et al., 1989; Shivers et al., 1988).

Modifications in the APP expression pattern have been demonstradted in several central nervous system injuries such as kainic and ibotenic acid injection (Siman et al., 1989; Kawarabayashi et al., 1991b; Shigematsu et al., 1992; Nakamura et al., 1992), stab lesion (Otsuka et al., 1991), axotomy (Palacios et al., 1992; Solá et al., 1993b), and colchicine administration (Shigematsu and Mc Geer, 1992).

It is well known from structural and ultrastructural studies that hippocampal pyramidal neurons in the CA1 sector are selectively vulnerable to ischemia after both short and long survival times (ito et al., 1975; Kirino, 1982; Kirino and Sano, 1984a,b; Petito and Pulsinelli, 1984a,b; Yamamoto et al., 1986a; Mudrick and Baimbridge, 1989; Kirino et al., 1990; Bonnekoh et al., 1990; Yamamoto et al.,

1990; Schmidt-Kastner and Freund, 1991; Desphande et al., 1992; Bonnekoh et al., 1992). However, a small population of pyramidal neurons present in the CA1 region and GABAergic interneurons may escape injury (Nitsch et al., 1989; Bonnekoh et al., 1992; Tortosa and Ferrer, 1993). Post-ischemic neuronal damage is accompanied by astrocyte activation and increases in glial fibrillary acidic protein (GFAP) expression and immunoreactivity (De Leo et al., 1987; Hatakeyama et al., 1988; Yamamoto et al., 1986b; Yoshimine et al., 1985; Petito et al., 1990; Schmidt-Kastner et al., 1990; Rischke and Krieglstein, 1991; Kindy et al. 1992).

Up until now, limited information has been available on the participation and function of APP protein and its mRNA in post-ischemic changes (Abe et al., 1991; Stephenson et al., 1992; Wakita et al., 1992).

In the present study we have used a well characterized polyclonal antibody raised against the carboxy terminal sequence (Palacios et al., 1992) common to all known forms of APP, to examine selective changes in APP immunoreactivity following hippocampal ischemia in the gerbil.

III.6.3 MATERIALS AND METHODS

Animals surgery and tissue processing

Male Mongolian gerbils (Meriones unguiculatus) from our own colony (aged from 3 to 6 months) were used in this study, and kepts in conditions previously reported by Tortosa and Ferrer, (1993). The animals were initially anaesthetized with 3% halothane mixed with room air and subsequently reduced to 1.5% for manteinance during the operation. The carotid arteries were exposed through a midline cervical incision and were temporarily occluded with small clips for 20 min. Anaesthesia was discontinued whilst the clips were in place. The animals were the anaesthetized again, the clips removed and the incisions sutured with silk. The absence of blood flow during occlusion and the reperfusion periods were controlled visually. During the operation the body temperature was monitored and maintained at 36-37°C (Freund et al., 1990). Sham-operated controls were treated identically except that occlusion was not performed. Following ischemia, gerbils were allowed to survive for 2,7,15, and 28 days. The animals were divided in groups of two and under ether anasthesia the brains were perfussed transcardially firts with physiological saline for 2 min and then with 150 ml of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1M phosphate buffer pH 7.4 (PB) for 20 min. The brains were removed, further fixed for 3-4 h at 4°C by immersion in the same fixative, and left overnight in 5% sucrose in PB at 4°C. Coronal sections (40 μm) were cut with vibratome (Lancer) at the hippocampal region level. Sections were washed for 12 h in PB at 4°C.

Antisera

An antibody against APP was obtained by immunizing New Zeland female rabbits with synthetic peptide fragment of the C-terminal end of APP (APP 676-695), a sequence common to all knoen forms of APP. The specificities and characterization of this antibody have been previously described (Palacios et al., 1992). Polyclonal antibody against glial fibrillary acidic protein (GFAP) was obtained commercially from Dako (Denmark).

Histological and immunocytochemical procedures

Histological evaluation of the hippocampal damage was carried out on vibratome sections stained with Cresyl violet. Immunocytochemistry was performed using the avidin-biotin peroxidase system (ABC). Alternate adjacent coronal sections were separated and treated free floating with 0.3% H_2O_2 in 0.1M phosphate buffer saline (PBS) to block endogenous peroxidase. This was followed by incubation in normal goat serum (diluted 1:100 in PBS) for 90 min. The following protocol was then used: APP an GFAP antibodies (diluted 1:1000 and 1:2000 respectively in PBS containing 1% BSA and 0.4% Triton x-100) incubated for 48 h at 4°C, followed by biotinylated goat anti-rabbit IgG (1:200 dilution Vectastain, Vector) for 2 h at 21°C, the ABC complex was then used (1:100 dilution Vectastain, Vector) for 1 h at 21°C. Peroxidase activity in sections treated with APP antibody was finally visualized with 0.05% 3,3'diaminobenzidine (DAB) and 0.01% H_2O_2 in PBS for 5-10 min. GFAP treated sections were processed with 0.04% 1-naphthol in

PBS for 5 min. Controls were obtained using sections treated in the same way except that the primary antibodies were omitted. The specificity of the APP antibody was established by the blockade of specific staining with the antiserum preabsorbed with 20 ug of purified peptide (see Palacios et al., 1992). The immunostained sections were mounted on slides with Glicerol (Dako) for light microscope observation and photography.

Bloks obtained from punches of the hippocampal CA1 region in both control and post-ischemic animals were postfixed in 1% O_sO_4 for 1 h, block-stained in 1% uranylacetate veronal buffer, dehydrated and embedded in Durcupan (Fluka). Semithin sections (1μm) were cut on a LKB ultratome III and stained with 1% Toluidine blue for light-microscope photography.

III.6.4 RESULTS

Histology

Cresyl violet staining of control hippocampus is shown in Fig. 1A. Two days after ischemia some small areas of pyramidal cell necrosis were observed in the CA1 region (Fig.1B). Complete necrosis of the pyramidal cell in the CA1 subfield was seen 7 days after ischemia and persisted throughout the other reperfusion periods (15 and 28 days) (Figs. 1C,D). However, some surviving pyramidal neurons and well preserved interneurons were observed scattered in the damaged CA1 region as well as a diffuse gliosis in the stratum oriens and radiatum (Figs. 1B, C, D). The CA3, CA4 subfields and dentate gyrus showed no visible changes at any of the post ischemic time points studied (Figs. 1B, C, D).

GFAP immunoreactivity

GFAP immunoreactivity revealed an homogeneous pattern of astrocyte distribution in all hippocampalzones in control animals, showing a well-ordered pattern in dendritic layers (Fig. 2A). A slight astrocyt hypertrophy was seen two days after ischemia (Fig. 2B). GFAP-stained astrocytes appeared in the CA1 lesioned sectors distributed in the strata oriens and radiatum (Fig. 2B). No changes in astrocyte staining were observed in the CA3-CA4 areas (not shown) or in the dentate gyrus. In the stratum moleculare non-reactive astrocytes were observed (Figs. 2B, C, D). At seven day survival after ischemia an increse of GFAP-

immunoreactivity indicated a strong astrocyte hypertrophy in the damaged CA1 areas and this hypertrophy persisted in this region throughout the 4 weeks of this study (Figs. 2C, D). Evidence of increases in GFAP reactivity in cell bodies and their processes indicated the presence of glial hypertrophy in individual astrocytes or in coupled astrocytes frequently observed in the strata oriens and radiatum (Figs. 3A,B). Hypertrophic astrocytes were also more numerous in the pyramidal layer associated with neuronal destruction (Figs. 3A,C). Thick or long processes of the hypertrophic astrocytes frequently showed close contacts with adjacent vessels (Fig. 3C).

The study of semithin sections of plastic embedded tissue demonstrate, in the CA1 damaged areas, proliferative astrocytes with mitotic figures located in all strata. Hyperplasic activity was observed at 7 days after ischemia but not at earlier or later time points (Flg. 3D). Vacuolated hypertrophic astrocytes were specially abundant in animals surviving 28 days after ischemia (Fig. 3E).

APP immunoreactivity

Vibratome sections adjacent to those stained with GFAP were used for APP immunohistochemistry. In control animals, APP-immunoreactivity was seen in pyramidal and granule neurons in all regions of the hippocampus and dentate gyrus (not shown). In contrast, only a few weakly stained astrocytes were observed. Two days after reperfusion, no APP-like immunoreactivity was in focal circumscribed necrotic pyramidal areas of the CA1 region (Fig.4A). Some neurons

showed strong immunoreactivity in their basal processes in the damaged stratum pyramidale (Fig. 4B) and the overall staining pattern of astrocytes was comparables to that seen in controls (Fig. 4B). From 7 to 28 days after ischemia, necrosis of pyramidal neurons was extended to the entire CA1 sector which had lost their APP immunoreactivity (Figs. 4C, D and 5A, B, C). Astrocytes in the strata oriens, pyramidale and radiatum were strongly stained in the CA1 damaged sector (Figs. 4C, D and 5A, B, C) with an increased staining of cell bodies and processes indicating astroglial hypertrophy (Figs. 5A, B, C). Hypertrophic astrocytes were usually more numerous in the necrotic pyramidal cell layer (Figs. 5A, B). Often astrocytes with thick immunostained processes showed a close relationsfip with adjacent vessels (Fig. 5B). Hypertrophic astrocytes showing vacuolated cytoplasm were observed especially in animals left to survive for 28 days (Fig. 5D). This was also seen in semi-thin sections of these astrocytes (Fig 3E). Astrocytes in the stratum lacunosum-moleculare were also strongly stained (Fig. 4C). In the stratum moleculare of the dentate gyrus subjacent to the damaged CA1 region, normal non-reactive astrocytes appear with either faintly stained or non-immunostained processes (Figs. 4A, C and 6D). Intact neurons in the CA3-CA4 sector showed immunostained soma and dendritic processes with strong punctate or filamentous perinuclear structures corresponding to the localization of the golgi apparatus in these neurons (Figs. 6B, C). In the pyramidal layer of CA2 close to damaged CA1 neurons, some intact pyramidal neurons revealed increased staining of cell bodies (Fig 6A). Granule cells of the dentate gyrus also exhibited a weak punctate immunoreactivity (Fig 6D).

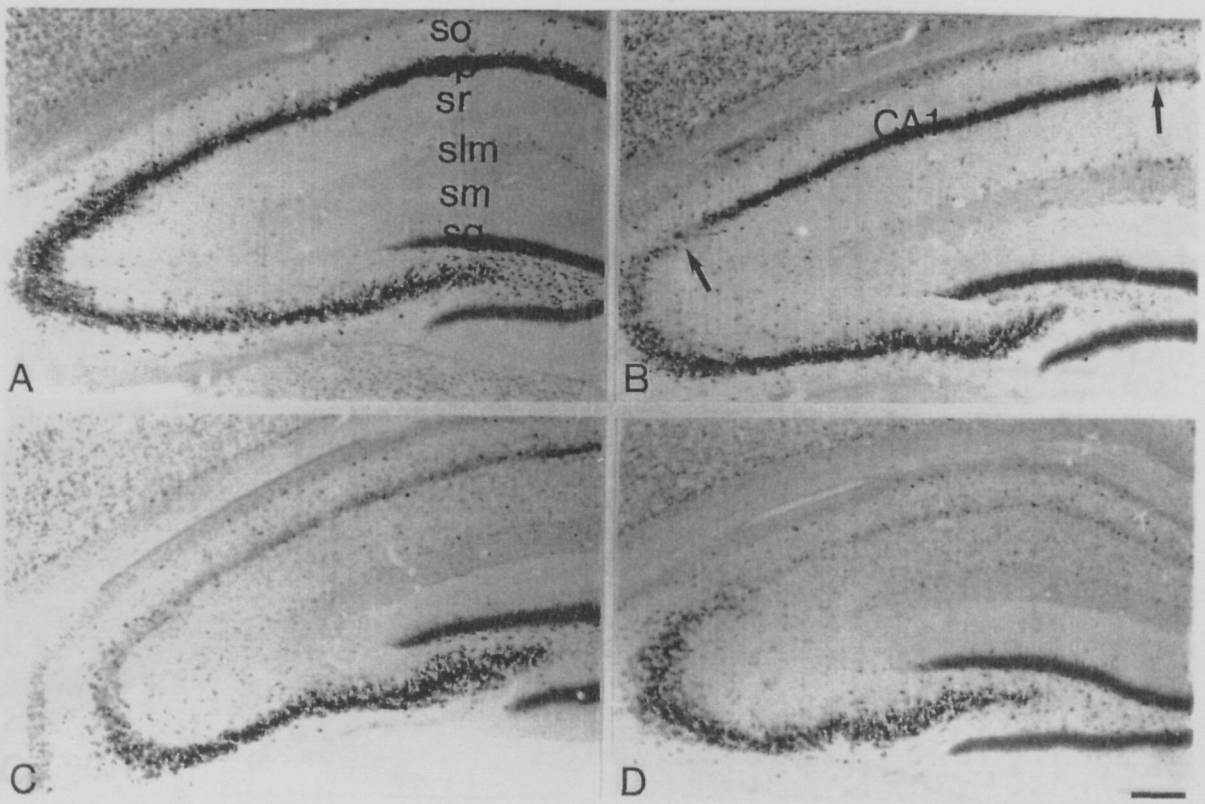

FIGURE 1: Representative micrographs of gerbil hippocampus. Coronal vibratome sections stained with cresyl violet. A: Animal control showing normal cell distribution and staining pattern throughout the CA regions of the hippocampus and dentate gyrus (so) stratum oriens, (sp) stratum pyramidale, (sr) stratum radiatum, (slm) stratum lacunosum moleculare, (sg) stratum granulosum. B: Focal pyramidal cell loss and shrinkage in the CA1 region 2 days post-ischemia (arrows). C and D: Severe neuronal damage is observed in the stratum pyramidale of the CA1 region 7 days and 28 days respectively after ischemia. Gliosis is also seen in the stratum oriens and radiatum of the CA1 damaged sector. Bar = 200 μm.

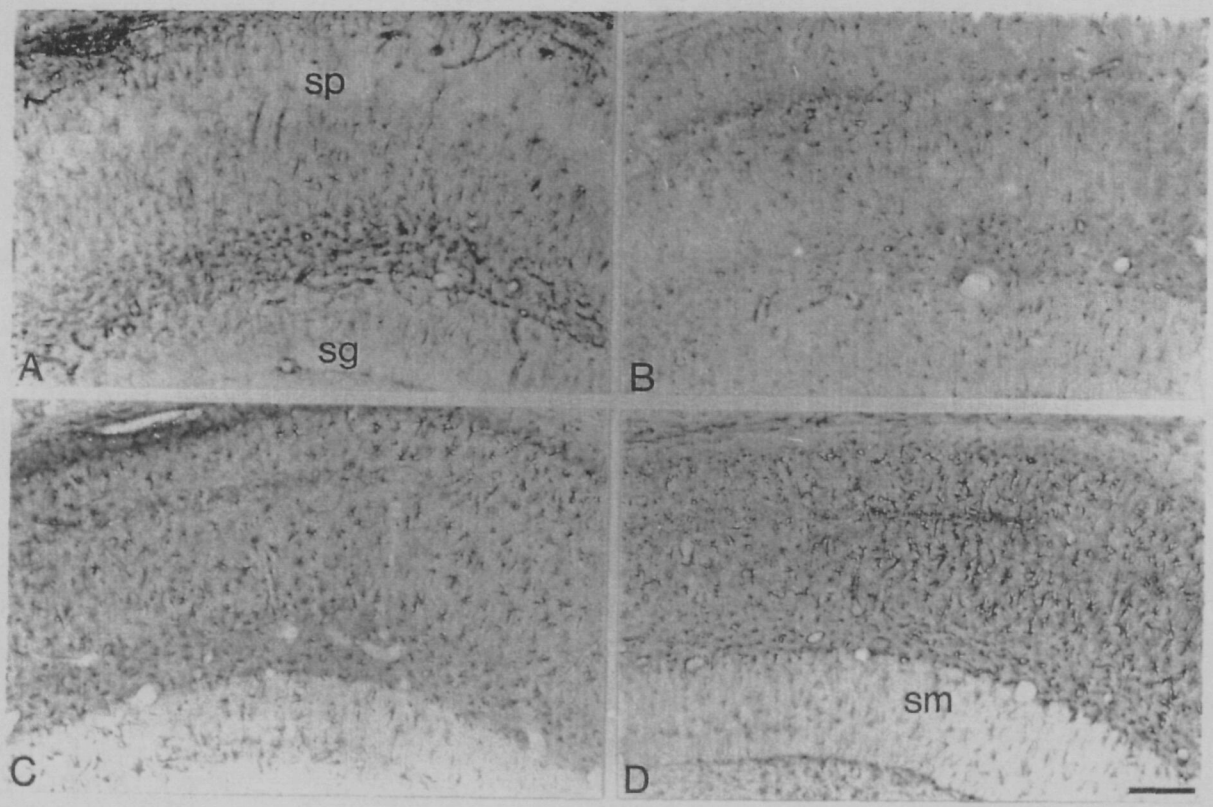

FIGURE 2: Immunohistochemical GFAP staining in the CA1 region of the hippocampus. A: Animal control showing normal astrocytic cells distributed in stratum oriens, radiatum and lacunosum-moleculare of the hippocampus and stratum moleculare of the dentate gyrus. Note the lack of staining in the stratum pyramidale (sp) and granular (sg). B: Moderate increase in astrocyte staining in the stratum oriens and radiatum of the CA1 sector 2 days after ischemia. C and D: Strong increase in staining intensity and apparent number of astrocytes in stratum oriens, pyramidale, and radiatum of the CA1 damaged sector 7 and 28 days respectively after ischemia. Note the normal pattern of astrocyte staining in the stratum moleculare (sm) of the dentate gyrus. Bar = 150 μm.

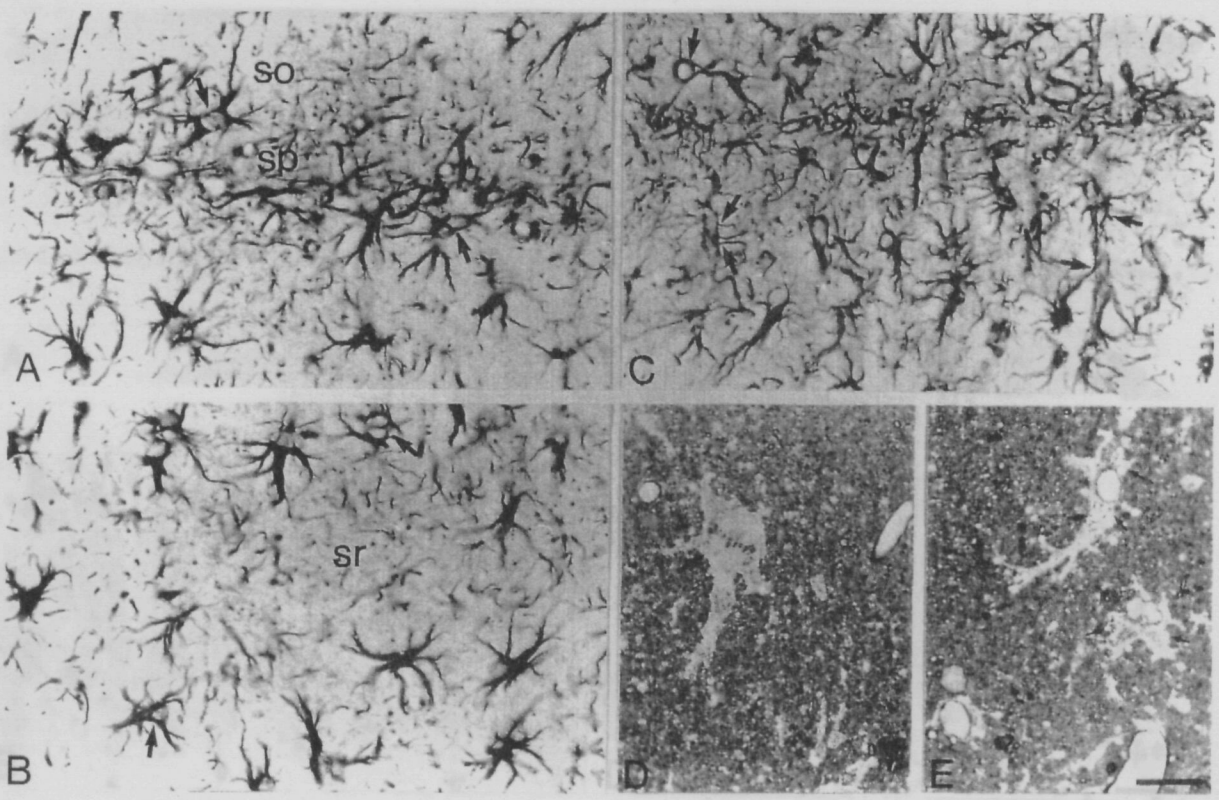

FIGURE 3: Glial fibrillary acid protein (GFAP) immunocytochemistry of CA1 damaged sector. A and B: At 15 days after ischemia hypertrophic astrocytes showed a strong staining in cell bodies and enlarged processes located in the stratum oriens (so), pyramidale (sp), and radiatum (sr). Some coupled or binucleated astrocytes can be seen (arrows). C: The same hypertrophic pattern and localization of astrocytes was demonstrated 28 days after ischemia. Note also some astrocyte processes in contact with adjacent vessels (arrows). D and E: Semi-thin sections obtained from plastic embedded material and stained with toluidine blue. D: Enlarged astrocyte in the stratum eadiatum showing a mitotic figure (metaphase) (arrow) 7 days after ischemia. E: At 28 days following ischemia cytoplasmic vacuolation of hypertrophic astrocytes was frequently found (small arrows). Bar = 30 μm in A, B, C; 15 μm i D, E.

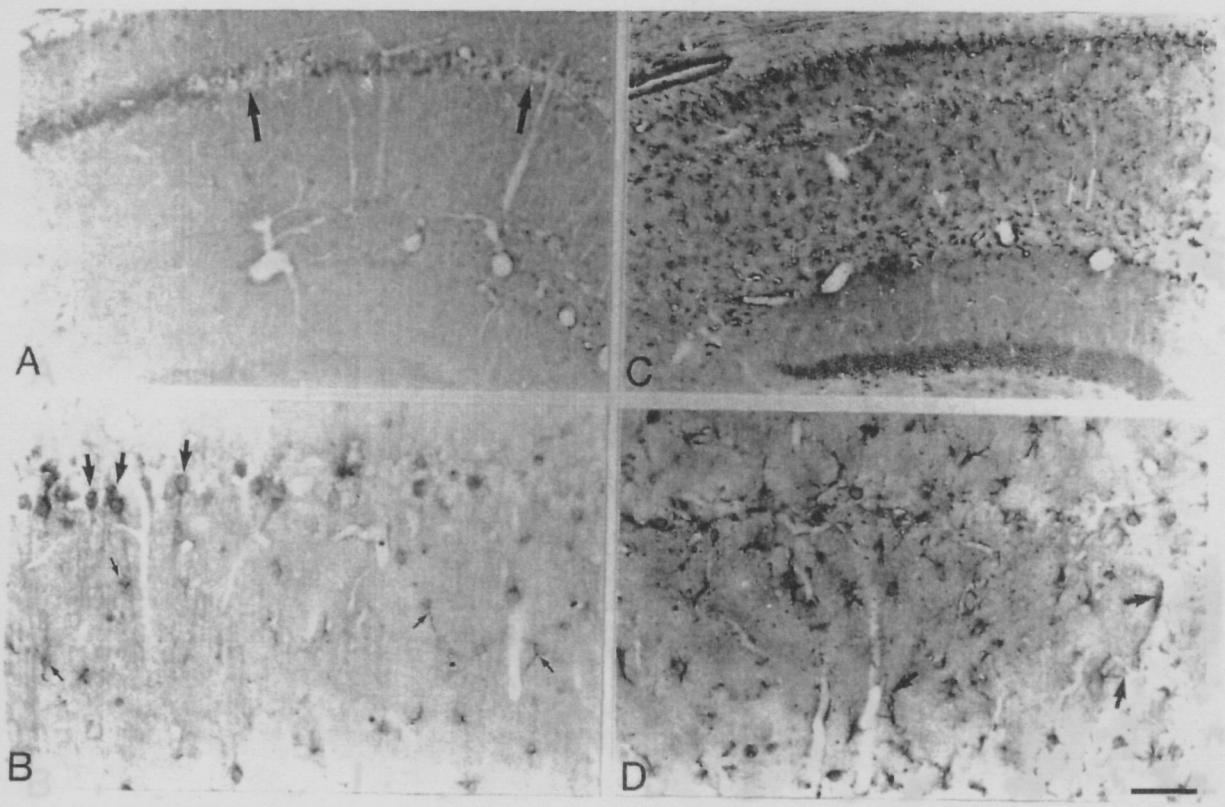

FIGURE 4: Immunocytochemical staining for the APP C-terminal in the hippocampal CA1 region. A: At 2 days after ischemia focal neuronal damage is seen in the stratum pyramidale (arrows). B: Detail at higher magnification showing some surviving pyramidal neurons with immunostained basal processes in the damaged CA1 sector (arrows). Note the presence of weakly immunoreactive cells with the morphology of astrocytes in the stratum radiatum (small arrows). C: At 28 days after ischemia a massive neuronal necrosis in stratum pyramidale of the CA1 region could be observed. Note the stromg increase in APP immunoreactivity in hypertrophic astrocytes in all strata of the CA1 region compared with the nonlesioned molecular and granular layer of the dentate gyrus. D: detail of the former micrograph showing the immunoreactive hypertrophic astrocytes in the stratum oriens, pyramidale and radiatum. Some astrocytes eshibited immunostained processes in contact with capillaries (arrows). Bar = 150 μm in A, C; 60 μm in B, D.

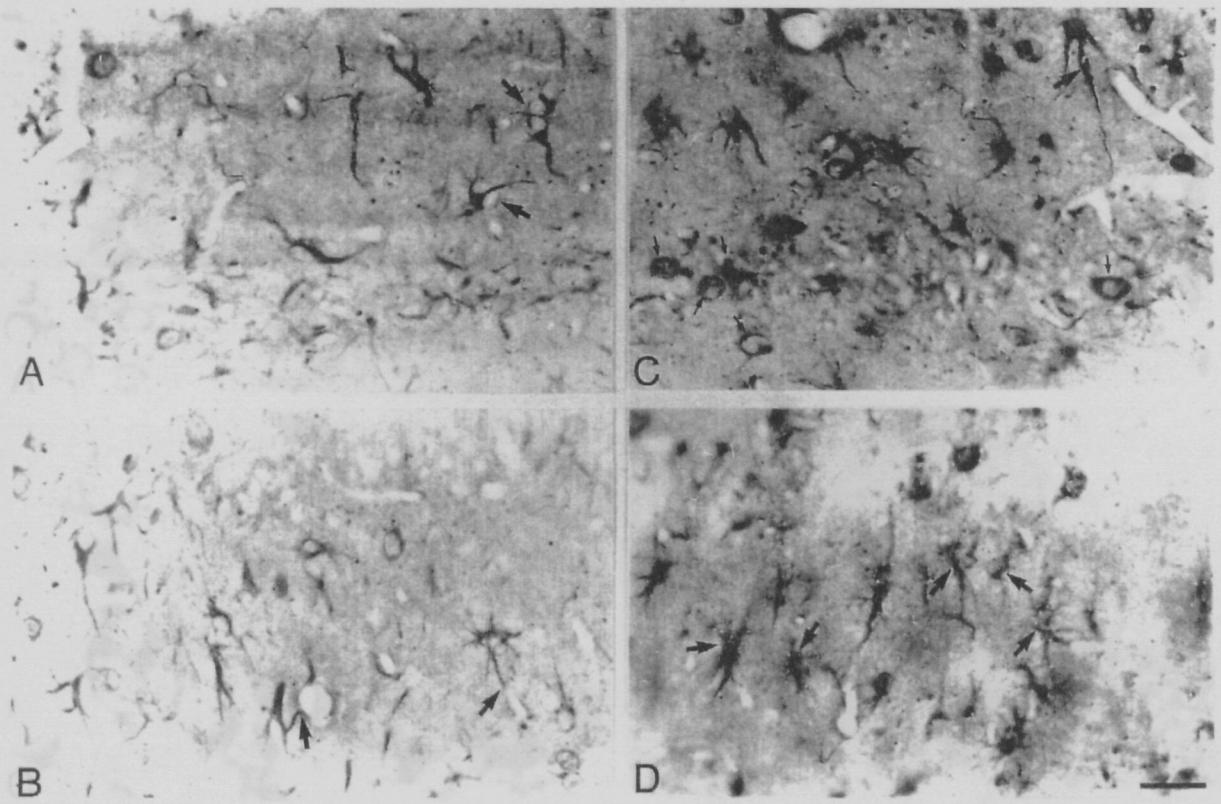

FIGURE 5: High power micrograph of the APP C-terminal immunoreactivity in different sectors of CA1 damaged region. A: At 15 days after ischemia the stratum oriens and pyramidale became occupied by immunostained astrocytes some showing coupled or binucleated figures (arrows). B: The subjacent stratum radiatum eshibited the same pattern with hypertrophic immunostained astrocytes some showing perivascular end-feet (arrows). C: At 28 days after ischemia hypertrophic astrocytes were intensely labeled and some showed long proceses (arrow). Note also some immunostained survival neurons in the pyramidal layer (small arrows). D: In the subjacent stratum radiatum most hypertrophic immunoreactive astrocytes exhibited a vacuolated cytoplasm (arrows). Bar = 30 μm.

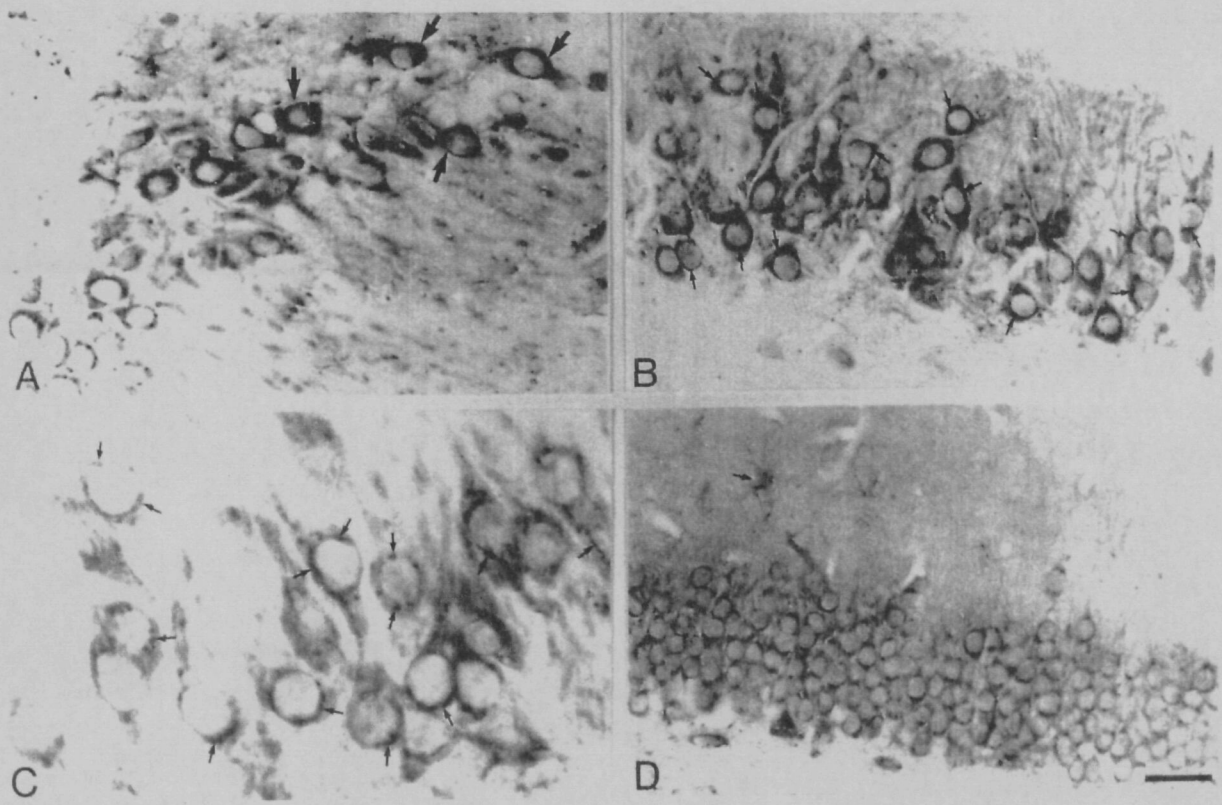

FIGURE 6: *Comparision of APP C-terminal immunoreactivity in different areas of the hippocampus* at 28 days after ischemia. A: Intense labeled pyramidal neurons (arrows) were observed in the CA2 sector adjacent to the necrosed pyramidal neurons of the CA1 region. B: Pyramidal intact neurons of the CA3 region showing immunostained somata and dendritic processes. Note the intense labeled perinuclear punctate structures (small arrows) in some neurons. C: Detail at higher magnification of the immunostained perinuclear or filamentous structures corresponding to the Golgi apparatus (small arrows). D: Dentate gyrus exhibiting APP immunoreactive intact granule neurons and some non reactive astrocyte weakly stained in the molecular layer (small arrow). Bar = 30 μm in A, B, D: 15 μm in C.

III.6.5 DISCUSSION

In the present study we provide structural data showing that long term survival after transient cerebral ischemia in the gerbil results in a dramatic increase in APP and GFAP immunoreactivities in astrocytes located in the CA1 sector of the hippocampus, a region particularly vulnerable to ischemic injury. These changes, especially in astrocyte APP imunoreactivity, are clearly visible 7 days after reperfusion, a period during which the same region also displayed a rapid increase in proliferation (mitotic) activity in astrocytes. These changes remained up to at least 28 days, the longest time studied after reperfusion.

Atroglial reactions after ischemia: GFAP immunohistochemistry

Complex morphological and pathophysiological events which are triggered by ischemia have been described in the hippocampal formation following short and long term reperfusion periods. Our data show that pyramidal neurons in the CA1 region of the hippocampus are selectively vulnerable after ischemia. This observation is in agreement with results found previously, after long and short term ischemia studies in Mongolian gerbils and rats (Ito et al., 1975; Kirino, 1982; Kirino and Sano, 1984a,b; Pulsinelli et al., 1982; Petito and Pulsinelli, 1984a,b; Yamamoto et al., 1986a; Smith et al., 1984; Mudrick and Baimbridge, 1989; Kirino et al., 1990; Bonnekoh et al., 1990; Yamamoto et al., 1990; Schmidt-Kastner and Freund, 1991; Deshpande et al., 1992; Bonnekoh et al., 1992; Tortosa and Ferrer, 1993).

Glial fibrillary acidic protein is a specific marker for astrocytes (Eng, 1985). Previous studies demonstrated an increase in GFAP mRNA and GFAP immunostaining in the first few days after ischemia (Yamamoto et al., 1986b; De Leo et al., 1987; Hatakeyama et al., 1988; Petito et al., 1990; Kindy et al., 1992). Reactive astrocytes in post-ischemic rat brain persist for prolonged periods of time (Petito et al., 1990).

In the present study we have demonstrated astrocytic mitotic proliferation, which was first observed 7 days after ischemia. Astrocytic proliferation has been the subject of some controversy in the ischemic model. Light microscopic studies after [^{3}H] thymidine incorportion or immunoelectron microscopic studies in the rat demonstrated early microglial cell proliferation following ischemia but no GFAP-staining astrocyte proliferation (Petito et al., 1990; Gehrmann et al., 1992). These studies, however, were only carried out at post-ischemia survival times of 3 days. Other autoradiographic studies (Schindler et al., 1987) with [^{3}H] thymidine incorportion have shown post-ischemic astrocyte proliferation in the gerbil. An hypertrophied astrocytes were apparent at 2 days and proliferated from 3 to 7 days post-ischemia in gerbils (du Bois et al., 1985). The results of our experiments suggest that such divergent findings are most probably due to the use of different cerebral ischemia models and/or different post-ischemic times.

APP immunoreactivity in post-ischemic hippocampus. Association to hyperplasic astrocytes

After ischemia a loos of APP in necrotic neuronal cells was observed, while in surviving neurons close to the lesional area APP immunoreactivity appeared increased. In astroglia, APP immunoreactivity was very low in the first 2 days after ischemia and increased dramatically in proliferating astrocytes. No APP immunoreactive microglial cells were observed in either control or injured hippocampus. Thus, a delay was observed between GFAP and APP expression, GFAP preciding that of APP. The expression of APP in immunoreactive astrocytes in different experimental models of degeneration and regeneration in the central nervous system is controversial. In the hippocampus and after kaiic injection Siman et al. (1989) showed an increase in APP and GFAP immunostaining in reactive astrocytes. APP immunoreactivity in astrocytes appears, first, 3 days after Ka administration and increases in intensity over the nest 4 weeks (Siman et al., 1989; Kawarabayashi et al., 1991b). Recent results from our laboratory (Solà et al., 1993a) confirm, in the same experimental conditions, an increase of expresion of APP, GFAP and their mRNAs in reactive astrocytes. Interestingly, increases in GFAP immunostaining and the hybrization signal for GFAP mRNA were observed 1 day after KA infusion while the increases in APP immunoreactivity and their mRNAs were observed 3 days after lesioning (Solà et al., 1993a). These differences in the time course of GFAP and APP mRNA induction have also been observed in the ischemic model. In the hippocampal model, the levels of GFAP and its mRNA in reactive astrocytes were already increased 4h after ischemia and reached peak levels 3 days after lesion (Kindy ey al., 1992). Although Abe et al.

(1991) reported induction of APP mRNA after local cerebral ischemia, recently Wakita et al. (1992) showed a lack of APP immunostaining in glial cells following transisnt cerebral ischemia and explained these negatives results in glial staining by the short period used (30 min - 7 days). However our study clearly demonstrates astrocyte APP immunoreactivity after 7 days of ischemia. On the other hand, we have shown (Palacios et al., 1992; Solà et al., 1993b) that following axotomy of cranial nerves, neurons show an increase in APP immunoreactivity and mRNA and protein expression is observed in association with the glial reaction around the cell bodies of the injured neurons. Surprisingly, the hypertrophic reactive astrocytes surrounding the chromatolytic neurons showed an increase in the expression of the GFAP gene transcript and GFAP immunoreactivity but no changes were observed in astrocyte APP gene expression or APP immunoreactivity (Palacios et al., 1992; Solà et al., 1993b). In agreement with these findings, it has been shown that astrocytes are able to proliferate following KA induced neuronal lesion in the hippocampus (Murabe et al., 1981; Murabe et al., 1982). In addition, astrocyte proliferation has also been shown in the hippocampal formation after the selective ischemic destruction of pyramidal neurons (Du Bois et al., 1985; Schilder et al., 1987). However, after axotomy, the regenerative response of motor neurons progresses showing an hypertrophic but not proliferative astrocyte response (Graeber et al., 1988).

APP and astrocytes: functional correlates

Although the physiological function of APP in the brain is unknown, some studies have demonstrated that these proteins could play a role in processes such as cell adhesion and cell proliferation (Shivers et al., 1988; Saitoh et al., 1989; Schubert et al., 19891, b; Breen et al., 1991; Le Blanc et al., 1992). Tanzi and coworkers (1988) also suggest that APP plays a role in cell growth and differentiation. A trophic effect of the protein on cortical neurons in cultures has also been demonstrated (Araki et al., 1991). Other data indicate that astrocytes cultured for 3 weeks expressed APP mRNA and that proliferation of glial cells promote the expression of APP mRNA (Forloni et al., 1992). APP proteins and mRNAs have been detected in astrocytes *in vivo* and *in vitro* preparations under basal and experimental conditions (Card et al., 1988; Siman et al., 1989; Mita et al., 1989; Golde et al., 1990; Berkenbosch et al., 1990; Ohyagi et al., 1990; Kawarabayashi et al., 1991a, b, Haass et al., 1991) showed that astrocytes and microglia synthesize substantial amounts of APP. Pulse labelling and immunocytochemical analysis demonstrated in these studies (Haass et al., 1991) that APP is turned over rapidly (with a half-life of about 30-45 min) in astrocytes and localized in intracellular vesicles with an apparent lack of insertion at the cell surface. These authors consider that APP in astrocytes may have an intracellular function rather than a role in ß-amyloid deposition throughout an aberrant processing. However, a recent report shows that soluble ß-amyloid peptides are normally secreted by a wide variety of cultured cells, human astrocytes being those that generate tha highest levels (Busciglio et al., 1993). In this sense, both reactive astrocytes and activated microglia have been associated with ß-amyloid

deposits produced in plaques occurring in Alzheimer's disease (Probst et al., 1987; Itagaki et al., 1989; Rozemuller et al., 1989; Wisniewski et al., 1989; Yamaguchi et al., 1991a, b; Mc Geer et al., 1992; Frederickson, 1992). The astrocytic ability to perform the proteolytic cleavage of APP proteins has been suggested by some authors (Rozemuller et al., 1986). In addition, recent experiment have shown a re-expression of the glial derived nexin (GDN), a serine protease inhibitor, in hippocampal astrocytes after transient ischemia in rats and gerbils (Hoffmann et al., 1992; Nitsch et al., 1993). GDN is an inhibitor of a secretase participating in APP normal cleavage, this inhibition may block the normal cleavage, producing accumulation of APP in reactive astrocytes and potentially pathological breakdown products such as ß-amyloid (Nitsch et al., 1993).

Several forms of APP are generate by alternative splicing (for a review see Selkoe, 1991). Two of these forms contain a domain homologous to the Kunitz protease inhibitors (Tanzi et al., 1988; Selkoe, 1991). Previous work has shown (Abe et al., 1991; Solà et al., 1993a) that after neuronal injury these kunitz containing forms are predominantly expressed and localized to astrocytes (Solà et al., 1993a). Although the antibody used in the present series of experiment does not differentiate among the different APP forms it is tempting to speculate that the observed increased in APP-like immunoreactivity could correspond to Kunitz-containing APP forms. Further work using selective probes or antibodies is necessary to prove these hypothesis.

In conclusion our results, taken together with previous data on APP expression in glial and nerve cells after different models of neuronal injury, point to an

association of enhanced APP expression in proliferating (hyperplasic) rather than hypertrophic astrocytes. These results further support the role for astrocytes in the synthesis and processing of APP in the pathway(s) leading to the formation of senile plaques in Alzheimer's disease.

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III.6.6 REFERENCES

Abe K, Tanki RE, Kogure K (1991) Selective induction of Kunitz-type protease inhibitor domain containig amyloid precursor protein mRNA after persistent focal ischemia in rat cerebral cortex. Neurosci Lett 125: 172-174.

Araki W, Kitaguchi N, Tokushima Y, Ishii K, Aratake H, Shimohama S, Nakamura S, Kimura J (1991) Trophic effect of ß-amyloid prcurson protein on cerebral cortical neurons in culture. Biochem Biophys Res Commun 181: 265-271.

Bahmanyar S, Higgins GA, Goldgaber D, Lewis DA, Morrison JH, Wilson MC, Shankar SK, Gajdusek DC (1987) Localization of amyloid ß-protein messenger RNA in brains from patients with Alzheimer's
disease. Science 237: 77-80.

Berkenbosch F, Refolo LM, Friedrich VL Jr, Casper D, Blum M, Robakis NK (1990) The Alzheimer's amyloid precursor protein is produced by type I astrocytes in primary cultures of rat neuroglia. J Neurosci Res 25: 431-440.

Bonnekoh P, Barbier A, Oschlies U, Hossmann K-A (1990) Selective vulnerability in the gerbil hippocampus: morphological changes after 5-min ischemia and long survival times. Acta Neuropathgol 80: 18-25.

Bonnekoh P. Oschlies U, Hossmann K-a (1992) Changes in hippocampal ultrastructure after ischemia with long survival times. In: Maturation phenomenon

in cerebral ischemia (Ito U, Kirino T, Kuroiwa T, Klatzo I, eds) Berlin, Springer-Verlag pp 33-39.

Breen KC, Bruce M, Anterton BH (1991) Beta amyloid precursor protein mediates neuronal cell-cell and cell-surface adhesion. J Neurosci Res 28: 90-100.

Busciglio J, Gabuzda DH, Matsudaira P, Yankner BA (1993) Generation of ß-amyloid in the secretory pathway in neuronal and nonneuronal cells. Proc Natl Acad Sci USA 90: 2092-2096.

Card JP, Meade RP, Davis LG (1988) Immunocytochemical localization of the precursor protein for ß-amyloid in the rat central nervous system. Neuron 1: 835-846.

DeLeo J., Toth L., Schubert P., Rudolphi K. and Kreutzberg G.W. (1987) Ischemia-induced neuronal cell death, calcium accumulation, and glial response in the *hippocampus of the Mongolian gerbil and protection by propentofylline* (HWA 285). J Cereb Blood Flow Metab 7: 745-751.

Deshpande J., Bergstedt K., Lindén T., Kalimo H. and Wieloch T. (1992) Ultrastructural changes in the hippocampal CA1 region following transient cerebral ischemia: evidence against programmed cell death. Exp Brain Res 88, 91-105.

Du Bois M, Bowman PD, Goldstein GW (1985) Cell proliferation after ischemic injury in gerbil brain. An immunocytochemical and autoradiographic study. Cell Tissue Res 242: 17-23.

Eng LF (1985) Glial fibrillary acidic protein (GFAP): the major protein of glial intermediate filaments in differentiated astrocytes. J Neuroimmunol 8: 203-214.

Forloni G, Demicheli F, Giorgi S, Bendotti C, Angeretti N (1992) Expression of amyloid precursor protein mRNAs in endothelial, neuronal and glial cells: modulation by interleukin-1. Mol Brain Res 16: 128-134.

Frederickson RCA (1992) Astroglia in Alzheimer's disease. Neurobiol Aging 13: 239-253.

Freund T.F., Buzsáki G., Leon A. and Somogyi P. (1990b) Hippocampal cell death following ischemia, effects of brain temperature and anaesthesia. Exp Neurol 108, 251-260.

Gehrmann J, Bonnekoh P, Miyazawa T, Oschlies U, Dux E, Hossmann K-A, Kreutzberg GW (1992) The micrtoglial reaction in the rat hippocampus following global ischemia: immuno-electronmicroscopy. Acta Neuropathol 84: 588-595.

Golde TG, Estus S, Usiak M, Younkin LH, Younkin SG (1990) Expression of ß-amyloid protein precursor m RNAs: recognition of a novel alternatively spliced form and quantification in Alzheimer's disease using PCR. Neuron 4: 253-267.

Graeber MR, Tetzlaff W, Streit WJ, Kreutzberg GW (1988) Microglial cells but not astrocytes undergo mitosis following rat facial nerve axotomy. Neurosci Lett 85: 317-321.

Hass C, Hung AY, Selkoe DJ (1991) Processing of ß-amyloid precursor protein in microglia and astrocytes favours an internal localization over constitutive secretion. J Neurosci 11: 3783-3793.

Hatakeyama T, Matsumoto M, Brengman JM, Yanagihara T (1988) Immunohistochemical investigation of ischemic and post-ischemic damage after bilateral carotid occlusion in gerbils. Stroke 19: 1526-1534.

Higgins GA, Lewis DA, Bahmanyar S, Goldgaber DC, Gajdusek C, Young WG, Morrison JH, Wilson MD (1988) Differential regulation of amyloid-ß-protein mRNA expression within hippocampal neuronal subpopulation in Alzheimer's disease. Proc Natl Acad Sci USA 85: 1297-1301.

Hoffmann M-C, Nitsch C, Scotti AL, Reinhard E, Monrad D (1992). The prolonged presence of glia-derived nexin, an endogenous protease inhibitor, in the hippocampus after ischemia-induced delayed neuronal death. Neurosciee 49: 397-408.

Itagaki S, Mc Geer PL, Akiyama H, Zhu S, Selkoe DJ (1989) Relationship of microglia anf astrocytes to amyloid plaques od Alzheimer disease. J Neuroimmunol 24: 173-182.

Ito U., Spatz M., Walker J.T. and Klatzo I. (1975) Experimental cerebral ischemia in Mongolian gerbils. I. Light microscopic observations. Acta Neuropathol 32, 209-223.

Joachim CL, Selkoe DJ (1992) The seminal role of ß-Amyloid in the pathogenesis of Alzheimer disease. Alzheirmer Disease and Associated Disorders 6: 7-14.

Kawarabayashi T, Shoji M, Harigaya Y, Yamamuchi H, Hirai S (1991a) Amyloid ß/A4 protein precursors is widely distributed in both the central and perpheral nervous systems of the mouse. Brain Res 552: 1-7.

Kawarabayashi T, Shoji M, Harigaya Y, Yamamuchi H, Hirai S (1991b) Expression of APP in the early stage of brain damage. Brain Res 563: 334-338.

Kindy MS, Bhat AN, Bhat NR (1992) Transient ischemia stimulates glial fibrillary acid protein and vimentin gene expression in the gerbil neocortex, striatum and hippocampus. Mol Brain Res 13: 199-206.

Kirino T. (1982) Delayed neuronal death in the gerbil hippocmpus following transient ischemia. Brain Res 239, 57-69.

Kirino T. and Sano K. (1984a) Selective vulnerability in the gerbil hippocampus following transient ischemia. Acta Neuropathol 62, 201-208.

Kirino T. and Sano K. (1984b) Fine structural nature of delayed neuronal death following ischemia in the gerbil hippocampus. Acta Neuropathol 62, 209-218.

Kirino T, Tamura A, Sano K (1990) Chronic maintenance of presynaptic terminals in gliotic hippocampus following ischemia. Brain Res 510: 17-25.

Le Blanc AD, Kovacs DM, Chen HY, Villare F, Tykocinski M, Gambetti A, Gambetti P (1992) Role of amyloid precursor protein (APP): study with antisense transfection of human neuroblastoma cells. J Neurosci Res 31: 635-645.

Manning RW, Reid CM, Lampe RA, Davis LG (1988) Identification in rodents and other species of an mRNA homologous to the human ß-amyloid precursor. Mol Brain Res 3: 293-297.

Mc Geer PL, Akiyama H, Kawamata T, Yamada T, Walker DG, Ischii T (1992) Immunohistochemical localization of beta-amyloid precursor protein sequences in Alzheimer and normal brain tissue by light and electron microscopy. J Neurosci Res 31: 428-442.

Mita S, Schon EA, Herbert J (1989) Long-term structural changes in the rat hippocampal formation following cerebral ischemia. Brain Res 493: 179-184.

Murabe Y, Ibata Y, Sano Y (1981) Morphological studies on neuroglia IV. Proliferative response of non-neuronal elements in the hippocampus of the rat to kainic acid-induced lesions. Cell Tissue Res 216: 569-580.

Nakamura Y, Takeda M, Niigawa H, Hariguchi S, Nishimura T (1992) Amyloid ß-protein precursor deposition in rat hippocampus lesioned by ibotenic acid injection. Neurosci Lett 136: 95-98.

Nitsch C., Goping G. and Klatzo I. (1989) Preservation of GABAergic perikarya and boutons after transient ischemia in the gerbil hippocampal CA1 field. Brain Res 495, 243-252.

Nitsch C, Scotti L, Monrad D, Heim C, Sontag K_H (1993) The glia-derived protease nexin 1 persists for over 1 year in rat brain areas selectively lesioned by transient global ischaemia. Eur J Neurosci 5: 292-297.

Ohyagi Y, Takahashi K, Kamegai M, Tabira T (1990) Developmental and differential expression of ß-amyloid protein precursor mRNA in mouse brain. Biochem Biophys Res Commun 167: 54-60.

Otsuka N, Tomonaga M, Ikeda K (1991) Rapid appearance of ß-amyloid precursor protein immunoreactivity in damaged axons and reactive glial cells in rat brain floowing needle stab injury. Brain Res 568: 335-338.

Palacios G, Palacios JM, Mengod G, Frey P (1992) ß-Amyloid precursor protein localization in the Glogi apparatus in neurons and oligodendrocytes. An immunocytochemical structural and ultrastructural study in normal and axotomized neurons. Mol Brain Res 15: 196-206.

Petito C.K. and Pulsinelli W.A. (1984a) Delayed neuronal recovery and neuronal death in rat hippocampus following severe cerebral ischemia: possible relationship to abnormalities in neuronal processes. J Cereb Blood Flow Metab 4, 194-205.

Petito C.K. and Pulsinelli W.A. (1984b) Sequential development of reversible and irreversible neuronal damage following cerebral ischemia. J Neuropathol Exp Neurol 43, 141-153.

Petito CK, Morgello S, Felix JC, Lesser ML (1990) The two patterns of reactive astrocytosis in post-ischemic rat brain. J Cereb Blood Flow Metab 10: 850-859.

Probst A, Brunnscheweiter H, Lautenschlager C, Ulrich J (1987) A special type of senile plaque, possibly an initial stage. Acta Neuropathol 74: 133-141.

Pulsinelli WA, Brierley JB, Plum F (1982) Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol 11: 491-499.

Rischke R, Krieglstein J (1991) Post-ischemic neuronal damage causes astroglial activation and increase in local cerebral glucose utilization of rat hippocampus. J Cereb Blood Flow Metab 11: 106-113.

Rozemuller JM, Eikelenboom P, Stam FC, Beyreuther K, Masters C (1989) A4 protein in Alzheimer's disease: primary and secondary cellular events in extreacellular amyloid deposition. J Neuropathol Exp Neurol 48: 674-691.

Saitoh T, Sundsmo M, Roch J-M, Kimura N, Cole G, Schubert D, Oltersdorf T, Schenk DB (1989) Secreted form of amyloid ß protein precursor is involved in the growth regulation of fibroblast. Cell 58: 615-622.

Schindler V, Javernick I, Trottnow D, Schraven E (1987) Cell proliferation in the gerbil hippocampus following brief ischemic episodis. J Cereb Blood Flow Metab 7 (suppl 1): S 18.

Schmidt-Kastner R. and Freund T.F. (1991) Selective vulnerability of the hippocampus in brain ischemia. Neuroscience 40, 599-636.

Schmidt-Kastner R, Szymas J, Hossmann K-A (1990) Immunohistochemical study of glial reaction and serum-protein extravasation in relation to neuronal damage in rat hippocampus after ischemia. Neuroscience 38: 527-540.

Schubert D, Cole G, Saitoh T, Olterdorf T (1989a) Amyloid beta protein precursor is a mitogen. Biochem Biophys Res Commun 86: 83-88.

Schubert D, Jin L-W, Saitoh T, Cole G (1989b) The regulation of amyloid ß protein precursor secretion and its modulatory role in the cell adhesion. Neuron 3: 689-694.

Selkoe DJ (1991) The molecular pathology of Alzheimer's disease. Neuron 6: 487-498.

Shigematsu K, Mc Geer PL, Walker DG, Ishii T, Mc Geer EG (1992) Reactive microglia/macrophages phagocytose amyloid precursor protein produced by neurons following neural damage. J Neurosci Res 31: 443-453.

Shigematsu K, Mc Geer PL (1992) Accumulation od amyloid precursor protein in neurons after intraventricular injection of colchicine. Amm J Pathol 140: 787-794.

Shivers BD, Hilbich C, Multhaup G, Salbaum M, Beyreuther K, Seeburg PH (1988) Alzheimer's disease amyloidogenic glycoprotein: expression pattern in rat brain suggest a role in cell contact. EMBO J 7: 1365-1370.

Siman R, Card JP, Nelson RB, Davis LG (1989) Expression of ß-amyloid precursor protein in reactive astrocytes following neuronal damage. Neuron 3: 275-285.

Smith M-L, Aver RN, Siesjo BK (1984) The density and distribution of ischemia brain injiry in the rat following 2-10 min of forebrain ischemia. Acta Neuropathol 64: 319-322.

Solà C, Garcia-Ladona FJ, Mengod G, Probst A, Frey P, Palacios JM (1993a) Increased levels of the kunitz protease inhibitor-containing ßAPP mRNAs in rat brain following neurotoxic damage. Mol Brain Res 17: 41-52.

Solà C, Garcia-Ladona FJ, Sarasa M, Mengod G, Probst A, Palacios G, Palacios JM (1993b) ßAPP gene expression is increased in the rat brain after motor neurons axotomy. Eur J Neurosci (in press).

Stephenson DT, Rash K, Clemen JA (1992) Amyloid precursor protein accumulates in regions of neurodegeneration following focal cerebral ischemia in the rat. Brain Res 593: 128-135.

Tanzi RE, Mc Clatchey AI, Lamperti ED, Villa-Komaroff LL, Gusella JF, Neve RL (1988) Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. Nature 331: 528-530.

Tortosa A, Ferrer I (1993) Parvalbumin immunoreactivity in the hippocampus of the gerbil after transient forebrain ischemia: a qualitative and quantitative sequential study. Neuroscience 55: 33-43.

Wakita H, Tomimoto H, Akiguchi I, Ohnishi K, Nakamura S, Kimura J (1992) Regional accumulation of amyloid ß/A4 protein precursor in the gerbil brain following transient cerebral ischemia. Neurosci Lett 146: 135-138.

Wisniewski HM, Wegiel J, Wang KC, Kujawa M, Lach B (1989) Ultrastructural studies of the cells forming amyloid fibers in classical plaques. Can J Neurol Sci 16: 535-542.

Yamamoto K, Morimoto K, Yanagihara T (1986a) Cerebral ischemia in the gerbil: Transmission electron microscopic and immunoelectron microscopic investigation. Brain Res 384, 1-10.

Yamamoto K, Yoshimine T, Homburger HA, Yanagihara T (1986b) Immunohischemical investigation of regional cerebral ischemia in the gerbil: occlusion of the posterior communicating artery. Brain Res 371: 244-252.

Yamamoto K, Hayakawa T, Mogami H, Akai F, Yanagihara T (1990) Ultrastructural investigation of the CA1 region of the hippocampus after transient cerebral ischemia in gerbils. Acta Neuropathol 80, 487-492.

Yoshimine T, Morimoto K, Brengman JM, Homburger HA, Mogami H, Yanagihara T (1985) Immunohistochemical investigation of cerebral ischemia during recirculation. J Neurosurg 63: 922-928.

IV.DISCUSSIO GENERAL

Aquesta darrera part de discussió general pretén donar una visió conjunta dels treballs aquí presentats. Tot i que cadascun d'ells consta d'una discussió pròpia, és ara de menester una visió global que ens retorni als objectius inicialment plantejats.

En aquesta tesi s'ha utilitzat un model experimental d'isquèmia global transitòria en el jerbu. Aquest model havia estat prèviament validat i s'ha demostrat molt útil donades les seves característiques anatòmiques, les quals permeten reproduir lesions similars en els diferents animals (Levine i Sohn, 1969; Kahn, 1972; Ito i cols., 1976; Dodson i cols. 1977; Ginsberg i Busto, 1989).

Tots els jerbus utilitzats varen ésser sotmesos a un test de comportament postisquèmia (Escala de McGraw, 1977) (IV.1 Annex) per tal de posar de manifest les alteracions clíniques secundàries a la isquèmia. Així, tal com s'ha comentat en el capítol 1, les anomalies més freqüents en els jerbus adults varen ser el comportament giratori i, el tremolor i pèl rugós, durant les primeres 8 hores de postoclusió carotídea. Un grup de jerbus va presentar, dins les primeres 6 hores postisquèmia, crisis rotatòries repetides que condicionaven la seva mort a la majoria d'ocasions. A partir del primer dia de supervivència postisquèmia, els jerbus no presentaven cap manifestació clínica.

El primer objectiu d'aquesta tesi va ser analitzar les repercusions anatomopatològiques de la isquèmia a l'hipocamp del jerbu. Els primers canvis morfològics en els jerbus adults van ser visibles per microscopia òptica a partir de les 24-48 hores postisquèmia. Aquestes alteracions eren molt més evidents a partir

del 3r-4t dia de supervivència, sobretot a la regió CA1 de l'hipocamp (Capítol 1, Figura 2; Capítol 2, Figura 1). Les alteracions morfològiques de les neurones de la regió CA1 de l'hipocamp al cap de 4 dies de supervivència es caracteritzaven per una condensació puntejada de la cromatina nuclear i un encongiment citoplasmàtic. Així mateix, un petit grup de neurones disperses per la regió CA1, mostraven un nucli uniformement condensat i de coloració molt fosca (Capítol 1, Figura 2; Capítol 2, Figura 1). En cap cas es va observar fragmentació del nucli, la qual cosa és característica de l'apoptosi. Aquestes troballes eren similars a les observades en estudis previs (Ito i cols., 1975; Kirino, 1982; Kirino i Sano, 1984; Kirino i cols., 1984).

Per tal d'estudiar la relació entre la presència de parvalbúmina en determinades neurones i el seu efecte en front a la isquèmia, el segon i tercer objectius d'aquesta tesi van ser l'anàlisi de les neurones de l'hipocamp que contenen parvalbúmina en el jerbu normal i després de la isquèmia. La primera troballa a destacar va ser les diferències entre els jerbus i les altres espècies (rates, eriçons, gats i humans) en quan a la distribució de les neurones que contenen parvalbúmina a l'hipocamp (Capítol 1, Figura 1). En primer lloc, els jerbus presenten un número inferior de neurones amb immunoreactivitat per a parvalbúmina a la regió CA1 de l'hipocamp en relació a la regió CA3 i al *gyrus dentatus* (Scotti i Nitsch, 1991). Tenint en compte que la parvalbúmina s'ha associat amb neurones *fast spiking* (Kawaguchi i cols., 1987), la disminució de neurones parvalbúmina a la regió CA1 pot alterar la inhibició mediada per les interneurones GABA-èrgiques. Per altra part, una segona característica que diferencia als jerbus de les altres espècies és la presència de neurones amb immunoreactivitat per a la parvalbúmina a la via

perforant, una projecció excitadora provinent del còrtex entorrinal (Seto-Oshima i cols., 1990). En aquest sentit, la presència de neurones parvalbúmina a la via perforant pot estar relacionada amb la generació i manteniment de crisis epilèptiques, a les quals és tant susceptible aquesta espècie (Loskota i cols., 1974).

Per altra part, a l'estudiar la immunoreactivitat per a parvalbúmina a l'hipocamp del jerbu adult després d'isquèmia cerebral transitòria es va observar, a totes les regions de l'hipocamp, una disminució d'immunoreactivitat, amb un màxim al cap de 6 hores de supervivència postisquèmia. Posteriorment, a partir de les 24 hores postisquèmia, s'observà una progressiva recuperació de la immunoreactivitat, que es va iniciar a la regió CA3 i fascia dentada afectant finalment també a la regió CA1 (Figura 3, capítol 1). Aquest augment condiciona que al cap de 7 dies de supervivència postisquèmia, el número de neurones amb immunoreactivitat per a parvalbúmina era superior a l'observat en el grup control (Capítol 1, Taula 1 i Figura 5). Un patró similar de pèrdua transitòria de la immunoreactivitat per a parvalbúmina amb recuperació posterior s'havia observat en un estudi previ amb isquèmia transitòria en rates, encara que la disminució tenia lloc de forma més retardada a partir del 4^t dia postisquèmia, amb una recuperació en els dies posteriors (Johansen i cols., 1990). La variabilitat en quan al temps d'aparició dels canvis en la immunoreactivitat per a parvalbúmina entre aquests dos estudis podria estar en relació a diferències entre les dues espècies. Es per això que aquestes troballes, conjuntament amb els resultats obtinguts prèviament en rates (Johansen i cols., 1990), suggereixen que després de la isquèmia es produeix una disminució transitòria de la immunoreactivitat per a la parvalbúmina a l'hipocamp.

Per tal de descartar altres factors a més de la isquèmia que poguessin estar implicats en la disminució d'immunoreactivitat per a parvalbúmina, es van realitzar diversos estudis. En primer lloc, es van analitzar els efectes que podien ser deguts a l'anestèsic utilitzat. Així, un grup de jerbus van ser anestesiats amb èter i un altre amb ketamina associada a diazepam. Els estudis d'immunoreactivitat per a parvalbúmina no varen demostrar diferències en relació al grup anestesiat amb halotane. Per altra part, un quart grup va ser sotmès solament a anestèsia amb halotane i cirurgia sense lligadura de les caròtides. A l'estudiar la immunoreactivitat per a parvalbúmina, tampoc mostrava cap diferència en relació al grup control. Aquests resultats suggereixen que els canvis observats en les neurones que contenen parvalbúmina no són deguts a l'anestèsic emprat.

Per altra part, tenint en compte que la parvalbúmina és una proteïna captadora de calci i que aquest element exerceix un paper important en el dany neuronal postisquèmia, els canvis en la immunoreactivitat per a parvalbúmina podrien estar relacionats en part amb diferències entre la fixació i la duració del període postfixació, ja que el tampó utilitzat no contenia calci. No obstant, les alteracions observades es van repetir sempre de forma similar en els diferents animals, a varis temps de supervivència, i afectaven únicament a l'hipocamp. A més, al realitzar estudis quantitatius de les neurones amb immunoreactivitat per a parvalbúmina en el còrtex somatosensorial (Capítol 1, Taula I) no es van observar diferències en quant al número de cèl.lules parvalbúmina en relació al temps de supervivència postisquèmia. Aquests resultats fan molt poc probable que el factor fixació i temps de postfixació siguin la causa dels canvis observats en la immunoreactivitat per a la parvalbúmina en les neurones de l'hipocamp.

Un altre factor a tenir en compte són els canvis en les concentracions intracel·lulars de calci. Estudis previs han demostrat que després de la isquèmia es produeix una entrada ràpida de calci a les neurones de l'hipocamp. Posteriorment s'observa una recuperació dels nivells de calci seguit d'un nou increment que apareix de forma retardada (Dienel, 1984; Simon i cols., 1984; Suzuki i cols., 1985; Kaas i Lipton, 1986; Sakamoto i cols., 1986; Tsuda i cols., 1986; Deshpande i cols., 1987; Martins i cols., 1988). L'anticòs utilitzat en aquesta tesi (Capítol 1) probablement reconeixia la forma de proteïna lligada al calci, de la mateixa manera que succeïa en un estudi previ amb tècniques *in vitro* (Pfyffer i cols., 1987). Així, l'increment d'immunoreactivitat per a parvalbúmina 15 minuts després de la isquèmia podria ser conseqüència d'un augment de la forma de la proteïna unida al calci, en relació a l'increment ràpid de calci en les fases inicials postisquèmia. Per altra part, la disminució de parvalbúmina posterior podria relacionar-se amb un alliberament del calci per part d'aquesta proteïna, en relació a la disminució dels nivells intracel·lulars de calci. Finalment, a partir de les 24 hores postisquèmia, l'increment secundari de la immunoreactivitat per a la parvalbúmina podria relacionar-se amb l'augment de calci a l'interior de la cèl·lula, que te lloc de forma retardada, produint-se una major proporció de la fracció de parvalbúmina lligada al calci.

Per altra part, la disminució d'immunoreactivitat per a parvalbúmina podria estar relacionada amb alteracions inespecifiques de la regió CA1 de l'hipocamp com a consequència directa de la isquèmia. No obstant, al realitzar estudis amb altres anticossos demostren que les neurones de la regió CA1 de l'hipocamp no presentaven disminució d'immunoreactivitat per a GABA (Capítol 1, Figura 4) ni

MAP (Capítol 2, Figura 2), malgrat algunes també contenien parvalbúmina. Tot això suggereix que les modificacions en la immunoreactivitat per a parvalbúmina no són degudes a canvis generals de la isquèmia sobre l'hipocamp, sinó que es tracta d'un efecte específic sobre aquestes neurones.

Per altra part, l'augment de neurones que contenen parvalbúmina a la regió CA1 a partir de les 24 hores postisquèmia, podria ser degut a l'encongiment del teixit com a conseqüència de la mort cel·lular. No obstant, aquest fet és poc probable ja que la mort neuronal a la regió CA1 s'observa a partir del 3^{r} dia postisquèmia i l'augment de parvalbúmina té lloc a partir de les 24 hores de supervivència (Capítol 1, Figura 5).

Finalment, l'augment d'immunoreactivitat per a parvalbúmina podria ser degut a un increment en la síntesi d'aquesta proteïna a conseqüència de la isquèmia. De totes maneres, malgrat no hi ha evidències clares que ho suggereixin caldrien estudis amb tècniques d'hibridació *in situ* per poder demostrar aquesta possibilitat.

En conclusió, els resultats d'aquesta tesi (Capítol 1) suggereixen que les neurones de la regió CA1 de l'hipocamp del jerbu adult que contenen parvalbúmina presenten una major supervivència en relació a la isquèmia que les neurones de projecció.

El quart objectiu d'aquesta tesi va ser investigar la implicació de la síntesi de proteïnes en la gènesi de la MCR, així com analitzar les similituds i diferències entre MCR i apoptosi. Per això, es va administrar un inhibidor de la síntesi de proteïnes,

la cicloheximida, a diferents dosis i pautes després de la isquèmia (Capítol 2). Els resultats ens mostraren que la cicloheximida administrada a dosis altes (Capítol 2, grup I) produïa un augment significatiu del número de cèl·lules mortes. No obstant, si s'administrava a dosis baixes (Capítol 2; grup II) disminuïa de forma molt discreta el número total de cèl·lules mortes a la regió CA1 (Capítol 2, Figura 3).

Per avançar en l'estudi dels mecanismes relacionats amb la MCR, es varen realitzar estudis d'immunohistoquímica amb l'anticòs anti-MAP després de la isquèmia. Així, es va observar una disminució precoç de la immunoreactivitat per a MAP (Capítol 2, Figura 2) al cap de 12 hores de supervivència postisquèmia. Aquest resultat suggereix que en la MCR es produeix una alteració inicial de les dendrites, prèvia als canvis nuclears i citoplasmàtics de mort cel.lular, els quals és possible de visualitzar per microscopia òptica a partir del 3^r-4^t dia de supervivència (Capítol 2, Figura 1). En aquest sentit, estudis previs amb microscopia electrònica havien demostrat que el primer que s'afecta en la MCR és la part distal de les dendrites i, posteriorment, el soma i el nucli (Kirino i Sano, 1984b; Yamamoto i cols., 1986; -90; Deshpande i cols., 1992). Així, la pèrdua inicial de la immunoreactivitat per a MAP es correlacionaria amb una afectació primarenca de les dendrites. Aquesta seria una diferència important entre la MCR i l'apoptosi, ja que les alteracions cel·lulars inicials en aquest segon tipus de mort tenen lloc en el nucli (Oppenheim i cols., 1990; Oppenhein, 1991).

El cinquè objectiu de la present tesi va ser analitzar els mecanismes de mort induïda per radiacions durant el desenvolupament com a model d'apoptosi (Capítol 5). Així, es va observar en rates d'un dia de vida, que una dosis de 200 cGy de

raigs-X ocasinava un augment de la mort tipus apoptosi a l'hipocamp, amb un màxim a les 6 hores de la irradiació. L'administració de cicloheximida a dosis baixes després de la irradiació, disminuïa de forma molt significativa la mort induïda per radiacions (Capítol 5, Figura 4). Contrariament, la mort induïda per radiacions no es va modificar després de l'administració intratecal de NGF (Capítol 5, Figura 5). Per altra part, les mateixes dosis de radiacions en rates de 15 dies de vida no ocasionava mort cel·lular.

La morfologia i la distribució regional de les cèl·lules mortes és molt similar en la mort natural i en la mort induïda per radiacions durant el desenvolupament. Les troballes d'aquest estudi suggereixen que la mort induïda per radiacions és una mort tipus apoptosi, la qual està mediada per la síntesi de proteïnes i probablement no depèn del NGF.

El sisè objectiu d'aquesta tesi va ser comparar els mecanismes implicats en la MCR i en la mort induïda per radiacions durant el desenvolupament. En aquest sentit, els resultats del capítol 4 suggereixen que la mort induïda per radiacions és del tipus apoptosis i la seva resposta en front a la cicloheximida recolza el fet de que es tracti d'un procés actiu mediat per la síntesi de proteïnes. Per altra part, la MCR presenta una seqüència d'afectació d'afectació de les organel·les intracel·lulars diferents de l'apoptosi, així com també una diferent morfologia d'afectació nuclear i de resposta en front a la cicloheximida. Tot això suggereix que la MCR induïda per isquèmia presenta característiques morfològiques diferents de l'apoptosi.

El setè objectiu d'aquesta tesi va ser l'estudi dels efectes de la fructosa-1,6-bisfosfat (FBP) com a protector de la MCR (Capítol 3). En aquest sentit, treballs previs realitzats amb conills (Farias i cols., 1990) i jerbus (Trimarchi i cols., 1990) havien demostrat una milloria del dany cerebral després de l'administració de FBP. El mecanisme d'acció proposat per explicar el seu efecte és, en ocasions, contradictori. Alguns autors han suggerit que la FBP és capaç de travessar membranes biològiques i actuar com a restaurador de l'activitat glicolítica, intervenint a la via glicolítica no tan sols com un regulador metabòlic sinó també com a substracte. No obstant, aquestes explicacions s'han posat en dubte tenint en compte que els sucres bifosforilats difícilment travessen les membranes cel·lulars. Un altre possible mecanisme d'acció seria que la FBP interaccionés amb les membranes cel·lulars modificant la permeabilitat iònica. De totes formes, en aquest moment el mecanisme d'acció de la FBP com a protector cel·lular en front a la isquèmia presenta múltiples punts obscurs i, fins i tot alguns treballs han posat en dubte el seu efecte beneficiós en la isquèmia (Eddy i cols., 1981; Leblanc i cols., 1989).

A la present tesi s'han utilitzat diferents dosis i vies d'administració (intraperitoneal i intratecal) de la FBP en el jerbu després d'isquèmia cerebral transitòria per tal d'avaluar els seus efectes sobre la MCR. Els resultats del capítol 3 no demostren diferències significatives entre el grup control i els grups que havien rebut FBP en quant al número de cèl·lules mortes a la regió CA1 de l'hipocamp (Capítol 3, Figura 1).

Els resultats d'estudis previs realitzats amb jerbus (Trimarchi i cols., 1990) i conills (Farias i cols., 1990) són contradictoris amb els observats en aquesta tesi. Una possible explicació per aquesta discrepància podria ser l'existència de diferències entre els models utilitzats. En aquest sentit, Trimarchi i cols., (1990) utilitzen també jerbus, però realitzen un temps d'isquèmia de 15 minuts i mesuren els nivells de putrescina al cap de 24 hores de supervivència. Aquests autors varen observar que els nivells de putrescina disminuïen després de l'administració de FBP, però no varen realitzar estudis neuropatològics per a confirmar la milloria histològica secundaria a la FBP. Per altra part, l'estudi de Farias i cols., (1990), es va dur a terme amb un model d'hipòxia-isquèmia-hipotensió durant 5-8 minuts en conills. Les diferències entre les espècies, el protocol utilitzat i el temps d'isquèmia poden explicar les discrepàncies amb el present estudi.

Per tot això, els resultats obtinguts en la present tesi (Capítol 3) suggereixen que la FBP no ofereix cap efecte beneficiós sobre la MCR en jerbus després d'isquèmia global transitòria.

El vuitè objectiu d'aquesta tesi va ser l'estudi dels canvis secundaris a la isquèmia durant el desenvolupament postnatal de l'hipocamp dels jerbus (Capítol 4). Des del punt de vista clínic, els jerbus de 7 i 15 dies no presentaven cap manifestació després de la isquèmia carotídea. A partir dels 21 i 30 dies de vida postnatal, els jerbus presentaven una posició ajupida durant aproximadament una hora. A més, els jerbus de 30 dies presentaven posteriorment un comportament rotatori durant aproximadament 6 hores, al igual que els jerbus adults. Les diferències clíniques observades entre els jerbus adults i els petits es van

correlacionar també amb diferències en les troballes anatomopatològiques. Els jerbus de 7 dies de vida no presentaven cap anomalia després de la isquèmia. En els jerbus de 15 dies s'observaren alteracions en les neurones de la capa granular interna de la fascia dentada i en algunes neurones aïllades de la capa piramidal de CA3. Quan els jerbus tenien 21 dies, les alteracions secundàries a la isquèmia eren menys evidents a la regió CA3 (Capítol 4, Figura 3). Finalment, en els jerbus de 30 dies el patró de destrucció cel·lular secundari a la isquèmia era igual que l'observat en els adults (Capítol 4, Figura 3).

El novè objectiu d'aquesta tesi va ser estudiar el desenvolupament de proteïnes fixadores de calci, parvalbúmina i calbindina D28K, i relacionar-lo amb els canvis secundaris a la isquèmia durant aquest període (Capítol 4). En relació a la immunoreactivitat per a parvalvúmina es va observar que apareixia, a partir del dia 15 postnatal, a la capa piramidal de CA3 i en algunes neurones aïllades de la regió CA2. Posteriorment, en els jerbus de 21 dies s'observaren de forma transitòria cèl·lules amb immunoreactivitat per a parvalbúmina a la capa piramidal de CA1. També es visualitzava immunoreactivitat per a parvalbúmina a la via perforant, la qual cosa diferencia al jerbu d'altres espècies (Seto-Oshima et al., 1990; Scotti i Nitsch, 1991). El patró adult d'immunoreactivitat per a parvalbúmina no s'assoleix fins al final del primer mes de vida postnatal (Capítol 4, Figura 1).

Per altra part, el desenvolupament de calbindina-D28K s'inicià al 5è dia de vida postnatal, amb immunoreactivitat per a calbindina-D28K a algunes neurones de la capa granular interna de la fascia dentada. Progressivament, la immunoreactivitat es distribueix per tota la capa granular interna en els jerbus de

7 dies. Així mateix, s'observà a les fibres *mossy* de CA3. En els jerbus de 15 dies es visualitza immunoreactivitat per a calbindina-D28k a la capa plexiforme de la regió CA3 de l'hipocamp i posteriorment també a la regió CA2 i CA1. El patró adult s'assoleix en els jerbus de 21 dies (Capítol 4, Figura 2).

Aquests patrons de desenvolupament són molt similars al d'altres espècies encara que en el jerbu tant la parvalbúmina com la calbindina apareixen amb més retard (Seto-Oshima et al., 1990; Baimbridge et al., 1992). Així mateix, una altra diferència és la presència d'immunoreactivitat per a parvalbúmina a la via perforant del jerbu (Scotti i Nitsch, 1991).

La presència de parvalbúmina a la regió CA1 de l'hipocamp s'ha relacionat amb una major resistència en front a la isquèmia global transitòria en els jerbus adults (Capítol 1). No obstant, altres estudis no havien trobat cap relació entre la presència de parvalbúmina i una menor vulnerabilitat en front a la isquèmia (Freund i cols., 1990; -91). Tot això demostra que no es coneix amb exactitud la relació entre el contingut de parvalbúmina i l'efecte beneficiós en front a la isquèmia. Per altra part, el patró de vulnerabilitat en els jerbus petits és molt diferent de l'observat en els jerbus adults. Així, els jerbus de 7 dies no mostren cap alteració després de la isquèmia. A partir del dia 15 postnatal, les àrees més vulnerables a la isquèmia són la capa granular de la fascia dentada i la regió CA3. Fins que els jerbus no tenen 30 dies no s'afecta la regió CA1 a l'igual que en els jerbus adults. Tenint en compte aquest patró de vulnerabilitat no es pot correlacionar el contingut intracel·lular de parvalbúmina amb la mort induïda per isquèmia durant el desenvolupament de l'hipocamp en el jerbu (Capítol 4).

En relació a la calbindina, Godmann i cols. (1993) varen observar en un model d'hipoxia-isquèmia en rates, que les neurones de la capa granular interna de la fascia dentada que contenien calbindina presentaven una menor vulnerabilitat. Els resultats del Capítol 4 han posat de manifest que, en els jerbus de 7 dies, les neurones immadures de la capa granular de la fascia dentada són resistents a la isquèmia i no contenen calbindina. Per altra part, en els jerbus de 30 dies, algunes cèl·lules de la capa granular són vulnerables a la isquèmia i, en canvi, contenen calbindina. Aquestes troballes suggereixen que tampoc hi ha relació entre el contingut de calbindina i la vulnerabilitat a la isquèmia de l'hipocamp durant el desenvolupament postnatal del jerbu.

El darrer objectiu d'aquesta tesi va ser analitzar els canvis en la immunoreactivitat per a APP a l'hipocamp del jerbu després d'isquèmia global transitòria i relacionar-los amb la proliferació astroglial (Capítol 6). Mitjançant l'anticòs anti-GFAP, es va observar que 2 dies després de la isquèmia hi havia una hipertròfia astroglial, mentre que no és fins a partir del setè dia de supervivència quan s'observa una hiperplàsia d'astròcits (Capítol 6, Figura 2 i 3). Per altra part, a l'analitzar la immunoreactivitat per a l'APP es va observar que els astròcits hipertròfics que es visualitzaven a partir del segon dia de supervivència postisquèmia, presentaven una immunoreactivitat molt dèbil (Capítol 6, Figura 4). D'altra banda, els astròcits hiperplàsics visibles a partir del setè dia de supervivència presentaven una forta immunoreactivitat per a l'APP (Capítol 6, Figura 4 i 5). Per tant, aquests resultats suggereixen que la producció d'APP després de la isquèmia està associada amb l'hiperplàsia dels astròcits però no amb una hipertrofia astroglial.

Fins l'actualitat, no es coneix amb exactitud quina és la funció de la APP a nivell cerebral. Alguns autors han observat que la proliferació astroglial promou l'expressió de mRNA de APP en cultius d'astròcits (Forloni i cols., 1992). Els resultats d'aquesta tesi (Capítol 6), conjuntament amb estudis previs, fan suposar que l'augment en la síntesi d'APP està associat a la proliferació d'astròcits i no a la hipertròfia.

V. CONCLUSIONS

1. Després d'isquèmia global transitòria durant 20 minuts, les neurones de la capa piramidal de la regió CA1 de l'hipocamp presenten, al cap de 3-4 dies, signes de mort evidents per microscopia òptica, que es caracteritzen per una condensació puntejada de la cromatina nuclear i un encongiment citoplasmàtic.

2. Al cap de set dies de supervivència postisquèmia s'observa una disminució important del número de neurones totals a la regió CA1 de l'hipocamp.

3. El jerbu presenta, a diferència d'altres espècies, un menor número de neurones amb immunoreactivitat per a parvalbúmina a la regió CA1 de l'hipocamp. Així mateix, presenta cèl·lules amb immunoreactivitat per a parvalbúmina a la via perforant. Aquestes diferències poden explicar la major vulnerabilitat dels jerbus a les crisis epilèptiques.

4. Després d'isquèmia cerebral transitòria en el jerbu es produeix una disminució precoç i transitòria del número de neurones que contenen parvalbúmina a l'hipocamp.

5. A partir de les 24 hores de supervivència postisquèmia s'observa un progressiu augment del número de cèl.lules que contenen parvalbúmina a l'hipocamp del jerbu, principalment a la regió CA1, assolint al cap de 7 dies de supervivència nivells superiors a les del grup control.

6. L'augment d'immunoreactivitat per a parvalbúmina després de la isquèmia té lloc de forma precoç en relació a la mort de neurones de la capa piramidal de CA1, el que suggereix que aquest augment no és degut a un encongiment del teixit. Els canvis en la immunoreactivitat per a parvalbúmina poden estar relacionats amb modificacions dels nivells intracel·lulars de calci en les fases postisquèmia.

7. La preservació de cèl.lules amb immunoreactivitat per a parvalbúmina a la regió CA1 de l'hipocamp del jerbu adult suggereix que la presència de parvalbúmina està associada a una major supervivència després de la isquèmia.

8. La cicloheximida, administrada a dosis baixes després de la isquèmia, disminueix de forma discreta el número de cèl.lules mortes a la regió CA1 de l'hipocamp. Aquests resultats suggereixen que la cicloheximida podria afavorir un reequilibri de la síntesi de proteïnes en la fase postisquèmia i prevenir la mort neuronal.

9. Dosis altes de cicloheximida, administrades després d'isquèmia global transitòria, augmenten el número de cèl·lules mortes a la regió CA1 de l'hipocamp, el que suggereix que la cicloheximida a dosis altes pot inhibir la síntesi de proteïnes associades a supervivència i afavorir la mort neuronal.

10. Després de la isquèmia s'observa una disminució precoç d'immunoreactivitat per a MAP, el que indica una alteració inicial de les dendrites, prèvia als canvis nuclears i citoplasmàtics.

11. La mort induïda per radiacions és una mort tipus apoptosi que s'inhibeix de forma significativa amb l'administració de cicloheximida però no amb la de NGF. Aquests resultats suggereixen que es tracta d'un procés mediat per la síntesi de proteïnes.

12. La seqüència d'afectació de les organel·les intracel·lulars així com la morfologia de la degeneració nuclear i la resposta en front a la cicloheximida suggereixen que la mort cel·lular retardada i l'apoptosi són dos tipus de mort de característiques diferents.

13. L'administració de fructosa-1,6-bisfosfat no protegeix les neurones de la regió CA1 de l'hipocamp de mort cel·lular retardada després d'isquèmia global transitòria en el jerbu.

14. Els jerbus de 7 dies no presentan cap anomalia després d'isquèmia global transitòria.

15. Els jerbus de 15 i 21 dies mostren alteracions a la fascia dentada i capa piramidal de CA3 després d'isquèmia transitòria.

16. Els jerbus de 30 dies presenten un patró de vulnerabilitat en front a la isquèmia molt similar als jerbus adults. Aquests resultats suggereixen una menor vulnerabilitat en front a la isquèmia en els jerbus durant el desenvolupament de l'hipocamp.

17. La immunoreactivitat per a parvalbúmina en l'hipocamp del jerbu s'inicia a la regió CA3 en el 15è dia de vida postnatal, visualitzant-se també a la via perforant a partir de la tercera setmana de vida. El patró adult s'assoleix al final del primer mes de vida.

18. No s'ha trobat cap relació entre el contingut de parvalbúmina i el patró de vulnerabilitat en front a la isquèmia durant desenvolupament de l'hipocamp.

19. Les primeres neurones amb immunoreactivitat per a calbindina en l'hipocamp del jerbu són visibles a la fascia dentada a partir del 5è dia postnatal. El patró adult s'assoleix al final de la tercera setmana de vida.

20. No es pot correlacionar el contingut intracel·lular de calbindina-28K amb la mort induïda per isquèmia durant el desenvolupament de l'hipocamp en el jerbu.

21. La producció de proteïna precursora beta-amiloide després de la isquèmia està associada a la hiperplàsia astroglial i no a la hipertròfia d'astròcits.

VI. ANNEXOS

ANNEX I, ESCALA DE Mc GRAW, 1977

	INDEX STROKE	1 H	2H	5 H	24 H	48 H	3D	4 D	5D	6D	7D
PEL AIXECAT O TREMOLOR	1										
APAGAT	1										
POBRESA MOVIMENT	1										
CAP GIRAT D o E	3										
ULL OBERT FIX D-E-B	3										
PTOSIS D o E	2										
ROTACIO EXTREMA EXTREMITATS POSTERIORS D-E-B	3										
COMPORTAMENT GIRATORI D-E-B	3										
CRISIS	2										
CRISIS GIRATORIES	3										
DEBILITAT EXTREMA COMATOS	6										
MORT	+3										
TOTAL											

VII. BIBLIOGRAFIA

Albers GW, Goldberg MP, Choi DW. N-methyl-D-aspartate antagonists: ready for clinical trial in brain ischemia?. Ann Neurol 1989; 25: 398-403.

Allsop D, Wong CW, Ikeda SI, Landon M, Kidd M, Glenner GG. Immunohistochemical evidence for the derivation of a peptide ligand from the amyloid beta-protein precursor of Alzheimer disease. Proc Natl Acad Sci USA 1988; 85: 2790-2794.

Altman, J. Programmed cell death: the paths to suicide, Trends Neurosci 1992; 15: 278-280.

Andersen P, Eccles JC, Loyning Y. Location of postsinaptic inhibitory synapses on hippocampal pyramidals cells. Neurophysiol 1964; 27: 592-707.

Anderson C, Stewart-Wynne E, Jamrozik K, Burvill P, Chakera T. Perth community stroke study: desin and preliminary results. Clin Exp Neurol 1990; 8: 125-129.

Andiné P, Jacobson J, Hagberg H. Calcium uptake evoked by electrical stimulation in enhanced postischemically and precedes delayed neuronal death in CA1 of rat hippocampus: involvement of N-methyl-D-aspartate receptors. J. Cerb Blood Flow Metab 1988; 8: 799-807.

Astrup J, Symon L, Branston NM, Lasen NA. Cortical evoked potential and extracellular K^+ and H^+ at critical levels of brain ischemia. Stroke 1977; 8: 51-57.

Astrup J, Siesjo BK, Symon L. Thresholds in cerebral ischemia. The ischemic penumbra. Stroke 1981; 12: 723.

Araki T, Kato H, Kogure K. Selective neuronal vulnerability following transient cerebral ischemia in the gerbil: distribution and time course. Acta Neurol Scan 1989; 80: 548-553.

Araki T, Kato H, Kogure K. Neuronal damage and calcium accumulation following repeated brief cerebral ischemia in the gerbil. Brain Research 1990a; 528: 114-122.

Araki T, Kato H, Inoue T, Kogure K. Regional impaiment of protein synthesis following brief cerebral ischemia in the gerbil. Acta Neuropathol 1990b; 79: 501-505.

Baimbridge KG, Celio MR, Rogers JH. Calcium-binding proteins in the nervous system. Trends Neurosci 1992; 15: 303-308.

Baker PF, DiPolo R. Axonal calcium and magnesium homeostasis. Curr Topics Membr Transp 1984; 22: 95-147.

Balaguer Vintró I. La importancia de la epidemiología cardiovascular como base de la organización eficiente de la cardiologia. Rev Esp Card 1984; 37: 303-306.

Barnes DM. NMDA receptors trigger excitement. Science 1988; 239: 254-256.

Beck T, Wree A, Sauer DD. Chronic infusion of nerve growth factor does not rescue pyramidal cells after transient forebrain ischemia in the rat. Neurosci Lett 1992; 135: 252-254.

Berger L, Hakim AM. Calcium chanel blockers correct acidosis in ischemic rat brain without altering cerebral blood flow. Stroke 1988; 19: 1257-1261.

Berry K, Wisniewski HM, Svarzbein L, Baez S. On the relationship of brain vasculature to production of neurological deficit and morphological changes following acute unilateral common carotid artery ligation in gerbils. J Neurol Sci 1975; 25: 75-92.

Betz AL. Identification of hypoxanthine transport and xanthine oxidase activity in brain capillaries. J. Neurochem 1985; 44: 574-579.

Bickler PE, Kelleher JA. Fructose-1,6-biphosphate stabilizes brain intracellular calcium during hypoxia in rats. Stroke 1992; 23: 1617-1622.

Bland BH, Andersen P, Ganes T, Sveen O. Automated analysis of rhythmicity of physiologically identified hippocampal formation neurons. Exp Brain Res 1980; 38: 205-219.

Blümcke I, Hof PR, Morrison JH, Celio MR. Parvalbumin in the monkey striate cortex: a quantitative immunoelectron-microscopy study. Brain Res 1991; 554: 237-243.

Bonnekoh P, Kuroiwa T, Kloiber O, Hossmann K Time profile of calcium accumulation in hippocampus, striatum and frontoparietal cortex after transient forebrain ischemia in the gerbil. Acta Neuropathol 1992; 84: 400-406.

Braak E, Strotkamp B, Braak H. Parvalbumin-immunoreactive structures in the hippocampus of the human adult. Cell Tissue Res 1991; 264: 33-48.

Brierley JB, Graham DI. Hypoxia and vascular disorders of the central nervous system. A: Hume Adams J, Corsellis JAN, Duchen LW, Eds. Greenfield's Neuropathology, London 1984; 125-207.

Buck CR, Martinez HJ, Chao MD, Black IB. Differential expression of the nerve growth factor receptor in multiple brain areas. Dev Brain Res 1988; 44: 259-268.

Buchan AM, Williams L, Bruederlin B. Nerve growth factor: pretreatment ameliorates ischemic hippocampal neuronal injury. Stroke 1990; 21: 177.

Butterfield JD, McGraw CP. Free radical pathology. Stroke 1978; 9: 443-444.

Celio MR, Heizmann CW. Calcium-binding protein parvalbumin as a neuronal marker. Nature 1981; 293: 300-302.

Celio MR. Parvalbumin in most gamma-aminobutyric acid-containing neurons of the rat cerebral cortex. Science 1986; 231: 995-997.

Celio MR, Baier W, Schärer L, De Viragh PA, Gerday CH. Monoclonal antibodies directed against the calcium binding protein parvalbumin. Cell Calcium 1988; 9: 81-86.

Celio MR. Calbindin D-28k and parvalbumin in the rat nervous. Neuroscience 1990; 35: 375-475.

Cohn R. Convulsive activity in gerbils subjected to cerebral ischemia. Exp Neurol 1979; 65: 391-397.

Crain BJ, Westerkam WD, Herrison AH, Nadler JV. Selective neuronal death after transient forebrain ischemia in the mongolian gerbil: a silver impregnation study. Neurosci 1988; 27: 387-402.

Curtis DR, Felix D, McLennan H. GABA and hippocampal inhibition. Br J Pharmacol 1970; 40: 881-883

Curtis DR, Johnston GAR. Amino acid transmitters in the mammalian central nervous system. Ergeb Physiol 1974; 69: 98-188.

Chalfie M, Wolinsky E. The identification and suppression of inherited neurodegeneration in *Canorhabditis elegans*. Nature 1990; 345: 410-416.

Charriaut-Marlangue C, Pollard H, Kadri-Hassani N, i cols. Increase in specific proteins and mRNA following transient anoxia-aglycaemia in rat CA1 hippocampal slices. Eur J neurosci 1992; 4: 766-776.

Cheung WY. Calmodulin plays a pivotal role in cellular regulation. Science 1980; 207; 19-27.

Cheung JY, Bonventre JV, Malis CD, Leaf A. Calcium and ischemic injury. N Engl J Med 1986; 314: 1670-1676.

Choi DW, Maulucci-Gedde MA, Kriegstein AR. Glutamate neurotoxicity in cortical cell culture. J. Neurosci 1987; 7: 357-368.

Choi DW. Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. Trends Neurosci 1988; 11: 465-469.

Chopp M, Li Y, Dereski MO, Levine SR, Yoshida Y, Garcia JH. Neuronal injury and expression of 72-kDA heat-shock protein after forebrain ischemia in the rat. Acta Neuropathol 1991; 83: 66-71.

Chronwall B, Wolff JR. Prenatal and postnatal development of GABA-accumulating cells in the occipital neocortex of rat. J Comp Neurol 1980; 190: 187-208.

Church J, Zeman S, Lodge D. The neuroprotective action of ketamine and MK-801 after transient cerebral ischemia in rats. Anesthesiology 1988; 69: 702-709.

DeFelipe J, Hendry SHC, Jones EG. Visualization of chandelier cell axons by parvalbumin immunoreactivity in monkey cerebral cortex. Proc Natl Acad Sci 1989; 86: 2093-2097.

DeLeo J, Toth L, Schubert P, Rudolphi K, Kreutzberg GW. Ischemia-induced neuronal cell death, calcium accumulation, and glial response in the hippocampus of the Mongolian gerbil and protection by propentofylline (HWA 285). J Cereb Blood Flow Metab 1987; 7: 745-751.

De OLiveira JR, Rosa JL, Ambrosio S, Bartrons R. Effect of galactosamine on hepatic carbohydrate metabolism: protective role of fructose 1,6-biphosphate. Hepatology 1992; 15: 1147-1153.

Deshpande JK, Siesjö BK, Wieloch T. Calcium accumulation and neuronal damage in the rat hippocampus following cerebral ischemia. J Cereb Blood Flow Metab 1987; 7: 89-95.

Deshpande J, Bergstedt K, Lindén T, Kalimo H, Wieloch T. Ultrastructural changes in the hippocampal CA1 region following transient cerebral ischemia: evidence against programmed cell death. Exp Brain Res 1992; 88: 91-105.

Didleke R, Kirchner KA, Lewin J, Bower JD, Markov A. Protection from ischemic renal injury by fructose-1,6-diphosphate infusion in rat. Circ Shock 1985; 16: 205-212.

Dienel GA. Regional accumulation of calcium in postischemic rat barin. J. Neurochem 1984; 43: 913-925.

Dienel GA, Cruz NF, Rosenfeld SJ. Temporal profiles of proteins responsive to ischemia. J Neurochem 1985; 44: 600-610.

Dienel GA, Kiessling M, Jacewicz M, Pulsinelli WA. Synthesis of heat-shock proteins in rat brain cortex after transient ischemia. J Cereb Blood Flow Metab 1986; 6: 505-510.

Dodson RF, Chu LWF, Welch KMA, Achar VS. Acute tissue response to cerebral ischemia in the gerbil. An ultrastructural study. J Neurol Sci 1977; 33: 161-170.

Drejer J, Benveniste H, Diemer NH, Schousbe A. Cellular origin af ischemia-induced glutamate release from brain tissue in vivo and in vitro. J Neurochem 1985; 45: 145-151.

Driscoll M, Chalfie M. Developmental and abnormal cell death in *C. elegans*. Trends Neurosci1992; 15: 15-19.

Dwyer BE, Nishimura RN, Powell CL, Mailheau SL. Focal protein synthesis inhibition in a model of neonatal hypoxic-ischemic injury. Exp Neurol 1987; 95: 277-289.

Ebashi S. Calcium-binding and relaxation in actomyosin system. J Biochem, 1960;48 150-151.

Ellis RE, Yuan J, Horvitz HR. Mechanisms and functions of cell death. Annu Rev Cell Biol 1991; 7: 663-698.

Fairén A, DeFelipe J, Regidor J. Nonpyramidal neurons. En: Pelers A, Jones EG eds. Cerebral Cortex. Vol 1. Plenum Press. New York/London 1984; 201-253.

Farias LA, Sun J, Markov AK. Improved brain metabolism with fructose 1-6 diphosphate during insulin-induced hypoglycemic coma. Am J Med Sci 1989; 296: 294-299.

Farias La, Smith EE, Markov AK. Prevention of ischemic-hypoxic brain injury and death in rabbits with fructose-1,6-diphosphate. Stroke 1990; 21: 606-613.

Ferrer I, Serrano T, Soriano E. Naturally occurring cell death in the subicular complex and hippocampus in the rat during development. Neurosci Res 1990; 8; 60-66.

Ferrer I, Soriano E, Tuñon T, Fonseca M, Guionnet N. Parvalbumin immunoreactive neurons in normal human temporal neocortex and in patients with Alzheimer's disease. J Neurol Sci 1991; 106: 135-141.

Ferrer I, Tuñon T, Soriano E, Del Rio A, Iraizoz I, Fonseca M, Guionnet N. Calbindin immunoreactivity in normal human temporal neocortex. Brain Res 1992; 572: 33-41.

Ferrer I. The effect of cycloheximide on natural and X-ray-induced cell death in the developing cerebral cortex. Brain Res 1992; 588: 351-357.

Ferrer I, Zújar MJ, Admella C, Alcántara S. Parvalbumin and calbindin immunoreactivity in the cerebral cortex of the hedgehog (Erinaceus europaeus). J Anat 1992; 180: 165-174.

Flamm ES, Demopoulos HB, Seligman ML et al. Free radicals in cerebral ischemia. Stroke 1978; 9: 445-447.

Freund TF, Buzsáki G, Leon A, Baimbridge KG, Somogyi P. Relationship of neuronal vulnerability and calcium binding protein immunoreactivity in ischemia. Exp Brain Res 1990; 83: 55-66.

Freund TF, Ylinen A, Miettinen R, Pitkänen A, Lahtinen H, Baimbridge KG, Riekkinen PJ. Pattern of neuronal death in the rat hippocampus after status epilepticus. Relationship to calcium binding protein content and ischemic vulnerability. Brain Res Bull 1991; 28: 27-38.

Fridovich I. The biology of oxigen radicals. Science 1978; 2018: 875.

Gamrani H, Onteniente B, Seguela PH, Geffars M, Calas A. Gamma-aminobutiric acid-inmunoreactivity in the rat hippocampus. A light and electron microscopic study with anti-GABA antibodies. Brain Res 1986; 364: 30-38.

Gaudet R, Welch KMA, Chabi E et al. Effect of transient ischemia on monoamine levels in the cerebral cortex of gerbils. J Neurochem 1978; 30: 751.

Gehrmann J, Bonnekoh P, Miyazawa T, Hossmann K-A, Kreutzberg GW. Immunocytochemical study of an early microglial activation in ischemia. J cereb Blood Flow Metab 1992; 12: 257-269.

Gerschenson LE, Rotello RJ. Apoptosis: a different type of cell death. FASEB 1992; 6: 2450-2455.

Giacosa A, Sukkar GS, Chiti D, Marchetti M. Effects of fructose-1,6-diphosphate on the hypoglycemic response to intravenous glucose load. Curr Therap Res 1987; 41: 874-880.

Gill R, Kemp JA. Protection of CA1 pyramidal cell function by MK-801 following ischaemia in the gerbil. Neurosci Lett 1989; 105: 101-106.

Ginsberg MD, Busto R. Rodent models of cerebral ischemia. Stroke, 1989: 20: 1627-1642.

Goldstein M, Barnett HJM, Orgogozo JM, Sartorius N, Symon L, Vereshchagin NV. Recommendations on stroke prevention, diagnosis and therapy. Stroke 1989; 20: 1407-1431.

Goto K, Ishige A, Sekiguchi K i cols. Effects of cycloheximide on delayed neuronal death in rat hippocampus. Brain Res 1990; 534: 299-302.

Graham DI. Hypoxia and vascular disorders. En: Adams JH, Duchen LW, eds. Greenfield's Neuropathology. E. Arnold, London 1992; 153-268.

Gregory GA, Yu ACH, Chan PH. Fructose-1,6-biphsphate protects astrocytes from hypoxic damage. J Cerb Blod Flow Metab 1989; 9: 29-34.

Gregory GA, Welsh FA, Yu ACH, Chan PH. Fructose-1,6-biphsphate reduces ATP loss from hypoxic astrocytes. Brain Res 1990; 516: 310-312.

Greenberg DA. Calcium channels and calcium channel antagonists. Ann Neurol 1987; 21: 317-330.

Grotta JC. Current medical and surgical therapy for cerebrovascular disease. N Engl J Med 1987; 317; 1505-1516.

Grotta JC, Picone CM, Dedman JR i cols. Neuronal protection correlates with prevention of calcium-calmodulin binding in rats. Stroke 1990; 21 (S III): 28-31.

Gustafson I, Miyauchi Y, Wieloch T. Modulation of ischemic brain damage by noradrenaline. Protection by idazoxan, an alpha-2-receptor antagonist. En: Ginsberg MD, Dietrich WD eds. Cerebrovascular Diseases. New York. Raven Press 1989; 117-121.

Hara H, Ozaki A, Yoshidomi M, Sukamoto T. Protective effect of KB-2796, a new calcium antagonist, in cerebral hypoxia and ischemia. Arch int Pharmacodyn 1990; 304: 206-218.

Harmayer J, De Luca HF. Calcium-binding protein and calcium adsorption after Vitamin D-administration. Archs Biochem Byophys 1969; 133: 247-254.

Hashimoto Y, Kawatsura H, Shiga Y, Furukawa S, Shigeno T. Significance of nerve growth factor content levels after transient forebrain ischemia in gerbils. Neurosci Lett 1992; 139: 45-46.

Hass WK. The cerebral ischemic cascade. Neurol Clin 1983; 1: 345.

Haynes RC. Agents affecting calcification: calcium, parathyroid hormone, calcitonin, vitamin D and other compounds. En: Goodman A, Rall TW, Nies AS, Taylor P, eds. The pharmacological basis of therapeutics. New York, 1991; 1496-1522.

Heffez DS, Passonneau JV. Effect of nimodipine on cerebral metabolism during ischemia and recirculation in the Mongolian gerbil. J Cerb Blood Flow Metab 1985; 5: 523-528.

Hendrickson AE. The ortograde axoplasmic transport autoradiographic technique and its implications for additional neuroanatomical analysis of the striate cortex. En: Chan-Palay ^Palay SL, eds. Cytochemical Methods in Neuroanatomy. Liss. New York 1982; 1-16.

Hendry SHC, Jones EG. Sizes and distributions of intrinsic neurons incorporating tritiated GABA in monkey sensory-motor cortes. J Neurosci 1981; 1: 390-408.

Hendry SHC, Houser CR, Jones EG, Vaughn JE. Synaptic organization of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex. J Neurocytol 1983; 12: 639-660.

Hendry SHC, Jones EG, Emson PC, Lawson DEM, Heizmann CW, Streit P. Two clases of cortical GABA neurons defined by differential calcium binding protein immunoreactivities. Exp Brain Res 1989; 76: 467-472.

Hewitt K, Corbet D. Combined treatment with MK-801 and nicardipine reduces global ischemic damage in the gerbil. Stroke 1992; 23: 82-86.

Höchli M, Zetzsche T, Chan-Palay V. Parvalbumin-immunoreactive neurons in the normal human hippocampus. Dementia 1991; 2: 243-258.

Hökfelt T, Ljungdahl A. Autoradiographic identification of cerebral and cerebellar cortical neurons accumulating labeled gamma-aminobutyric acid (^{3}H-GABA). Exp Brain Res 1972; 14: 354-362.

Horvitz HR, Ellis HM, Sternberg PW. Programmed cell death in nematode development. Neurosci Comment 1982; 1: 56-65.

Hossmann KA. Pathophysiology of cerebral infarction. En: Vinken PJ, Bruyn GW, Klawans HL, Toole JF eds. Handbook of Clinical Neurology. Vascular Diseases. New York. Elsevier Science Publishing Co. Inc, 1988; 107-153.

Houser CR, Hendry SHC, Jones EG, Vaughn JE. Morphological diversity of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex. J Neurocytol 1983; 12: 617-638.

Inuzuka T, Tamura A, Sato S i cols. Changes in the concentrations of cerebral proteins following occlusion of the middle cerebral artery in rats. Stroke 1990; 21: 917-922.

Ito U, Walter JT jr, Spatz M, Klatzo I. Experimental cerebral ischemia in mongolian gerbils.I Ligth microscopic observations. Acta Neuropath 1975; 32: 209-223.

Iversen LL. Biochemical psychopharmacology of GABA. En Lipton MA, Di Mascio A, William KF eds. Psycopharmacology: A Generation of Progress. Raven Press New York 1978; 25-28.

Jacewicz M, Kiessling M, Pulsinelli WA. Selective gene expression in focal cerebral ischemia. J cereb Blood Flow Metab 1986; 6: 263-272.

Johansen FF, Tonder N, Zimmer J, Baimbridge KG, Diemer NH. Short-term changes of parvalbumin and calbindin immunoreactivity in the rat hippocampus following cerebral ischemia. Neurosci Lett 1990; 120: 171-174.

Joó F, Ikeda J, Lohr J, Nagashima G, Mies G, Nowak TS, Ruetzler C, Klatzo I. (1992) Possible role of intracellular calcium translocation in the maturation of ischemic damage. In Maturation phenomenon in cerebral ischemia (ed. Ito U, Kirino T, Kuroiwa T, Klatzo I), pp 41-47. Springer-Verlag, Berlin, Heidelberg, New York.

Jones JW, Gionis TA, Nichols RL, Markov AK, Webb WR. Myocardial preservation with fructose-1,6-diphosphate: energy without oxygen. Surg Forum 1980; 31: 307-309.

Kaas IS, Lipton P. Calcium and long-term transmission damage following anoxia in dentate gyrus and CA1 regions of the rat hippocampal slice. J Physiol 1986; 378: 313-334.

Kahn K. Tha natural course of experimental cerebral infarction in the gerbil. Neurology 1972; 22: 510-515.

Kang J, Lemaine HG, Unterbeck A i cols. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell surface receptor. Nature 1987; 325: 733-736.

Kaplan TM, Lasner TM, Nadler JV, Crain BJ. Lesions of excitatory pathways reduce hippocampal cell death after transient forebrain ischemia in the gerbil. Acta Neuropathol 1989; 78: 283-290.

Katsumaru H, Kosaka T, Heizmann CW, Hama K. Immunocytochemical study of GABAergic neurons containing the calcium-binding protein parvalbumina in the rat hippocampus. Exp Brain Res 1988; 72: 347-362.

Kawaguchi Y, Katsumaru H, Kosaka T, Heizmann CW, Hama K. fast spiking cells in rat hippocampus (CA1 region) contain the calcium-binding protein parvalbumin. Brain Res 1987; 416: 369-374.

Kemp JA, Foster AC, Wong EH. Non-competitive antagonists of excitatory amino acid receptors. Trends Neurosci 1987; 10: 294-298.

Kerr JFR, Searle J, Harmon BV, Bishop CJ (1987) Apoptosis. In C.S. Potten (Edit.) Perspectives on Mammalian Cell Death, Oxford Univ. Press, Oxford, pp. 93-128.

Kerwin J, Morris C, Oakley A, Perry r, Perry E. Distribution of nerve growth factor receptor immunoreactivity in the human hippocampus. Neurosci Lett 1991; 121: 178-182.

Kiessling M, Dienel GA, Jacewicz M, Pulsinelli WA. Protein synthesis in postischemic rat brain: a two dimensional electrophoretic analysis. J Cereb Blood Flow Metab 1986; 6: 642-649.

Kirinino T. Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Research 1982; 239: 57-69.

Kirino T, Sano K. Fine structural nature of delayed neuronal death following ischemia in the gerbil hippocampus. Acta Neuropathol 1984; 62: 209-218.

Kirino T, Tamura A, Sano K. Delayed neuronal death in the rat hippocampus following transient forebrain ischemia. Acta Neuropathol 1984; 64: 139-147.

Kirino T, Tsujita Y, Tamura A. Induced tolerance to ischemia in gerbil hippocampal neurons. J. Cereb Blood Flow Metab 1991; 11: 299-307.

Kita H, Kosaka T, Heizmann CW. Parvalbumin-immunoreactive neurons in the rat neostriatum: a light and electron microscopic study. Brain Res 1990; 536; 1-15.

Kitagawa K, Matsumoto M, Handa N i cols. Prediction of stroke-prone gerbils and their cerebral circulation. Brain Res 1989; 479: 263-269.

Kitagawa K, Matsumoto M, Niinobe M i cols. Microtubule-associated protein 2 as a sensitive marker for cerebral ischemic damage-immunohistochemical investigation of dendritic damage. Neuroscience 1989; 31: 401-411.

Kleihues P, Hossmann KA. Protein synthesis in the cat brain after prolonged cerebral ischemia. Brain Res. 1971; 35: 409-418.

Kontos HA. Oxygen radicals in cerebral ischemia. En Ginsberg MD, Dietrich WD, ed. Cerebrovascular Diseases. New York, Raven Press, Publishers,1989; 365-371.

Koo EH, Sisodia SS, Archer DR i cols. Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport. Proc Natl Acad Sci USA 1990; 87: 1561-1565.

Kosaka T, Katsumaru K, Hama K, Wu JY, Heizmann CW. GABAergic neurons containing the Ca^{2+} binding protein parvalbumin in the rat hippocampus and dentate gyrus. Brain Res 1987; 419: 119-130.

Kosaka T, Wu JY, Benoit R. GABAergic neurons containing somatostatin-like immunoreactivity in the rat hippocampus and dentate gyrus. Exp Brain Res 1988; 71: 388-398.

Kostron H, Sperl W, Murr C, Pillwein K. Effect of the calcium entry blocker nimodipine on the metabolism of nuclei acids in rat brain ischemia. A: Long D et al. eds. Advances in Neurology. Vol 52. Raven Press 1990; 133-139.

Krnjevic K. Chemical nature od synaptic transmission in vertebrates. Physiol Rev 1974; 54: 418-540.

Kucharczyk J, Chew W, Derugin N i cols. Nicardipine reduces ischemic brain injury: magnetic resonance imaging/spectroscopy study in cats. Stroke 1989; 20: 268-274.

Kudo T, Tada K, Takeda M, Nishimura T. Learning impairment and microtubule-associated protein 2 decrease in gerbils under chronic cerebral hypoperfusion. Stroke 1990; 21: 1205-1209.

Kurtzke JF. Epidemiology of cerebrovascular disease. En: cerebrovascular survey report for Joint Council Subcommitee on cerebrovascular disease. NINCDS and NHLI (Revised) January 1980. Whiting Press Rochester 1980; 135-176.

LeBlanc MH, Farias LA, Evans OB, Vig V, Smith EE, Markov AK. Fructose-1,6-biphosphate, when given immediately before reoxygenation, or before injury, does not ameliorate hypoxic ischemic injury to the central nervous system in the newborn pig. Crit Care Med 1990; 19: 75-83.

Levine S, Sohn D. Cerebral ischemia in infant and adult gerbils. Arch Path 1969; 87:315-317.

Levy DE, Duffy TE. Cerebral energy metabolism during transient ischemia and recovery in the gerbil. J Neurochem 1977; 28: 63-70.

Li Y, Chopp M, Garcia JH, Yoshida Y, Zhang Z, Levine SR. Distribution of the 72-kd heat-shock protein as a function of transient focal ischemia in rats. Stroke 1992; 23: 1292-1298.

Lightfoote WE, Molinari GF, Chase TN. Modification of cerebral ischemic damage by anesthetics. Stroke 1977; 8: 627-628.

Lindquist S, Craig EA. The head shock proteins. Annu Rev Genet 1988; 22: 631-677.

Lockshin RA, Zakeri FZ. Programmed cell death: new thoughts and relevance to aging. J Geron Biol Sci 1990; 45: B135-140.

Lorente de Nó R. Studies on the structure of the cerebral cortex.II. Continuation of the study of the ammonic system. J Psychol Neurol 1934; 46: 113-117.

Loskota WJ, Lomax P, Rich ST. The gerbil as a model for the study of epilepsies. Epilepsia 1974; 15: 109-115.

Lysko GP, Yue T-L, Gu JL, Feuerstein G. Neuroprotective mechanism of (+)SKF 10,047 in vitro and in gerbil global brain ischemia. Stroke 1992; 23: 1319-1324.

Marcoux FW. Goodrich JE, Dominick MA. Ketamine prevents ischemic neuronal injury. Brain Res 1988; 452: 329-335.

Marchionni N, Conti A, De Alfieri W i cols. Hemodynamic and electrocardiographic effects of fructose-1,6-Diphosphate in acute myocardial infarction. Am L Cardiol 1985; 56: 266-269.

Markov AK, Oglethorpe NC, Blake TM, Lehan PH, Hellems HK. Hemodynamic, electrocardiographic and metabilic effects of fructosa diphosphate on acute myocardial ischemia. Amm Heart J 1980; 100: 639-646.

Markov AK, Oglethorpe N, Jones J, Young DB, Lehan PH, Hellens HK. Prevention of arrhythmias with fructose diphosphate in acute myocardial ischemia (abs) Circulation 1984; 62: 143A.

Markov AK. Hemodynamic and metabolic effects of fructose 1-6 diphosphate (FDP) in ischemia and shock-Experimental and clinical observations. Ann Emerg Med 1986; 15: 1470-1477.

Markov AK, Finch CD, Hellens HK. Prevention of superoxide generation and inhibition of oxygen burst in human and canine neutrophils with fructose 1-6 diphosphate (FDP). En Tsuchiya M, Asano M, Mishina Y, Oda M eds. Microcirculation: an update. New York, Amsterdam, Elsevier Science Publishing Co, Inc 1987: 691-696.

Matsumoto M, Hatakeyama T, Fumiharu A, Brengman JM, Yanagihara T. Prediction of stroke before and after unilateral occlusion of the common carotid artery in gerbils). Stroke 1988; 19: 490-497.

Martin DP, Schmidt RE, DiStefano PS, Lowry OH, Carter JG, Johnson EM. Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by nerve growth factor deprivation. J Cell Biol 1988; 106: 829-844.

Meldrum B. Excitotoxicity in ischemia: an overview. En: Ginsberg MD, Dietrich WD eds. Cerebrovascular Diseases. New York. Raven Press 1989; 47-64.

Mies G, Kawai K, Saito N i cols. Protein synthesis and calcium uptake following complete cerebral ischemia of rat brain. A. Ito U, Kirino T, Kuroiwa T, Klatzo I eds. Maturation phenomenon in cerebral ischemia. Springer-Verlag. Berlin/Heidelberg 1992: 129-138.

Miller MW. Deveolpment of projection and local circuit neurons in neocortex. En: Peters A, Jones EG eds. Cerebral cortex. Vol 7. Plenur Press, New York 1988; 133-285.

Miller RJ. The control of neuronal Ca^{2+} homeostasis. Progress in Neurobiology 1991; 37: 255-285.

Mitani A, Imon H, Iga K, Kubo H, Kataoka K. Gerbil hippocampal extracellular glutamate and neuronal activity after transient ischemia. Brain Res Bull 1990; 25: 319-324.

Morimoto K, Yanagihara T. Cerebral ischemia in gerbils: polyribosomal function during progresion and recovery. Stroke 1981; 12: 105-110.

Morioka T, Kalehua AN, Streit WJ. The microglial reaction in the rat dorsal hippocampus following transient forebrain ischemia. J Cerb Blood Flow Metab 1991; 11: 966-973.

Mouritzen A, Bajorek JC, Lomax P. Hippocampal neuron density and seizures in the Mongolian gerbil. Epilepsia 1981; 22: 667-674.

Nagai T, McGeer PL, McGeer EG. Distribution of GABA-T intensive neurons in the rat forebrain and midbrain. J Comp Neurol 1983; 218: 220-238.

Nakamura Y, Takeda M, Niigawa H, Hariguchi S, Nishimura T. Amyloid ß-protein precursor deposition in rat hippocampus lesioned by ibotenic acid injection. Neurosci Lett 992; 136: 95-98.

Nedergaard M. Mechanisms of brain damage in focal cerebral ischemia. Acta Neurol Scand 1988; 77: 81-101.

Nieto-Sampedro M, Lewis ER, Cotman CW. Brain injury causes a time-dependent increase in neuronotrophic activity at the lesion site. Science 1982; 217. 860-861.

Nitsch C, Goping G, Klatzo I. Preservation of GABAergic perikarya and boutons after transient ischemia in the gerbil hippocampal CA1 field. Brain Res 1989a; 495: 243-252.

Nitsch C, Scotti A, Sommacal A, Kalt G. GABAergic hippocampal neurons resistant to ischemia-induced neuronal death contain the Ca^{2+}-binding protein parvalbumin. Neurosci Lett 1989b; 105: 263-268.

Nitsch R, Soriano E, Frotscher M. The parvalbumin-containing nonpyramidal neurons in the rat hippocampus. Anat Embryol 1990; 181: 413-425.

Nitsch C. Preservation of GABAergic perikarya and boutons after transient ischemia in the gerbil hippocampal CA1 field. Stroke 1990; 21: 194.

Nitsch C. Reorganization in the gerbil hippocampus after ischemia-induced delayed neuronal death: fate of parvalbumin-containing neurons. A: Ito U, Kirino T, Kuroiwa T, Klatzo I eds. Maturation phenomenon in cerebral ischemia. Springer-Verlag. New York 1992: 23-31.

Nowak TS. Synthesis of a stress protein following transient ischemia in the gerbil. J Neurochem 1985; 45: 1635-1641.

Nowak TS. Localization of 70 kDa stress protein mRNA induction in gerbil brain after ischemia. J Cereb Blood Flow Metab 1991; 11: 432-439.

Olney JW, Price MT, Fuller TA i cols. The anti-excitotoxic effects of certain anesthetics , analgesics and sedative-hypnotics. Neurosci Lett 1986; 68: 29-34.

Oppenheim RW, Prevette D, Tytell M, Homma S. Naturally occurring and induced neuronal death in the chick embrion in vivo requires protein and RNA synthesis: evidence for the role of cell death genes. Develop Biol 1990; 138; 104-113.

Oppenheim RW. Cell death during development of the nervous system. Annu Rev Neurisci 1991; 14: 453-501.

Palacios G, Palacios JM, Mengod G, Frey P. ß-Amyloid precursor protein localization in the Glogi apparatus in neurons and oligodendrocytes. An immunocytochemical structural and ultrastructural study in normal and axotomized neurons. Mol Brain Res 1992; 15: 196-206.

Papas S, Crépel D, Hasboun I, Jorquera I, Chinestra P, Ben-Ari Y. Cycloheximide reduces the effects of anoxic insult in vivo and in vitro. Eur J Neurosci 1992; 4: 758-765.

Parnavelas JG, McDonald JK. The cerebral cortex. En Emson PC ed. Chemical Neuroanatomy. Raven Press. New York 1983; 505-549.

Paul LA, Fried I, Watanabe K, Forsythe AB, Scheibel AB. Structural correlates of seizure behavior in the mongolian gerbil. Scienc 1981; 213: 924-926.

Peters T. Calcium in physiological and phatological function. Eur Neurol 1986; 25(S1): 27-41.

Petito CK, Morgello S, Felix JC, Lesser ML. The two patterns of reactive astrocytosis in postischemic rat brain. J Cereb Blood Flow Metab 1990; 10: 850-859.

Pfyffer GE, Faivre-Bauman A, Tixier-Vidal A, Norman AW, Heizmann CW. Developmental and functional studies of parvalbumin and calbindin D28k in

hypothalamic neurons grown in serum-free medium. J Neurochem 1987; 49: 442-451.

Pulsinelli WA, Sigsbee B, Rawlinson D et al. Experimental hyperglycemia and diabetis mellitus worsen stroke outcome. Ann Neurol 1980; 8:91.

Pulsinelli WA, Brierley JB, Pulm F. Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol 1982; 11: 491-498.

Pulsinelli WA. Selective neuronal vulnerability: morphological and molecular characteristics. Prog Brain Res 1985; 63; 29-37.

Pulsinelli WA, Buchan A: The utility of animal ischemia models in predicting pharmacotherapeutic response in the clinical setting. En Ginsberg MD, Dietrich WD, ed. Cerebrovascular Diseases. New York, Raven Press, Publishers, 1989; 87-91.

Raff, M.C. (1992) Social controls on cell survival and cell death, Nature, 356: 397-400.

Ramon y Cajal S. Histologie du système nerveux de l'home et des vertebrés. Maloine. Paris 1911.

Rehncroma S, Folbergrova J, Smith DS et al. Influence of complete and pronounced incomplete cerebral ischemia and subsequent recirculation on cortical

concentrations of oxidized and reduced glutathione in the rat. J Neurochem 1980; 34: 477.

Ribak CE. Aspinous and sparsely-spinous stellate neurons in the visual cortex of rats contain glutamic acid decarboxilase. J Neurocytol 1978; 7: 461-478.

Ribak CE, Harris AB, Vaughn JE, Roberts E. Inhibitory, GABAergic nerve terminals decrease at sites of focal epilepsy. Science 1979; 205: 211-214.

Ribak CE, Bradburne RM, Harris AB. A preferencial loss of GABAergic symmetric synapses in epileptic foci: a quantitative ultrastructural analysis of monkey neocortex. J Neuoscu 1982; 2: 1725-1735.

Rosenbaum D, Zabramski J, Frey J i cols. Early treatment of ischemic stroke with a calcium antagonist. Stroke 1991; 22: 437-441.

Rothman SM, Synaptic activity mediates death of hypoxic neurons. Science 1983; 220: 536-537.

Rothman SM, Olney JW. Glutamate and the pathophysiology of hypoxic-ischemic brain damge. Ann Neurol 1986; 19: 105-111.

Rothman SM, Thurston JH, Hauhart RE, Clark GD, Solomon JS. Ketamine protects hippocampal neurons from anoxia in vitro. Neurosci 1987; 21: 673-678.

Sancho J, Chamorro R, Sancho A, Laínez JM, Rubio JM, Romaguera A, Morera J, Escudero JV. Evolución de la mortalidad por enfermedad cerebrovascular en España durante el decenio 1974-1983. Neurologia 1988; S3: 16-17.

Sakamoto N, Kogure K, Kato H, Ohtomo H. Disturbed Ca2+ homeostasis in the gerbil hippocampus following brief transient ischemia. Brain Res 1986; 364: 372-376.

Schanne FAX, Kane AB, Young EE Farber JL. Calcium dependence of toxic cell death: a final common pathway. Science 1979; 206: 700-702.

Scotti AL, Nitsch C. The perforant path in the seizure sensitive gerbil contains the Ca^{2+} binding protein parvalbumin. Exp Brain Res 1991; 85: 137-143.

Selkoe DJ, Podlisny MD, Joachim C i cols. Beta-amyloid precursor protein of Alzheimer disease occurs at 110- to 135-kilodalton membrane-associated proteins in neural and nonneural tissues. Proc Natl Acad Sci USA 1988; 85; 7341-7345.

Seto-Oshima A, Aoki E, Semba R, Emson PC, Heizmann CW. Appearance of parvalbumin-specific immunoreactivity in the cerebral cortex and hippocampus of the developing rat and gerbil brain. Histochemistry 1990; 94: 579-589.

Shigematsu K, Mc Geer PL. Accumulation od amyloid precursor protein in neurons after intraventricular injection of colchicine. Amm J Pathol 1992; 140: 787-794.

Shigeno T, Yamasaki Y, Kato G i cols. Reduction of delayed neuronal death by inhibition of protein synthesis. Neurosci Let 1990; 120: 117-119.

Shigeno T, Mima T, Takakura K i cols. Amelioration of delayed neuronal death in the hippocampus by nerve growth factor. J. Neurosci 1991; 11: 2914-2919.

Siesjö BK. Calcio, isquemia y muerte celular. Drugs of today 1988; 24(S); 67-79.

Siman R, Card JP, Nelson RB, Davis LG. Expression of ß-amyloid precursor protein in reactive astrocytes following neuronal damage. Neuron 1989; 3: 275-285.

Simon RP, Griffith T, Evans MC, Swan JH, Meldrum BS. Calcium overload in selectively vulnerable neurons of the hippocampus during and after ischemia: and electron microscopy study in the rat. J Cereb Blood Flow Metab 1984; 4: 350-361.

Sloviter RR. Calcium-binding protein (calbindin-D28K) and parvalbumin immunocytochemistry: localization in the rat hippocampus with specific reference to the selective vulnerability of hippocampal neurons to seizure activity. J Comp Neurol 1989; 280: 183-186.

Sloviter RS, Sollas AL, BArbaro NM, LAxer KD. Calcium-binding protein (CAlbindin-D28K) and parvalbumin immunocytochemistry in the normal and epileptic human hippocampus. J Comp Neurol 1991; 308: 381-396.

Spatz M, Kumani K, Ueki Y, Djuricic BD, Mrsulja BB. Cerebral ischemia in adult and young mongolian gerbils: delayed changes of monoamines. En Ito U, Kirino T, Kuroiwa T, Klatzo I eds. Maturation phenomenon in cerebral ischemia. Springer-Verlag, Berlin, Heidelberg, New York 1992: 169-178.

Soriano E, Nitsch R, Frotscher M. Axo-axonic chandelier cells in the rat fascia dentata: a Golgi-EM and immunocytochemical studies. J Comp Neurol 1990; 293: 1-25.

Steen PA, Gisvold SE, Milde JH i cols. Nimodipine improves outcome when given after complete cerebral ischemia in primates. Anesthesiology 1985; 62: 406-414.

Stephenson DT, Rash K, Clemen JA. Amyloid precursor protein accumulates in regions of neurodegeneration following focal cerebral ischemia in the rat. Brain Res 1992; 593: 128-135.

Strubley RG, Desmond NL, Levy WB. Anatomical evidence for interlamelar inhibition in the fascia dentata. Brain Res 1978; 152: 580-585.

Suzuki R, Yamaguchi T, Inaba Y, Wagner HG. Microphysiology of selectively vulnerable neurons. Prog Brain Res 1985; 63:59-68.

Takahashi K, Bodsch W, Hossmann KA. Susceptibility of hippocampal protein syntesis to transient forebrain ischemia of adult and infant gerbil brain. Drug Dis 1984; 1: 72-78.

Trimarchi GR, De Luca R, Campo GM, Scuri R, Caputi AP. Protective effects of fructose-1,6-biphosphate on survival and brain putrescine levels during ischemia and recirculation in the Mongolian gerbil. Stroke 1990; S IV: 171-173.

Tomei LD, Cope FO. Apoptosis: The molecular Basis of Cell Death, Cold Springer Harbor Laboratory Press, 1991; New York.

Tsuda T, Kogure K, Ishii K, Orihara H. Postischemic changes of calcium and endogenous antagonist in the rat hippocampus studied by proton-induced X-ray emission analysis. Brain Research 1989; 484: 228-233.

Ueda Y, Obrenovitch TP, Lok SY, sarna GS, Symon L. Efflux of glutamate produced by short ischemia of varied severity in rat striatum. Stroke 1992; 23: 253-259.

Uemura Y, Kowall NW, Beal MF. Global ischemia induces NMDA receptor-mediated c-fos expression in neurons resistant to injury in gerbil hippocampus. Brain Res 1991; 542: 343-347.

Urban L, Neill KH, Crain BJ, Nadler JV, Somjen GG. Postischemic synaptic excitation and N-Methyl-D-aspartate receptor activation in gerbils. Stroke 1990; 21 (S-II): 23-27.

Van Brederode JFM, Helliesen MK, Hendrickson AE. Distribution of the calcium-binding proteins parvalbumin and calbindin-D28k in the sensorimotor cortex of the rat. Neurosci 1991; 44: 157-171.

Van Neuten JN, Vanhoutte PM. Improvements of tissue perfusion with inhibitors of calcium ion influx. Biochem Pharmacol 1980; 29; 479-481.

Vass K, Welch WJ, Nowak TS. Localization of 70 kDA stress protein induction in gerbil brain after ischemia. Acta Neuropathol 1988; 77; 128-135.

Waener MA, Neill KH, Nadler JV, Crain BJ. Regionally selective effects of NMDA receptor antagonists against ischemic brain damage in the gerbil. J Cereb Blood Flow 1991; 11: 600-610.

Wassermann RH, Taylor AN. Vitamin D_3 induced calcium-binding protein in chick intestinal mucosa. Science 1966; 152: 791-793.

Watkins JC, Olverman HJ. Agonists and antagonists for excitatory amino acid receptors. Trends Neurosci 1987; 10: 265-272.

Welch KMA, Barkley GL. Biochemistry and pharmacology of cerebral ischemia. En: Barnett HJM, Mohr JP, Stein BM, Yatsu FM eds. Stroke. New York: Churchill Livingstone, 1986; 75-90.

Welsh FA, Moyer DJ, Harris VA. Regional expression of heat shock protein-70 mRNA and c-fos mRNA following focal ischemia in rat brain. J Cereb Blood Flow Metab 1992; 12: 204-212.

White BC, Winegar CD, Wilson RF i cols. Possible role of calcium blockers in cerebral resuscitation: a review of the literature and synthesis for future studies. Crit Care Med 1983; 11: 202-207.

Whitson JS, Glabe CG, Shintani E, Aber A, Cotman CW. Beta-amyloid protein promotes neuritic branching in hippocampal cultures. Neurosci Lett 1990; 110: 319-324.

Widmann R, Kuroiwa T, Bonnekoh P, Hossmann KA. [^{14}C]Leucine incorporation into brain proteins in gerbils after transient ischemia: relationship to selective vulnerability of hippocampus. J Neurochem 1991; 56: 789-796.

Wieloch T, Harris RJ, Siesjo BK. Brain metabolism and ischemia: mecanisms of cell damage and principles of proteccion. J Cereb Blood Flow Metab 1982; 2: S5.

Wyllie AH. Cell death: a new classification sepa rating apoptosis from necrosis. En I.D. Bowen and R.A. Lockshin (Eds.) Cell death in Pathology and Biology, Chapman and Hall, New York 1981: 9-34.

Wyllie AH, Morris RG, Smith AL, Dunlop D. Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. J Path 1984; 142: 67-78.

Yamamoto K, Morimoto K, Yanagihara T. Cerebral ischemia in the gerbil: Transmission electron microscopic and immunoelectron microscopic investigation. Brain Res 1986; 384: 1-10.

Yamamoto K, Hayakawa T, Mogami H, Akai F, Yanagihara T. Ultrastructural investigation of the CA1 region of the hippocampus after transient cerebral ischemia in gerbils. Acta Neuropathol 1990; 80: 487-492.

Yamamoto S, Yoshimine T, Fujita T i cols. Protective effect of NGF atelocollagen mini-pellet on the hippocampal delayed neuronal death in gerbils. Neurosci Lett 1992; 141: 161-165.

Yanagihara T, Brengman JM, Mushynski EW. Differential vulnerability of microtubule components in cerebral ischemia. Acta Neuropathol 1990; 80: 499-505.

Yankner BA, Duffy LK, Kirschner DA. Neurotrophic and neurotoxic effects of amyloid beta-protein: reversal by tachykinin neuropeptides. Science 1990; 250: 279-282.

Yoshimi K, Takeda M, Nishimura T i cols. An immunohistochemical study of MAP2 and calthrin in gerbil hippocampus after cerebral ischemia. Brain Res 1991; 560: 149-158.