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Astroglial responses in photochemically induced focal ischemia of the rat cortex

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Abstract This study investigated astroglial responses after focal cerebral ischemia in the rat cortex induced by photothrombosis. Astrocyte activation was studied at various time points by immunocytochemistry for glial fibrillary acidic protein (GFAP) and vimentin (VIM). We found a dual astrocytic response to focal ischemia: In the border zone of the infarct, GFAP-positive astrocytes were present within 2 days and persisted for 10 weeks. These astrocytes additionally expressed VIM. Remote from the ischemic lesion, cortical astrocytes of the entire ipsilateral hemisphere transiently expressed GFAP, but not VIM, beginning on day 3 after photothrombosis. This response had disappeared on day 14. By recording DC potentials, five to seven spreading depressions (SD) could be detected on the cortical surface during the first 2 h after photothrombosis. Treatment with MK801, a non-competitive NMDA-receptor antagonist, completely abolished SD and remote ipsilateral astrocytic activation, while the reaction in the border zone of the infarct remained unchanged. Functionally, persistent astrocytosis around the infarct might be induced by leukocyte-derived cytokines, while NMDA-receptor-mediated SD might cause remote responses.

Key words Spreading depression · GFAP · Astrocytes · Focal ischemia · Rat

Introduction

Astrocytes play a pivotal role in the CNS by maintaining ion and pH homeostasis and by regulating volume and glucose levels (Walz 1989). They are essential constituents of the blood-brain barrier (Risau and Wolburg 1990). Astrocytes respond to a wide range of pathological conditions in the CNS by cell hypertrophy and proliferation (Bignami and Dahl 1976; Latov et al. 1979; Eng 1985; Schiffer et al. 1986). Glial fibrillary acidic protein (GFAP), an established marker for astrocytes, is constitutively expressed in fibrous astrocytes of the white matter, but at much lower levels in protoplasmic astrocytes, which account for most astrocytes in the gray matter (Bignami and Dahl 1976; Miller and Raff 1984). The passage from the quiescent to the reactive state is accompanied by an increase in intermediate filaments, predominantly in GFAP and, under certain conditions, also vimentin (VIM) (Schiffer et al. 1986).

Astrocytes are activated after global cerebral ischemia (Petito et al. 1990; Schmidt-Kastner et al. 1993). In this study, we examined whether this also applies to focal ischemia. Focal ischemia in the rat parietal cortex was induced by local illumination after systemic application of rose bengal according to the method of Watson et al. (1985). This leads to photochemically stimulated platelet aggregation with ensuing occlusion of small intracerebral vessels. With this technique, focal cortical infarcts can be induced which are highly reproducible in location and size (Watson et al. 1985; Domann et al. 1993). Paraffin sections were stained immunocytochemically for GFAP and VIM at various stages after photothrombosis. We show that two types of astroglial responses occur which are differentially influenced by spreading depression (SD).

SD is characterized by a transient depression of EEG activity and a negative shift in the DC potential that spreads across the cortical surface (Leao 1944). MK801, a noncompetitive *N*-methyl-D-aspartate receptor antagonist, abolishes SD (Marrannes et al. 1988; Gill et al. 1992). SD also occurs after photothrombosis (Dietrich et al. 1994). After application of KCl to the cortical surface, SD can trigger astroglial activation (Kraig et al. 1991). To examine the contribution of SD to the activation of astrocytes after photothrombosis, SD was registered and astroglial responses were compared between sham- and MK801-treated animals.

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Materials and methods

Induction of photothrombosis and tissue processing

Focal ischemia was induced in the rat parietal cortex at the same location as in two previous studies (Domann et al. 1993; Jander et al. 1995) according to the method described by Watson et al. (1985). Briefly, male Wistar rats (250–300 g) were anesthetized with 1.3% halothane in O_2/N_2 (1:2) and placed in a stereotactic frame. A fiberoptic bundle was placed stereotactically onto the skull at 3.5–4 mm posterior to bregma and 3.5–4 mm lateral from the midline for a 20-min period of illumination. During the first minute of illumination, rose bengal (1.3 mg/100 g body weight at a concentration of 10 mg/ml in 0.9% NaCl) was injected into the femoral vein.

For immunocytochemistry, rats were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer transcardially in deep anesthesia (number of animals examined in parenthesis) at 4 h (2), days 1 (2), 2 (2), 3 (4), 6 (3), 14 (2), and 60 (2) after photothrombosis. Whole brains were embedded in paraffin. Coronal and sagittal serial sections 5 μm thick were cut through the center of the lesion and stained with monoclonal antibodies (mab) against GFAP (1:50) and VIM (1:20) (both mab Boehringer-Mannheim, Germany) using the avidin-biotin-peroxidase method as described elsewhere (Jander et al. 1995).

Registration of spreading depression

In another set of experiments, the astroglial responses in MK801- and sham-treated rats were compared. One group received 2 mg/kg MK801 intravenously 30 min prior to illumination; the control group received saline. For the detection of SD, a trepanation was made ipsilaterally 4 mm anterior to the illumination site. The dura was opened, and an electrode for DC recording was placed onto the brain surface, which was filled with artificial cerebrospinal fluid (NaCl 125; $NaHCO_3$ 25; Na_2HPO_4 0.5, $CaCl_2$ 1.1; $MgCl_2$ 0.8; KCl 3 [mmol/l]; pH 7.3). SD were recorded during illumination and within 2 h thereafter. Rats from each group were perfused at days 3 or 6, and paraffin sections stained for GFAP and VIM, as described above.

Results

Astroglial responses in photochemically induced ischemia

When paraffin sections of rat cortex were stained with mab against GFAP, only astrocytes located in the subcortical white matter and the glia limitans on the surface of the brain were labelled in untreated control animals. A similar staining pattern was observed with mab against VIM, except that in addition to astrocytes, ependymal, meningeal and some endothelial cells were VIM-positive. One day after photothrombosis, the GFAP and VIM staining pattern had not changed (Fig. 1A,B). By day 2, GFAP-/VIM-positive astrocytes started to form a ring around the ischemic lesion that was fully established at day 3 (Fig. 1C,D; Fig. 2C,D). At day 3, additional GFAP staining occurred on astrocytes in the entire ipsilateral cortex remote from the lesion (Fig. 2A). The contralateral hemisphere did not show any changes in GFAP immunoreactivity. GFAP-positive cortical astrocytes distant from the lesion were VIM-negative (Fig. 2B). Similar observations were made 6 days after photothrombosis, except that the ipsilateral astrocytic GFAP response was slightly diminished. By day 14 the infarct was surrounded by a broad band of GFAP-/VIM-positive astrocytes that had increased in comparison to day 6 (Fig. 1 E,F). However, no more GFAP staining in the remote ipsilateral cortex was observed. In the upper zone of the infarct, no GFAP immunoreactivity was seen, despite intense VIM staining (arrows in Fig. 1F). VIM-positive cells in this region most likely represent fibroblasts derived from the meninges that organize the infarct to form a scar. By day 60, the region of the infarct had shrunken, cortical atrophy had occurred, and astrocytes in this atrophic region still expressed GFAP/VIM immunoreactivity (Fig. 1G,H).

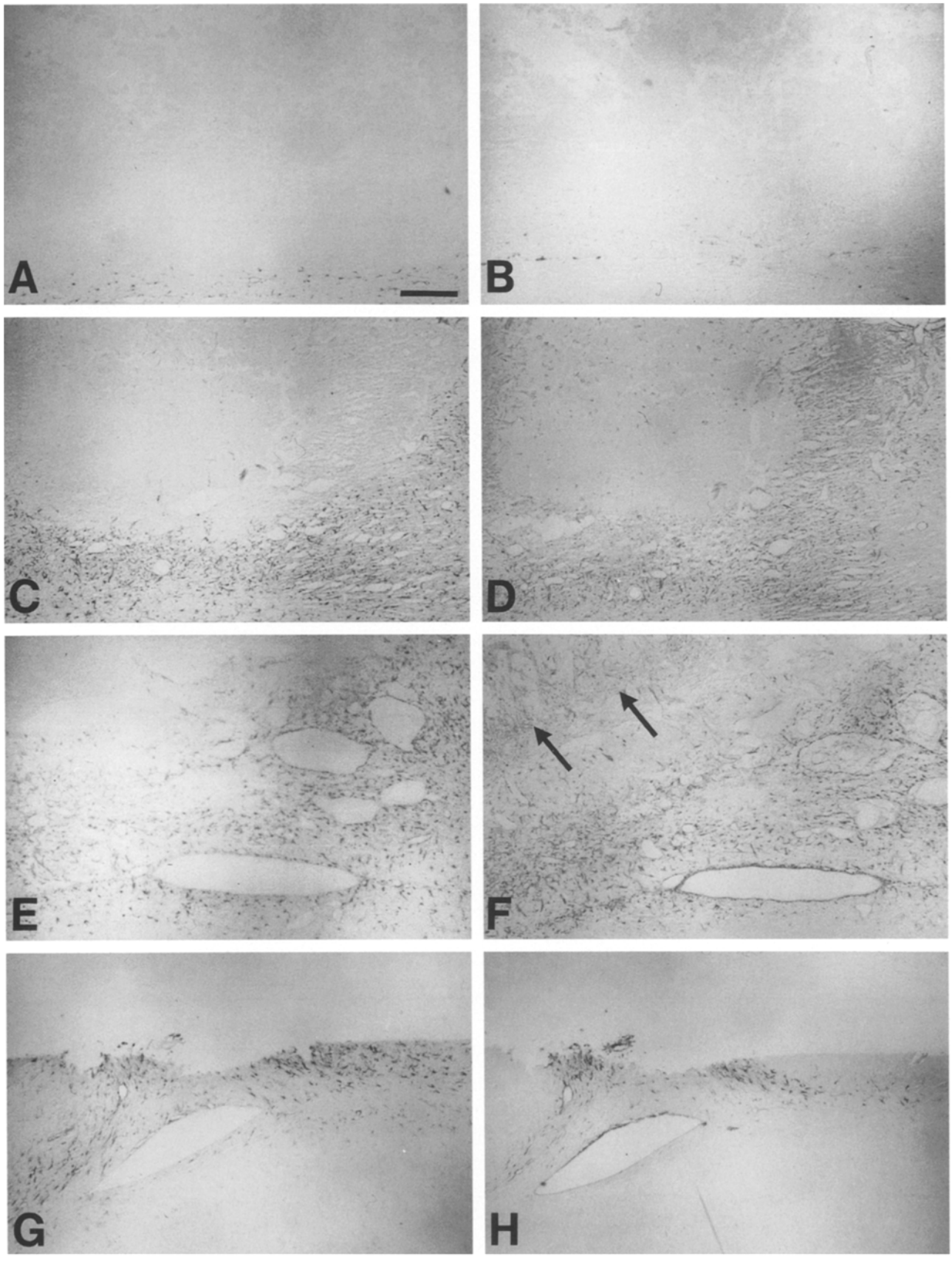
Influence of spreading depression on astroglial responses

To elucidate the mechanisms that lead to the remote ipsilateral and transient activation of astrocytes after photothrombosis, spreading depressions were registered electrophysiologically. Within the first 120 min, five to seven SDs lasting about 2 min could be detected (Fig. 3A). Systemic application of MK801 abolished SD (Fig. 3B). When paraffin sections of rats with photothrombosis that were treated with MK801 were stained for GFAP, the remote ipsilateral GFAP staining of astrocytes had disappeared at day 3 (short arrows in Fig. 2E). In contrast, astrocytes surrounding the infarct still expressed GFAP-immunoreactivity (arrows in Fig. 2E). Similar observations were made 6 days after photothrombosis (not shown).

Discussion

This study shows that two different types of astroglial responses occur in photochemically induced focal ischemia of the rat cerebral cortex: (1) a long-lasting astrocytosis surrounding the infarct zone; these astrocytes, in addition to GFAP, expressed VIM typical for reactive astrocytes (Schiffer et al. 1986); (2) a transient astrocytic reaction remote from the lesion affecting the entire ipsilateral cortex; these astrocytes were VIM-negative. This dual astrocytic reaction in the cortex has also been observed in global cerebral ischemia (Petito et al. 1990), after laser irradiation (Schiffer et al. 1986) and after stab wounds (Takamiya et al. 1988), which means that this response is fundamental to CNS injury independent of the

Fig. 1 Localization of GFAP (**A, C, E, G**) and VIM (**B, D, F, H**) in the lesion area at days 1 (**A, B**), 3 (**C, D**), 14 (**E, F**) and 60 (**G, H**) after photothrombosis: 5-μm serial coronal paraffin sections. The surface of the cortex is always on top. At day 1 GFAP/VIM staining is limited to the subcortical white matter, while by day 3 GFAP/VIM-positive reactive astrocytes form a ring around the lesion. By day 14 more parts of the ischemic lesion are covered by GFAP/VIM-positive astrocytes. In addition, fibroblasts on the surface of the infarct express VIM (*arrows* in **F**). By day 60, there is persistent expression of GFAP and VIM in the atrophic cortex. *Bar* 300 μm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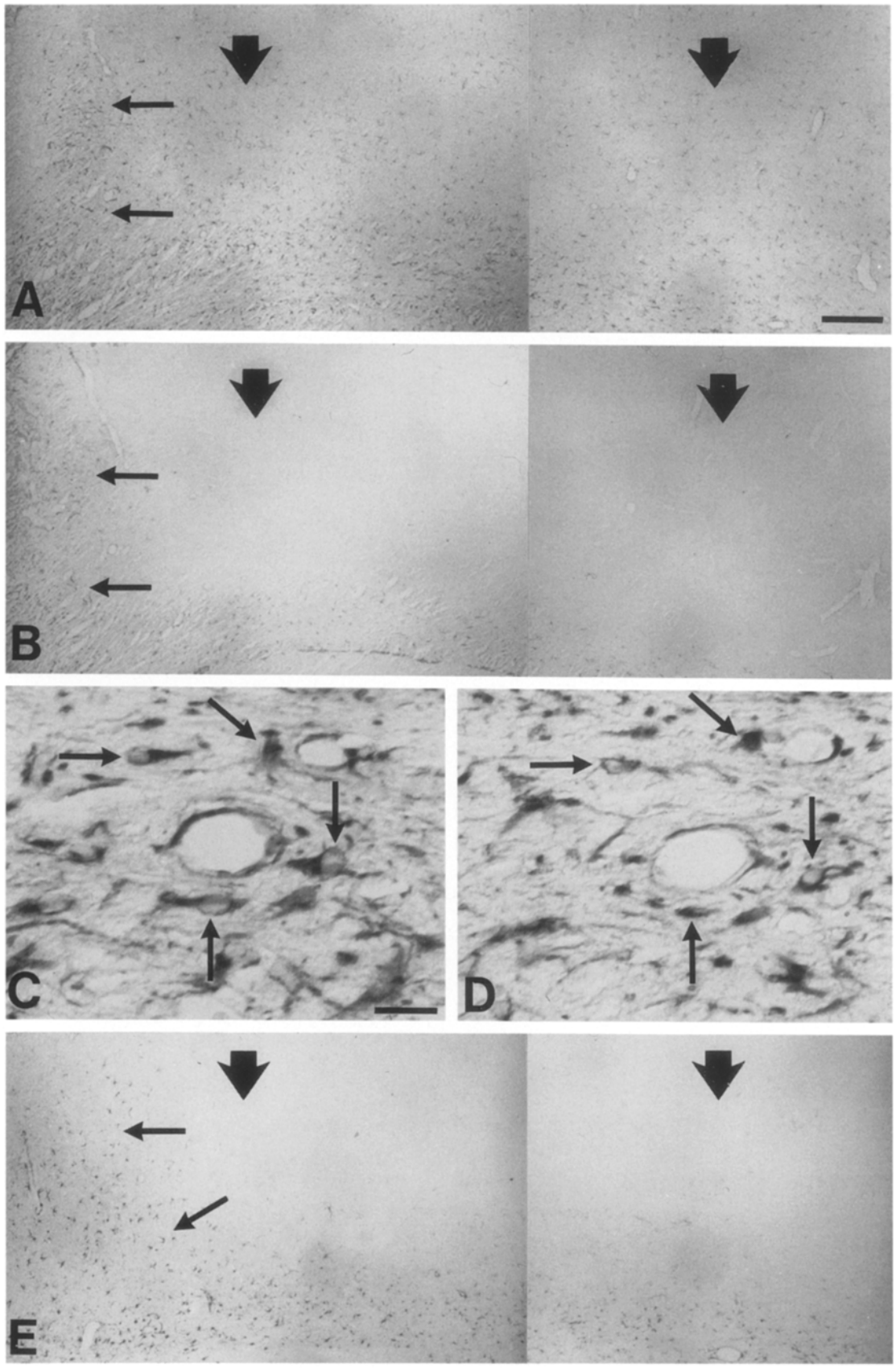
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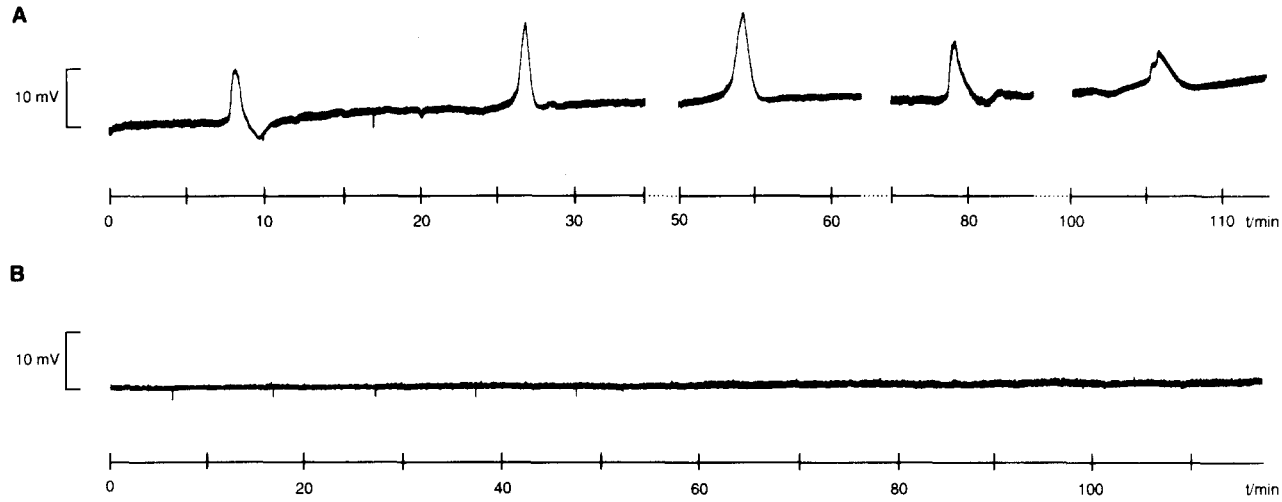

Fig. 3 Registration of spreading depressions (SDs) within 120 min after illumination onset. Note that the first DC deflection occurs as early as 7 min after beginning of illumination (**A**). After application of MK801, SDs are abolished (**B**)

mode of induction. It is interesting that only astrocytes close to the infarct expressed VIM in addition to GFAP. Similar results with limited colocalization of GFAP and VIM to areas close to the necrotic zone have been reported by Schiffer et al. (1986) after laser-induced CNS lesions. These authors concluded that VIM becomes stainable only in astrocytes that proliferate. Accordingly, it is conceivable that in photothrombosis astrocytes proliferate in the vicinity of the ischemic lesions, but show hypertrophy only in remote areas.

What are the mechanisms that trigger astrocyte activation after photothrombosis? The border zone of the infarct is heavily infiltrated by lymphocytes and macrophages, while the ipsilateral cortex remote from the lesion is spared (Jander et al. 1995). Macrophages can release a variety of cytokines, among them interleukin-1 (IL1) (reviewed in Nathan 1987). IL1 injected into mammalian brain stimulates astrogliosis and neovascularization (Guilian et al. 1988). Thus, astrocytosis in the border zone of the infarct may at least partly be the consequence of stimulation by cytokines derived from infiltrating leucocytes.

Fig. 2 Localization of GFAP and VIM in the ipsilateral cortex lateral to the ischemic lesion 3 days after photothrombosis. Same animal as shown in Fig. 1C,D. **A, B** At left the border zone of the infarct is shown (*thin arrows*). Note that astrocytes in the cortex outside the border zone of the lesion strongly express GFAP immunoreactivity (*thick arrows* in **A**), but are VIM-negative (*thick arrows* in **B**). **C, D** Representative astrocytes at higher magnification from the border zone stained for GFAP (**C**) and VIM (**D**). In this area GFAP and VIM are colocalized (*arrows* denote identical astrocytes in serial sections). After application of MK801, thereby suppressing SD (Fig. 3B), the ipsilateral astrocytic activation outside the lesion is abolished (*thick arrows* in **E**), while astrocytosis surrounding the lesion persists (*thin arrows*). Note that, in the area corresponding to **A**, GFAP staining is lost in this animal. Bars: **A, B, E** 300 μm; **C, D** 30 μm

After photothrombosis, cortical astrocytes not only express GFAP, but also the immediate early genes FOS and JUN are induced in the entire ipsilateral hemisphere (Gass et al. 1992). Moreover, electrophysiological investigations showed an impaired paired-pulse inhibition in this area (Domann et al. 1993). SDs occur in photochemically induced focal ischemia on the entire ipsilateral cortex, as described recently by Dietrich et al. (1994). These findings were confirmed in the present study. Treatment with MK801, a non-competitive NMDA receptor antagonist, abolished SD, but also remote astroglial responses. Similar results have been reported after topical application of KCl to the cerebral cortex, known to induce SD and activation of astrocytes in the entire ipsilateral cortex (Kraig et al. 1991; Herrera and Cuello 1992). Again, MK801 blocked SD and astrocytic activation. Since astrocytes do not possess NMDA receptors (Kettenmann and Schachner 1985), a direct effect of MK801 on astrocytes unrelated to blocking of spreading depression seems unlikely. It is conceivable that interstitial glutamate released by other cells after SD triggers activation of cortical astrocytes. In fact, astrocytes are able to rapidly metabolize glutamate (Hertz 1979). Alternatively, the activation of astrocytes is caused by the strong alterations of extra- and intracellular ion activities during SD which impose a strong metabolic stress upon astrocytes (Hansen and Zeuthen 1981).

In summary, there is concurring evidence from many studies that important differences exist in the reaction of astrocytes to a CNS lesion itself and to lesion-induced SD. Disclosure of the molecular mechanisms underlying these different responses will have implications for our understanding of astrogliosis in general.

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