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Peripheral Benzodiazepine Receptors Are Colocalized with Activated Microglia Following Transient Global Forebrain Ischemia in the Rat

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In mammalian brain the expression of peripheral benzodiazepine receptors (PBRs) can be markedly induced following different types of neuronal injury. PBRs are believed to be expressed on non-neuronal cells in the brain, yet the specific cell type that expresses these receptors following CNS insult has not been defined. In the present study, we investigated the effects of transient global forebrain ischemia on PBRs by autoradiographic localization of ^{3}H-PK11195 binding. The distribution of PBRs was compared to glial fibrillary acidic protein (GFAP) as a marker for astrocytes and OX42 as a marker for microglia. Five to 6 d following four-vessel occlusion (4-VO), an increase in PBRs was seen in the CA1 region of all 15 brains examined. In brains from rats subjected to 4-VO, microglia were selectively activated in stratum pyramidale of the CA1 layer. In contrast, astrocytes appeared to be activated in multiple hippocampal cell layers including stratum radiatum and stratum oriens. Activated astrocytes were also found in regions that did not exhibit increased ^{3}H-PK11195 binding. In some brains, selected regions of secondary lesion, specifically necrotic thalamic nuclei and the isocortex were found to be strongly immunoreactive for OX42 but lacked GFAP immunoreactive cells. In adjacent sections, these same regions displayed high densities of ^{3}H-PK11195 binding. These observations lend further support to the application of ^{3}H-PK11195 binding as a marker of neuronal injury in the brain. Furthermore, the data strongly suggest that activated microglia rather than astrocytes express PBRs following ischemic insults.

[Key words: four-vessel occlusion, ^{3}H-PK11195, autoradiography, astrocytes, hippocampus, immunocytochemistry]

Two main classes of benzodiazepine receptors exist in mammals. The predominant high affinity benzodiazepine receptor in the brain, the central benzodiazepine receptor, is a component of a macromolecular complex of the GABA receptor/chloride channel and mediates the anxiolytic and anticonvulsant effects of benzodiazepines (Mohler and Okada, 1977; Tallman et al., 1980; Tallman and Gallager, 1985). Peripheral benzodiazepine receptors (PBRs) can be distinguished from central benzodiazepine receptors by their differing pharmacological profiles. The isoquinoline carboxamide PK11195 selectively binds to the peripheral benzodiazepine receptor and is devoid of affinity for the central-type binding site. ^{3}H-PK11195 has been widely utilized as a marker for PBRs (Benavides et al., 1983; Doble et al., 1987). PBRs are distributed widely in the periphery but have a more restricted distribution in the CNS. In the brain, PBRs are mainly present in periventricular regions such as the choroid plexus, area postrema and ependyma (Richards and Mohler, 1984) while relatively low levels of binding sites are present in the brain parenchyma (Benavides et al., 1983). This anatomical distribution as well as binding studies *in vitro* (Syapin and Skolnick, 1979; McCarthy and Harden, 1981; Bender and Hertz, 1984; Sher and Machen, 1984; Olson et al., 1988; Black et al., 1990; Itzhak et al., 1993) strongly support the idea that PBRs are expressed on glial and other non-neuronal cells. Specifically, astrocytes (Syapin and Skolnick, 1979; Itzhak et al., 1993) and cells of monocytic lineage such as macrophages (Ruff et al., 1985; Zavala and Lenfant, 1987) are thought to express PBRs. Subcellular distribution studies indicate that PBRs are localized on the outer membrane of mitochondria in rat adrenal gland (Anholt et al., 1986), human and guinea pig lung (Mak and Barnes, 1990), rat brain (Basile and Skolnick, 1986), and human brain (Doble et al., 1987). In the periphery, PBRs have been shown to play a role in mitochondrial proliferation (Shiraishi et al., 1991), immunomodulation (Zavala and Lenfant, 1987), hormonal regulation (reviewed by Gavish et al., 1992), and modulation of intermediary metabolism (Anholt, 1986; Hirsch et al., 1988); however, the function of PBRs in the CNS is unknown.

The density of PBRs has been shown to increase in regions of brain which have undergone a primary lesion. Intrastriatal injection of excitotoxins results in marked increases in the number of PBRs in the striatum (Schoemaker et al., 1982; Owen et al., 1983; Benavides et al., 1987). In the rat, focal ischemia (Benavides et al., 1990a,b; Myers et al., 1991a,b), thiamine deficiency encephalopathy (Leong et al., 1994), gliomas (Starosta-Rubinstein et al., 1987), local administration of proinflammatory agents (Bourdiol et al., 1991), and bilateral olfactory bulbectomy (Beauchemin et al., 1994) all result in increased densities of binding sites for PBRs in the area of lesion. Increases in PBRs have been observed in lesioned brain areas in a wide range of human neuropathological states including cerebral infarction (Benavides et al., 1988; Ramsay et al., 1992), multiple sclerosis (Benavides et al., 1988), Huntington's disease (Schoemaker et al., 1982), Alzheimer's disease (Owen et al., 1983; McGeer et al., 1988), astrocytomas (Benavides et al., 1988), and AIDS

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(Hauw et al., 1989). Given that astrocytic proliferation, microglial activation and macrophage invasion are the consequences of virtually all conditions associated with neuronal injury and degeneration, increases in PBRs following lesion is thought to be due to the ensuing gliosis. The above observations have led to the hypothesis that high densities of PBRs in injured tissue can be used as an indicator of damage in the CNS. As a result, ^{11}C-PK 11195 has been developed as a ligand for use in positron emission tomography to detect ischemic (Ramsay et al., 1992) and neoplastic (Junck et al., 1989; Black et al., 1990) brain lesions in humans. While a large body of evidence suggests PBRs are a useful marker of neuronal injury, the specific cell type expressing PBRs has not been identified.

^{3}H-PK 11195 binding to tissue homogenates has been reported to be increased in ischemic brain regions in the rat four-vessel occlusion (4-VO) model (Demerle-Pallardy et al., 1991). In the present study, we investigated the distribution of PBRs in the brains from rats subjected to 4-VO and compared it to the distribution of activated astrocytes and microglia.

Materials and Methods

Four-vessel occlusion. Transient forebrain ischemia was induced by four-vessel occlusion according to the method of Pulsinelli and Brierley (1979). Briefly, male Wistar rats (250–270 gm) (Hilltop Laboratories, Scottsdale, PA) were prepared for forebrain ischemia under Halothane anesthesia by electrocauterizing the bilateral vertebral arteries and placing atraumatic clasps around the common carotid arteries without interrupting the arterial blood flow. On the following day, forebrain ischemia was induced by tightening the clasps for 20 min. The animals were unanesthetized during the ischemia. Fifteen 4-VO animals were evaluated in this study. Three animals were not subjected to ischemia and served as controls. At 5–6 d following ischemia, animals were decapitated and brains processed as described below.

Tissue preparation. Forebrains were rapidly frozen in isopentane chilled using dry ice and serially sectioned in the coronal plane throughout the rostrocaudal extent of the hippocampus. Twenty micrometer thick sections were thaw-mounted onto gelatin-coated slides and stored at −70°C until evaluation. Regions evaluated included only those that contained the same coronal plane as the hippocampus.

Immunocytochemistry. For anatomical localization of the lesioned areas, tissue sections were stained with cresyl violet for Nissl substance. Immunocytochemistry employed two different antibodies: antisera directed to GFAP (1:500, Sigma Immunochemicals, St. Louis, MO), a cytoskeletal marker for astrocytes, and monoclonal antibody OX42 (Bioproducts for Science, Indianapolis, IN) which identifies the CR3 complement receptor, expressed on activated microglia (Graeber et al., 1988; Gehrmann et al., 1992). Sections were postfixed in 3.7% formalin (5 min) followed by acetone (50% acetone 2 min, 100% 2 min, 50% 2 min) and then quenched for 30 min in 0.25% H_2O_2 in 10% nonbuffered formalin. Immunocytochemistry was performed using the avidin–biotin peroxidase system (ABC kit, Vector Labs, Burlingame, CA) and peroxidase enzymatic activity was revealed with 0.5 mg/ml 3,3′-diaminobenzidine in the presence of 0.003% H_2O_2 (DAB substrate kit, Vector Labs). Immunostained sections were lightly counterstained with hematoxylin to delineate cytoarchitecture. For each animal, a total of nine sections taken through three different levels of the hippocampus were stained with each marker.

To assure accuracy in comparing results from immunostained slides, multiple sections from both 4-VO and control brains were stained in parallel on the same day. Development times in DAB were held constant for all sections. These experimental conditions reduced the possibility that differences in immunoreactivity profiles might be accounted for on the basis of experimental variables (e.g., antibody titer/affinity, tissue postfixation, DAB development times, etc.). Quantitative immunocytochemistry using image analysis was not performed on these slides due to the robust differences in specific staining between ischemic vs control animals. This technique can be advantageous when immunoreactivity profiles are subtly different.

Receptor autoradiography. Binding experiments were carried out by incubating slides for 30 min at 23°C in Tris-HCl buffer (170 mM, pH 7.4) containing 1nM ^{3}H-PK11195 (specific activity 86.4 Ci/mmol, Du Pont-NEN, Boston, MA). Nonspecific binding was defined by incubating adjacent sections in the radioligand with the addition of 1 μM unlabeled PK11195 (RBI, Natick, MA). The incubation was terminated by rinsing sections two times for 5 min each in ice-cold incubation buffer. Sections were then dipped briefly in cold distilled water, dried rapidly and exposed to ^{3}H-Hyperfilm (Amersham, Arlington Heights, IL) for 1 week. A total of nine sections taken from three different levels of the hippocampus were used for autoradiography. These slides were near-adjacent to those used for immunocytochemistry. Slides from each experimental group were labeled with controls at the same time. Minimal variability was observed between sections from control animals.

Results

In agreement with previous reports, 20 min of transient global forebrain ischemia resulted in selective degeneration of neurons in the hippocampal CA1 region. At 5 and 6 d postischemia, necrotic neurons were present throughout the extent of the CA1 pyramidal cell layer in all 15 4-VO brains and could not be observed in the control animals (Fig. 1). In the hippocampus, the neuronal death was accompanied by intense glial activation. Astrocytes, as defined by GFAP immunocytochemistry, exhibited morphological features consistent with an activated state including swollen cell bodies, enlarged processes and increased staining intensity for GFAP (Fig. 2*C,F*) when compared to astrocytes detected in control brains. Activated astrocytes were observed in multiple hippocampal layers of the CA1 zone including not only stratum pyramidale but also stratum oriens, stratum moleculare and stratum radiatum as well as the hilus of the dentate gyrus (Fig. 2). The CA2–CA3 and CA4 regions also displayed astrocytes with an "activated" morphology but they were not as numerous and enlarged as those present in the CA1 region.

In contrast to the distribution of astrocytes in the hippocampus, the distribution of activated microglia in 4-VO brains was much more restricted. OX42, a monoclonal antibody that recognizes the rat CR3 receptor, defined a narrow zone of activated microglia that was concentrated within stratum pyramidale of the CA1 zone (Fig. 2*B,E*) in all the 4-VO animals evaluated. Furthermore, all 4-VO brains showed a significant number of OX42 immunoreactive cells in the hilus of the dentate gyrus. Increased OX42 staining was also observed in lacunosum moleculare (Fig. 2*B*). In sections developed longer with the chromogen, OX42 stained cells were scattered throughout stratum radiatum and stratum oriens. The majority of stained cells exhibited a typical amoeboid morphology although a few ramified microglia were also observed in the hippocampus, particularly those located in lacunosum moleculare (Fig. 2*B*). OX42 staining in control animals defined resting microglia that are predominantly in white matter tracts (not shown). Thus, in the hippocampus from 4-VO animals we observed that the distribution of activated astrocytes and microglia do not completely overlap.

^{3}H-PK11195 binding in sections from 4-VO rats was significantly increased in the ischemic hippocampus (Fig. 3*A*) as compared to controls (Fig. 3*C*). The increase in ^{3}H-PK11195 binding was observed in the CA1 region from all 4-VO brains evaluated. Increased binding was most prominent throughout the CA1 zone and was also present in the hilus of the dentate gyrus. In the CA2-CA3 cell layers, binding in ischemic brains was similar to control animals. A lighter exposure of an autoradiogram (Fig. 4*D*) revealed increased binding was observed throughout all layers within CA1; however, the highest level was present in the pyramidal cell layer. ^{3}H-PK11195 binding in the hippocampus was most similar to the distribution of OX42 immunoreactivity (Fig. 4). The CA1 pyramidal cell layer and the hilus of the

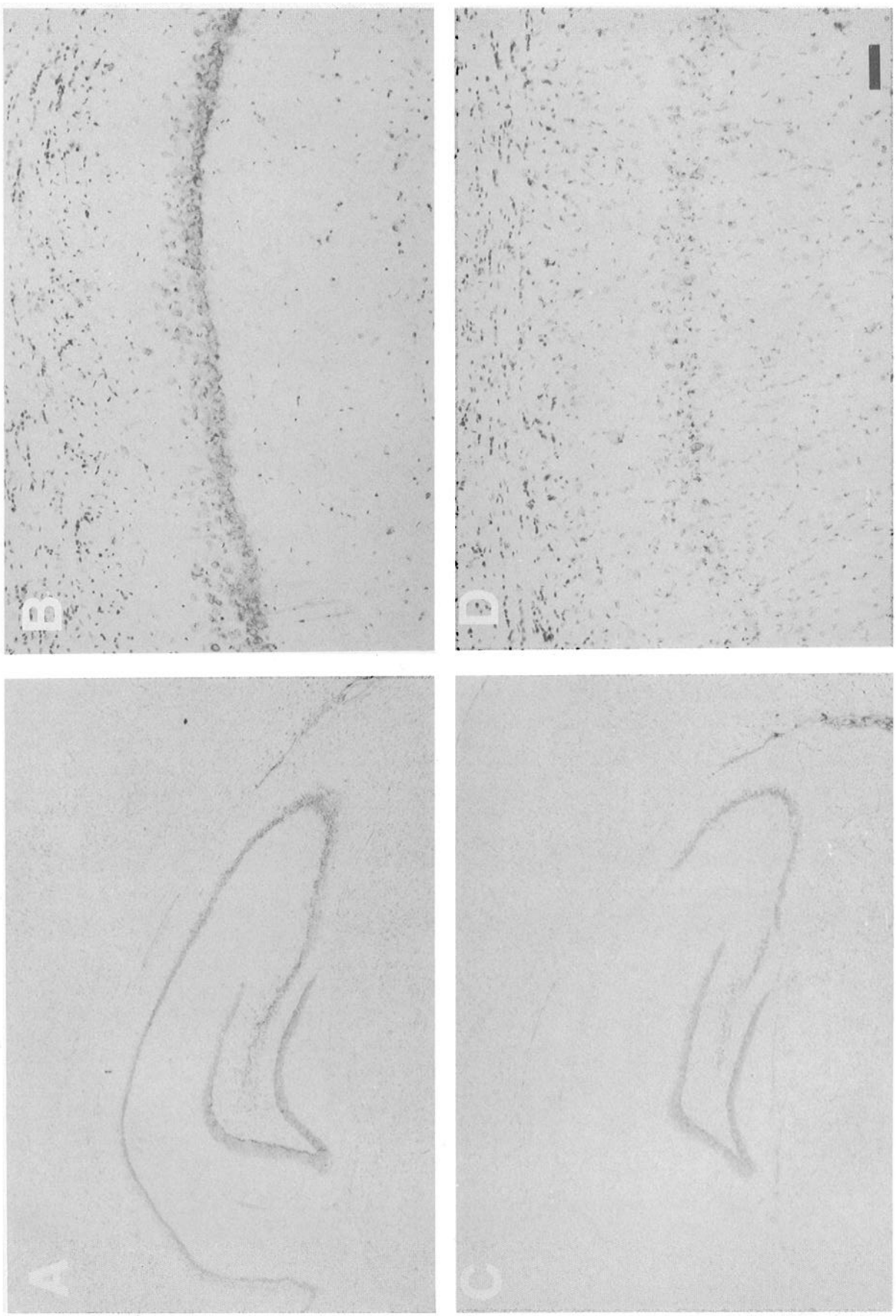

Figure 1. Nissl stained section through the dorsal hippocampus from a control rat (*A, B*) and from a rat subjected to 20 min of 4-VO (*C, D*). The CA1 pyramidal cell neurons, illustrated at higher magnification in *B* and *D*, undergo selective necrosis as a result of the ischemia. Scale bar: *A* and *C*, 240 μm; *B* and *D*, 50 μm.

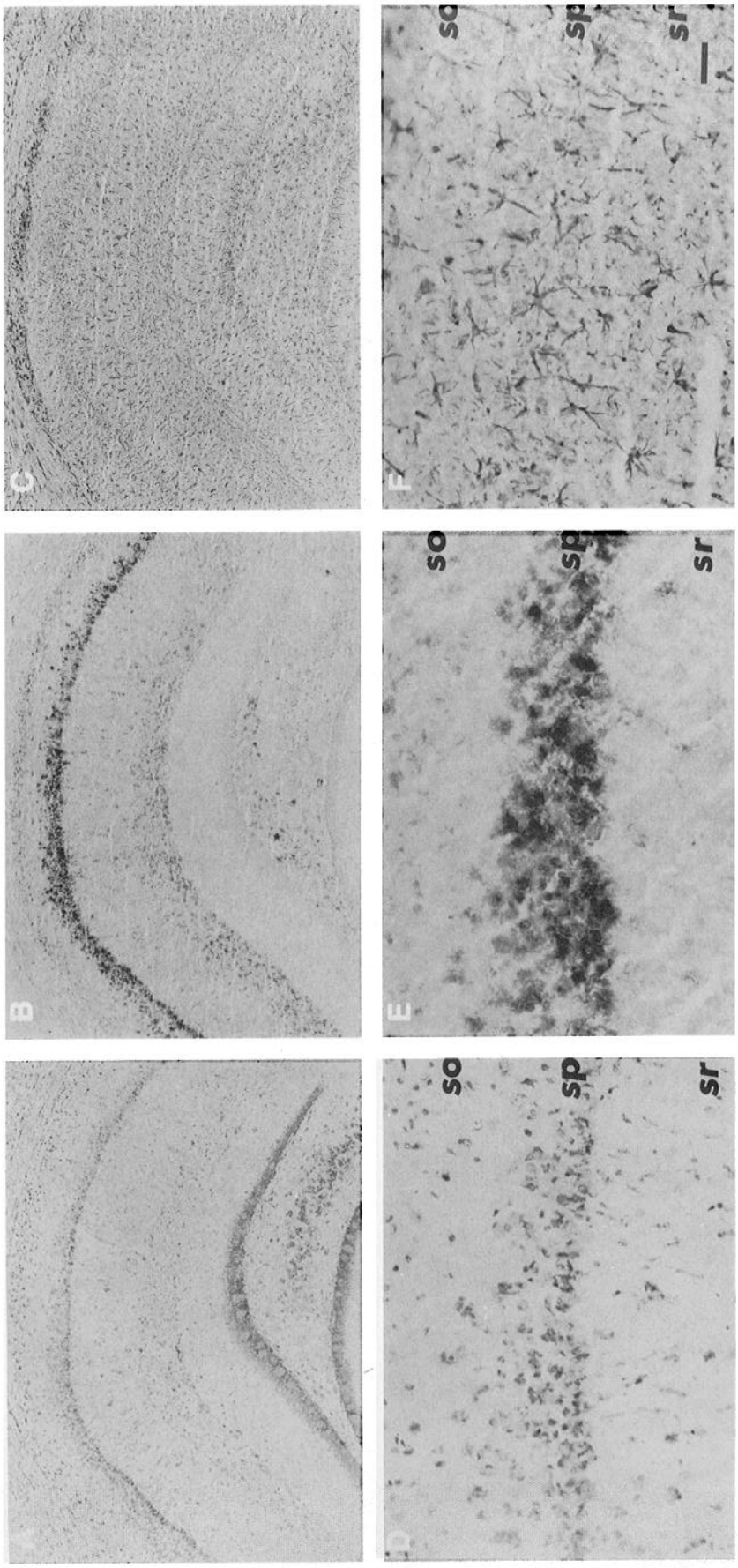

Figure 2. Dorsal hippocampus from a rat subjected to 4-VO stained for Nissl substance (*A, D*) and for OX42 (*B, E*) and GFAP (*C, F*) immunoreactivities. Activated astrocytes, defined by GFAP, are present above and below the CA1 layer (*so, sr*) while activated microglia, defined by OX42, are concentrated in the CA1 pyramidal cell layer (*sp*). *so*, Stratum oriens; *sp*, stratum pyramidale; *sr*, stratum radiatum. Scale bar: *A–C*, 120 μm; *D–F*, 40 μm.

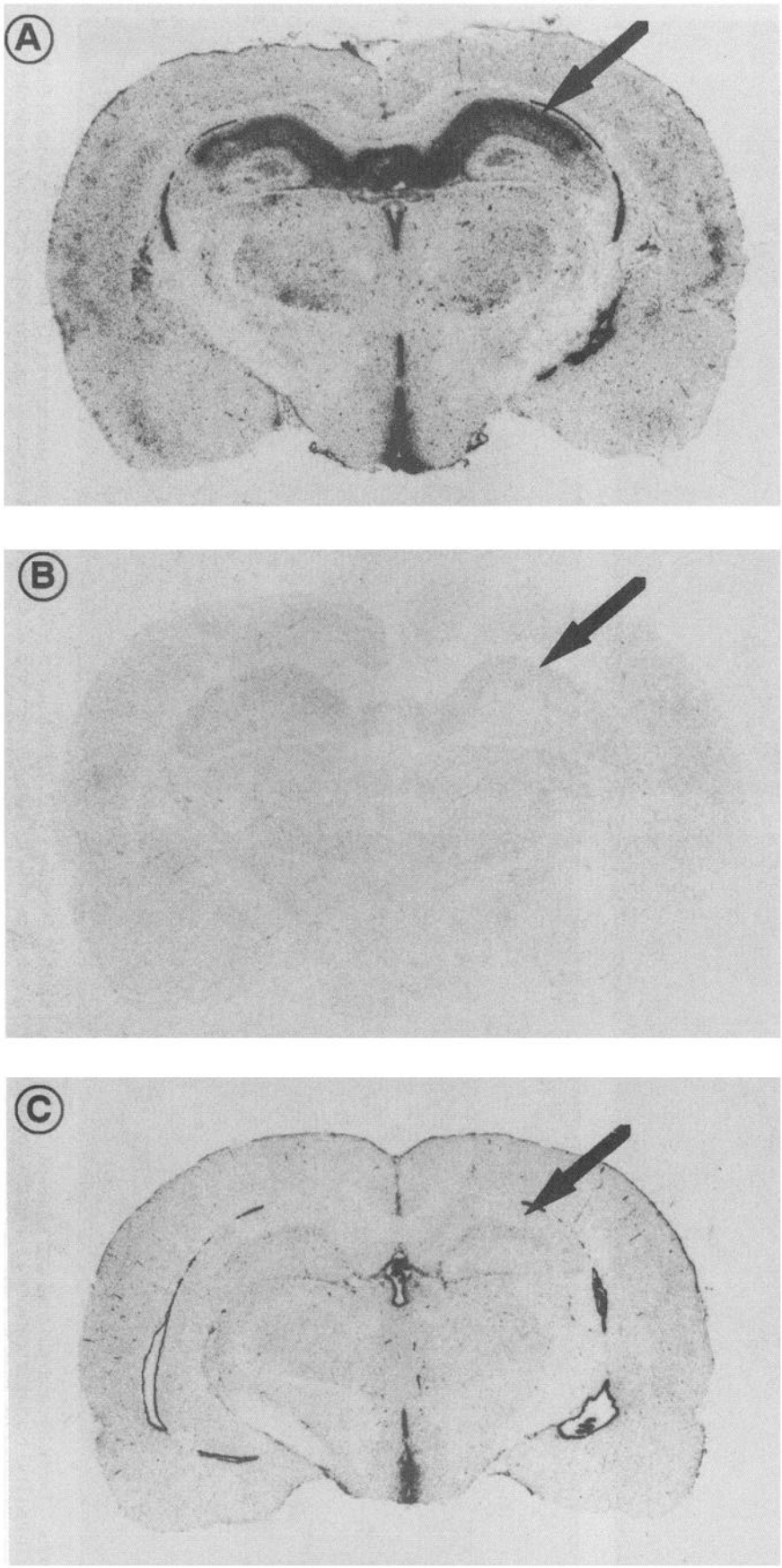

Figure 3. Coronal sections labeled with ^{3}H-PK11195 from a rat subjected to 4-VO (*A, B*) and from a control rat (*C*). ^{3}H-PK11195 binds specifically to the hippocampal CA1 region in animals subjected to 4-VO (*A*). The addition of excess unlabeled PK11195 produced autoradiograms representing nonspecific binding (*B*). Control animals show a pattern of binding known to occur in nonlesioned brain, for example, chorioid plexus, ependyma (*C*).

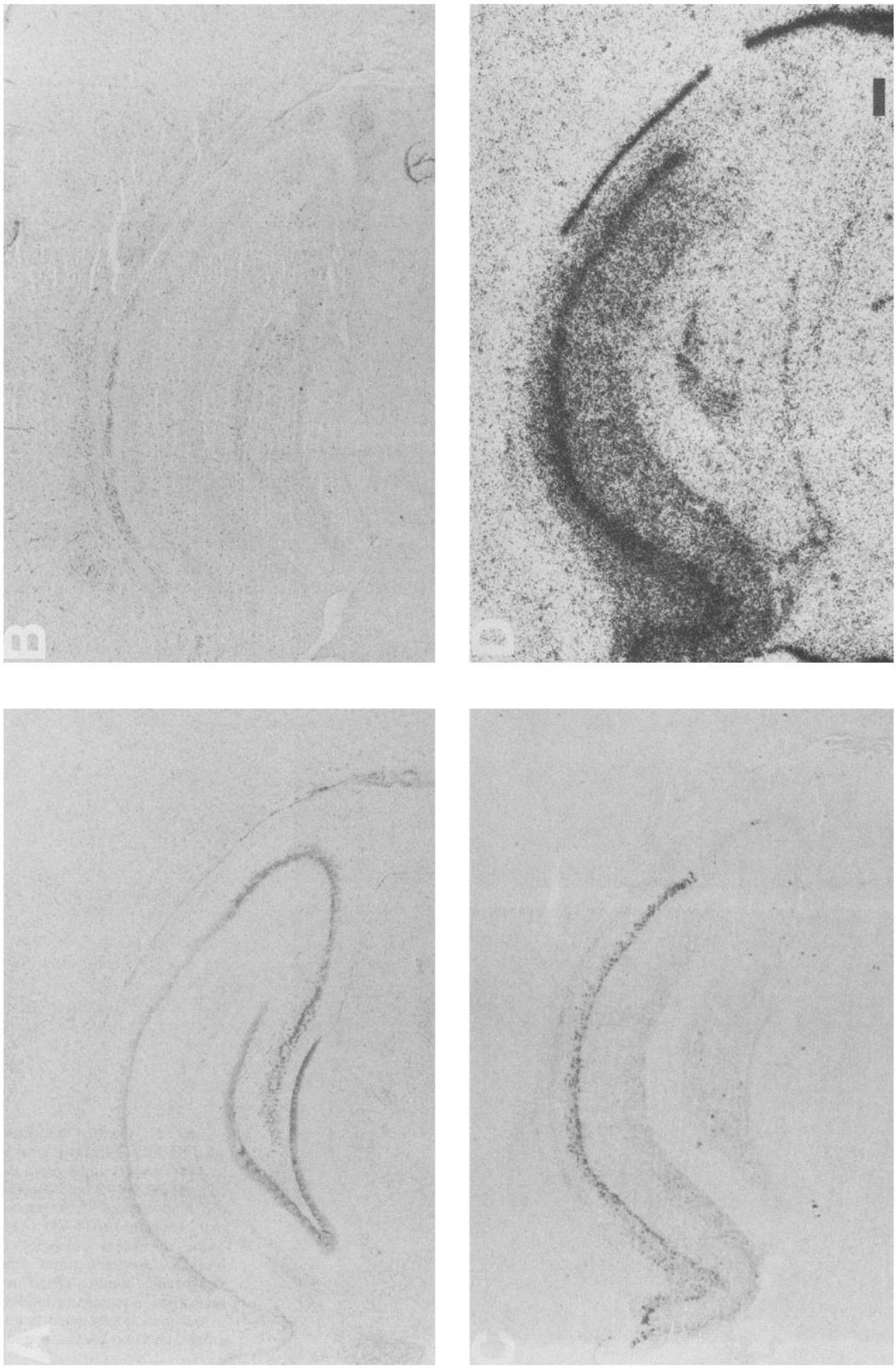

Figure 4. Dorsal hippocampus from a rat subjected to 4-VO stained for Nissl substance (*A*), GFAP immunoreactivity (*B*), OX42 immunoreactivity (*C*) and radiolabeled with ^{3}H-PK11195 (*D*). ^{3}H-PK11195 binding is concentrated in the CA1 pyramidal cell layer and the hilus, the same regions where OX42 reactivity is expressed (compare *C* and *D*). GFAP immunoreactivity is more widespread (*B*). Scale bar, 200 μm.

dentate gyrus contained OX42 reactive glia. In contrast, GFAP immunoreactive astrocytes appeared to be much more widespread. Activated astrocytes, as defined by GFAP staining, were observed in multiple hippocampal layers, including the molecular layer and the CA2–CA3 region.

In rats subjected to 4-VO, an abrupt transition can be observed at the CA1–CA2 boundary. In this region, CA1 necrotic neurons are observed immediately adjacent to morphologically intact neuronal perikarya of the CA2 region (Fig. 5*A*). The binding of ^{3}H-PK11195 precisely delineates this boundary (Fig. 5*D*). OX42 reactive microglia likewise define this border zone (Fig. 5*C*). In contrast, GFAP immunoreactive astrocytes can be observed throughout this region and do not clearly demark the CA1–CA2 transition (Fig. 5*B*).

In 10 of the 15 4-VO brains evaluated, increased specific ^{3}H-PK11195 binding was also found outside the hippocampus. Nonhippocampal areas with increased ^{3}H-PK11195 binding in the sections evaluated included the ventral posterior nuclei of the thalamus (consisting of ventral posterior medial nucleus, ventral posterior lateral nucleus and posterior thalamic nuclear group) (n = 10), the dorsal and medial geniculate nuclei (n = 3), the subthalamic nucleus (n = 2), medial and lateral dorsal thalamic nuclei (n = 3), the reticular thalamic nuclei (n = 4), lamina 3 of the temporal cortex (n = 8), the cingulate cortex (n = 2), entorhinal cortex (n = 2), parietal cortex (n = 2), occipital cortex (n = 1), the substantia nigra (n = 3), and the basolateral amygdala (n = 4). In five of the brains, the caudal most extent of the striatum could be observed in some sections and this region showed dense ^{3}H-PK11195 binding. Figure 6*A* illustrates an autoradiogram from a section that exhibited binding to multiple brain regions.

Patterns of glial reactivity outside the hippocampus of 4-VO animals were unlike that observed in control animals. Control animals showed patterns of resting astrocytes and microglia, predominantly located in white matter. In 4-VO brains, GFAP immunoreactivity highlighted reactive astrocytes throughout many regions of the same coronal section, including isocortex, thalamus, hypothalamus, and amygdala (not shown). These activated astroglia were not readily observed at low magnification (Fig. 6*E*). In contrast, OX42 immunoreactive microglia were localized in more restricted regions, including the ventroposterior nuclei of the thalamus (n = 10), the isocortex including temporal, somatosensory, cingulate cortices (n = 11), basolateral amygdaloid nuclei (n = 5), the reticular nucleus of the thalamus (n = 4), the subthalamic nucleus (n = 2), and the entorhinal cortex (n = 3). In sections that included the substantia nigra or the striatum, OX42 reactive cells were found to be associated with these regions. By comparing the autoradiograms with the sections processed for immunocytochemistry we found that the same animals that exhibited increased ^{3}H-PK11195 binding in nonhippocampal regions were the same animals that exhibited extrahippocampal OX42 immunoreactivity. In fact, the distribution of ^{3}H-PK11195 binding was found to overlap with the distribution of OX42 staining in these brains (Fig. 6). Again, GFAP immunoreactive astrocytes appeared to be much more widespread. For example, the hypothalamus, which contained many GFAP positive cells with an activated morphology, lacked ^{3}H-PK11195 binding and OX42 immunoreactivity in all animals evaluated.

Some nonhippocampal regions exhibited features of focal necrosis. This included the ventral posterior nuclei of the thalamus in four animals and the isocortex in two animals. It was these regions that most clearly illustrated that ^{3}H-PK11195 binding colocalized with activated microglia and not with activated astrocytes. Histological evaluation of the sections from these brains revealed the presence of focal necrotic regions characterized by the presence of necrotic neuronal somata and infiltration of small nuclei. These lesions spanned throughout many sections. Immunocytochemistry of near adjacent sections demonstrated that the center of these regions lacked GFAP immunoreactive cells; GFAP positive astrocytes were located around the periphery of the necrotic area (Fig. 6*F*). OX42 immunoreactive microglia, on the other hand, were concentrated in the center of the lesion and were relatively absent at increasing distances from the necrotic area (Fig. 6*D*). ^{3}H-PK11195 specifically bound to the central portion of these necrotic areas (Fig. 6*B*). Thus, the region enriched with ^{3}H-PK11195 binding lacked GFAP immunoreactivity and possessed numerous OX42 reactive cells in adjacent sections.

Discussion

Global cerebral ischemia results in the death of selected neuronal subpopulations in the brain, the hippocampal CA1 neurons being most susceptible. Other neuronal populations that undergo ischemic cell changes include the small and medium-sized cells of the dorsolateral striatum and some neurons in layers 3, 5, and 6 of the neocortex (Pulsinelli et al., 1982). The temporal profile of glial cell response to ischemia has been fully characterized (Schmidt-Kastner et al., 1990; Morioka at al., 1991; Gehrmann et al., 1992; Ordy et al., 1993; Petito and Halaby, 1993). The delay between the ischemic insult and ischemic cell change differs between susceptible populations of neurons. Changes in CA1 hippocampal neurons are not typically observed until 72 hr following reperfusion whereas ischemic cell change is seen within 24 hr in striatal and cortical neurons (Pulsinelli et al., 1982; Schmidt-Kastner and Freund, 1991). The mechanism of delayed neuronal death in the hippocampal region of rats subjected to 4-VO has been a topic of intense investigation. Recently, it has been proposed that microglia and macrophages may contribute to delayed neuronal death following ischemia (Banati et al., 1993; Giulian and Vaca, 1993; Lees, 1993).

The present study demonstrates increased PBR expression in selected brain regions from rats subjected to transient global forebrain ischemia. Increased binding of the radioligand ^{3}H-PK11195 was most frequently observed in the CA1 region of the hippocampus, the area of selective neuronal damage in this model of ischemia. Other regions that exhibited increased binding included the ventrolateral thalamus, the cortex, the substantia nigra and the amygdala. The majority of these regions undergo secondary or transynaptic degeneration in this model (C. S. Lin et al., 1990; B. Lin et al., 1992; Kato et al., 1993). Comparison of autoradiograms with nearby sections immunostained for glial cells revealed that ^{3}H-PK11195 binding overlapped consistently with the distribution of activated microglia in this model. In the hippocampus, ^{3}H-PK11195 binding and OX42 reactive microglia were concentrated in the pyramidal cell layer of the CA1 region while GFAP stained activated astrocytes were present in multiple hippocampal layers. Both ^{3}H-PK11195 binding and OX42 staining clearly defined the CA1–CA2 boundary while GFAP staining did not. Selected regions outside the hippocampus revealed elevated ^{3}H-PK11195 binding in focal necrotic areas that lacked GFAP immunoreactivity but possessed numerous OX42 reactive microglia.

Quantification of PBRs has been suggested to be a sensitive

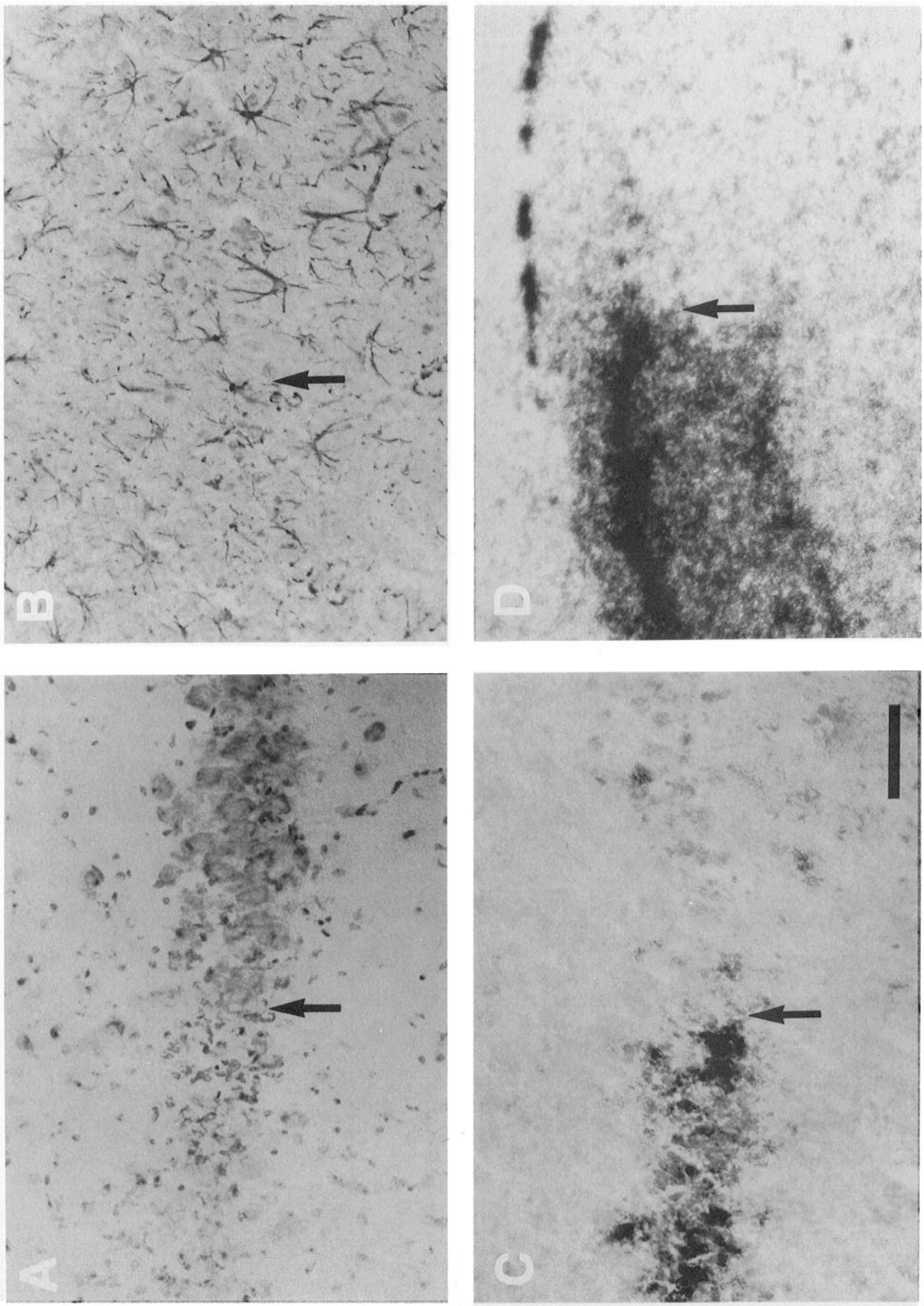

Figure 5. The CA1-CA2 boundary in the hippocampus from a rat subjected to 4-VO stained for Nissl substance (*A*), GFAP immunoreactivity (*B*), OX42 immunoreactivity (*C*) and radiolabeled with ^{3}H-PK11195 (*D*). Nissl staining, ^{3}H-PK11195 binding and OX42 staining clearly define the transition between CA1 and CA2 (*arrows*). GFAP immunoreactivity is similar throughout this region. *Arrow* delineates CA1–CA2 border. Scale bar, 70 μm.

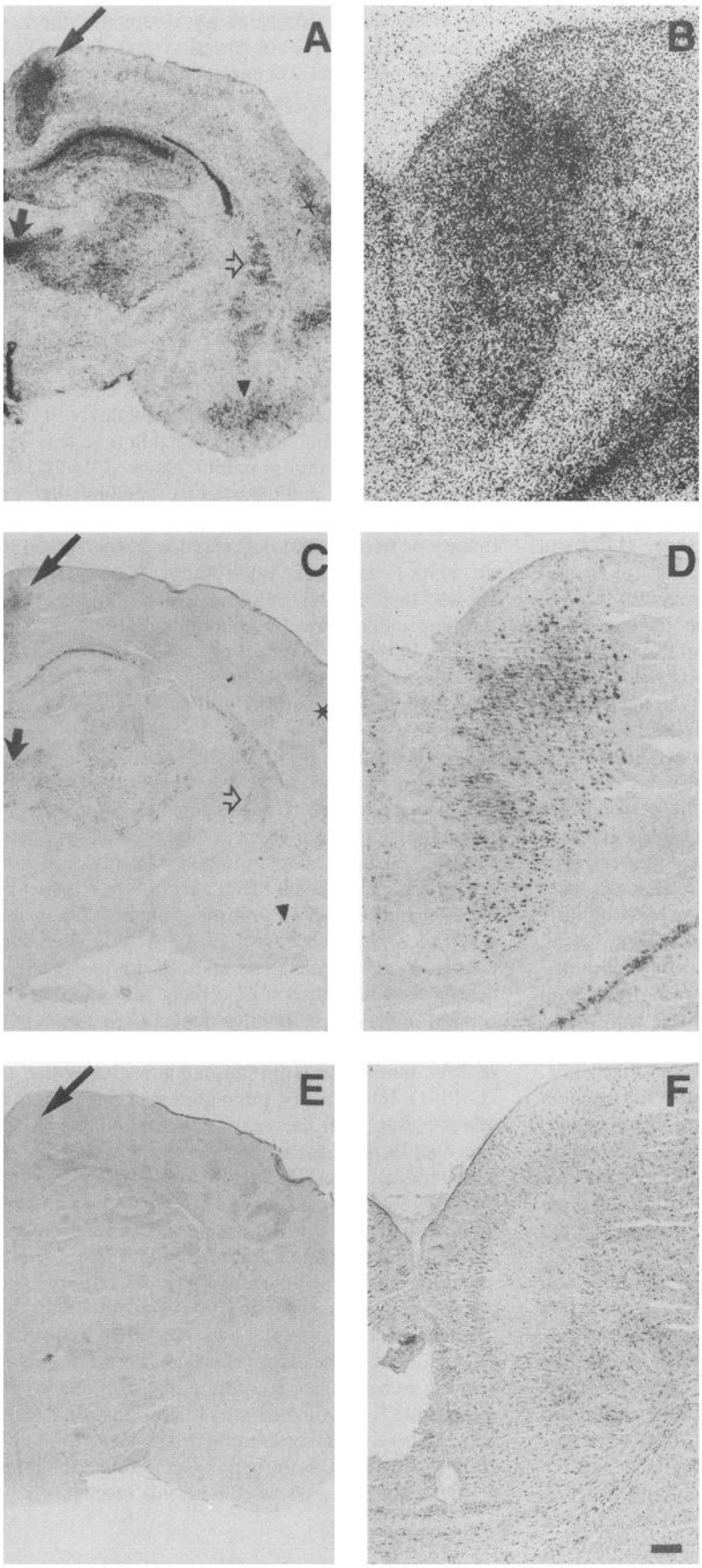

Figure 6. Comparison of the distribution of ^{3}H-PK11195 (*A, B*) with OX42 (*C, D*) and with GFAP (*E, F*) immunoreactivities in a rat subjected to 4-VO that exhibited widespread degeneration. At low magnification, multiple regions exhibit both ^{3}H-PK11195 binding and OX42 positive staining including ventral posterior thalamus, striatum (*open arrow*), basolateral amygdala (*arrowhead*), laminae 2-3 of temporal (*asterisk*) and somatosensory cortices, mediodorsal thalamus (*short arrow*), and cingulate cortex (*long arrow*). These regions cannot be discerned in the GFAP stained section (*E*) (areas of dark circular stain are a result of processing artifacts). Higher magnification of the cingulate cortex (*long arrows* in *A, C, E* are shown at high mag in *B, D, F*) illustrates the presence of dense ^{3}H-PK11195 binding and OX42 reactivity in a necrotic region where GFAP immunoreactive astrocytes are absent (*F*). Scale bar: *A, C,* and *E*, 400 μm; *B, D,* and *F* 100 μm.

marker of damage induced by global ischemia. Using *ex vivo* radioligand binding of ^{3}H-PK11195 to tissue homogenates, Demerle-Pallardy et al. (1991) demonstrated a significant increase in PBRs in the hippocampus, striatum, and cortex from rats subjected to 4-VO. The duration of ischemia and time following recirculation was similar to that used in our own studies. The limitation of receptor binding in tissue homogenates is a lack of anatomical correlation. Our results, using receptor autoradiography in combination with immunocytochemistry, support and extend the observations of Demerle-Pallardy et al. (1991). Not only did we observe increases in primary lesion sites, we also observed increased radioligand binding in regions of secondary lesion. Our results provide an anatomical framework for this observation and furthermore document PBR expression overlaps with activated microglia in this model.

The cell type that expresses PBRs following cerebral insult has not yet been established. Both astrocytes and macrophages have been postulated as sources of PBRs following neuronal injury. Utilizing models of cerebral ischemia some investigators have attempted to address this issue. Focal cerebral ischemia induced by occlusion of the middle cerebral artery (MCA) in rats (Benavides et al., 1990a,b), hemorrhagic lesions in spontaneously hypertensive stroke-prone rats (Benavides et al., 1990a,b) and cortical ischemia resulting from photochemical induction (Myers et al., 1991a,b) all produce increased PBR binding sites in the necrotic core and rim of the lesioned area. By emulsion autoradiography, Benavides et al. (1990a) demonstrated the association of the photoaffinity ligand ^{3}H-PK14105 with ependymal, glial, and phagocytic cells following MCA occlusion and in spontaneous brain lesions in stroke-prone rats. The authors concluded that macrophage-like cells in the necrotic core and astrocyte-like cells at the periphery of the lesions expressed PBR sites. Because no specific identification of the glial cell type by immunocytochemistry or other histologic techniques were performed in this study, the specific cell type(s) expressing PBR binding was not delineated. In a separate study, Myers et al. (1991a) did, however, attempt to determine the cellular localization of ^{3}H-PK11195 binding by comparing autoradiograms with adjacent sections processed immunocytochemically with markers for astrocytes (GFAP antibodies) and macrophages (monoclonal antibody ED1) at various time points post focal ischemia. The binding of ^{3}H-PK11195 was found to correlate in both time and spacial relationship with the appearance of macrophages around the lesion. Reactive astrocytes were found to occupy a separate region in the tissue surrounding the lesion and were located outside the region defined by ^{3}H-PK11195 binding. These latter results are consistent with the data reported in the present study.

We show that PBRs colocalized with activated microglia following 4-VO. It may be that activated microglia release a diffusible factor into the nearby parenchyma which then accounts for the increased ^{3}H-PK11195 binding. Alternatively, PBRs may be expressed on the microglia themselves. Because ^{3}H-PK11195 is a readily diffusible radioligand, we were unable to perform emulsion autoradiography in combination with OX42 immunocytochemistry to provide single cell colocalization. However, the observations made at the CA1–CA2 border zone suggest that this may be the case. If a diffusible factor released by activated microglia leads to increased PBR expression, one might predict that at the CA1–CA2 border, where OX42 reactivity halts, ^{3}H-PK11195 binding would be seen to diffuse out uniformly from this region in all directions. In contrast, we observed that ^{3}H-PK11195 clearly delineated an abrupt transition at the CA1–CA2 boundary. The observation that both OX42 and ^{3}H-PK11195 both clearly define an abrupt border zone in this region suggest that PBRs are expressed on the activated microglia themselves.

Several reports have shown that lesions occurring remote from the site of the primary insult can be detected autoradiographically using ^{3}H-PK11195. Following photochemically induced focal cortical ischemia, the ventrolateral nucleus of the thalamus, known to have reciprocal connections with the lesioned cortical area, expressed high densities of ^{3}H-PK11195 binding (Myers et al., 1991b). Following focal cerebral ischemia produced by MCA occlusion, distant lesions such as the substantia nigra and specific thalamic nuclei demonstrated a marked increase in PBR binding sites (Dubois et al., 1988). Induction of PBRs in these secondary lesions is thought to occur via transsynaptic degeneration and is delayed in time from the time point at which primary lesions exhibit elevated ^{3}H-PK11195 binding. The majority of nonhippocampal regions that exhibited increased ^{3}H-PK11195 binding are areas known to undergo secondary or transynaptic degeneration in this model (C. S. Lin et al., 1990; B. Lin et al., 1992; Kato et al., 1993). Our own results showed that ^{3}H-PK11195 binding can be detected not only in the hippocampus but in anatomical regions outside the hippocampus in the 4-VO model of ischemia. This is consistent with the suggestion that PBRs can be useful as an index of neuronal degeneration at primary as well as secondary lesion sites in the brain.

The mechanism by which PBRs become increased following insults is not known. It has been suggested that excitotoxic damage mediated by NMDA receptor activation may be important (Leong et al., 1994). Alternatively, there is strong evidence that immune mediators play an important part in the processes leading to the increase in PBRs that follows brain injury. In an experimental model of arthritis, inflammatory neutrophils display much higher levels of PBRs binding sites than quiescent circulating neutrophils (Scatton et al., 1990) suggesting that PBR density may be a marker of cellular activation. The finding that cortical injections of interleukin-1, tumor necrosis factor-alpha and lipopolysaccharide cause a marked increase in the density of PBR binding sites further supports this notion (Bourdiol et al., 1991). Interestingly, pretreatment of membranes from rat cerebral cortex with the enzyme phospholipase A_2 (PLA_2) produced an increase in the number of binding sites of ^{3}H-PK11195 (Havoundjian et al., 1986). PLA_2 is the key enzyme initiating the generation of eicosanoids and thus plays an important role in the initiation of the inflammatory cascade. Activation of PLA_2 can result in membrane destruction and is triggered by inflammation, ischemia or injury. We have recently demonstrated that cytosolic PLA_2 is expressed in astrocytes of the human brain (Stephenson et al., 1994). Given that PLA_2 activation as well as production of lymphokines are thought to be an important initial event in ischemia, the possibility that this may be a mechanism responsible for the increase in PBRs should be considered. Future studies evaluating the effects of selective inhibitors of microglial activation, PLA_2 activity or lymphokine production will be important pathways to address this mechanistic hypothesis.

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