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Regulation of the Heparan Sulfate Proteoglycan, Perlecan, by Injury and Interleukin-1α

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Abstract: Perlecan is a specific proteoglycan that binds to amyloid precursor protein and β-amyloid peptide, is present within amyloid deposits, and has been implicated in plaque formation. Because plaque formation is associated with local inflammation, we hypothesized that the mechanisms involved in brain inflammatory responses could influence perlecan biosynthesis. To test this hypothesis, we first studied perlecan regulation in mice after inflammation induced by a brain stab wound. Perlecan mRNA and immunoreactivity were both increased 3 days after injury. Interleukin-1α (IL-1α) is a cytokine induced after injury and plays an important role in inflammation. As such, IL-1α may be one of the factors participating in regulating perlecan synthesis. We thus studied perlecan regulation by IL-1α, in vivo. Regulation of perlecan mRNA by this cytokine was area-specific, showing up-regulation in hippocampus, whereas in striatum, perlecan mRNA was unchanged. To support this differential regulation of perlecan mRNA by IL-1α, basic fibroblast growth factor (bFGF), a growth factor also present in plaques, was studied in parallel. bFGF mRNA did not show any regional difference, being up-regulated in both hippocampus and striatum in vivo. In vitro, both astrocyte and microglia were immunoreactive for perlecan. Moreover, perlecan mRNA was increased in hippocampal glial cultures after IL-1α but not in striatal glia. These results show an increase in perlecan biosynthesis after injury and suggest a specific regulation of perlecan mRNA by IL-1α, which depends on brain area. Such regulation may have important implications in the understanding of regional brain variations in amyloid plaque formation. **Key Words:** Perlecan—Injury—Interleukin-1—Basic fibroblast growth factor—Alzheimer's disease—Inflammation.
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Heparan sulfate proteoglycans (HSPGs) are typical constituents of basement membranes together with collagen IV, laminin, and entactin/nidogen. Classically, the functions attributed to the HSPGs have been (a) molecular impermeability of the basement membranes through anionic interchange, (b) sequestration of growth factors that bind to heparin, and (c) basement membrane degradation during the process of tumor metastasis (Noonan et al., 1991). Perlecan is an HSPG formed by a core protein of 400–470 kDa in the human and 396 kDa in the mouse, attached to three chains of the glycosaminoglycan heparan sulfate. The role of HSPGs, specifically perlecan, in Alzheimer's disease (AD) plaque formation is being extensively investigated. Its presence has been demonstrated in several types of amyloidosis (Snow et al., 1990*b*). Furthermore, perlecan has been localized immunohistochemically not only in neuritic but also in diffuse plaques (Snow et al., 1988, 1990*a*,*b*, 1994*b*). Its early presence in AD pathological alterations suggests that perlecan could play an active role in plaque formation. It has been shown that perlecan binds with high affinity to β-amyloid peptide (Aβ) and amyloid precursor protein (APP) (Narindrasorasak et al., 1991; Buée et al., 1993*a*,*b*; Castillo et al., 1997) and to other molecules implicated in AD such as apolipoprotein E (Mahley et al., 1979). It has been demonstrated as well that heparin, a highly sulfated glycosaminoglycan related to perlecan heparan sulfate glycosaminoglycans, promotes β-secretase cleavage of APP (Leveugle et al., 1997). Moreover, in rodents, fibrillar amyloid deposition after the infusion of Aβ is increased and more persistent if Aβ is coinfused with HSPGs (Snow et al., 1994*a*). Finally, in vitro microglia phagocytosis of Aβ aggregates is inhibited by the presence of heparan sulfate glycosaminoglycans (Shaffer et al., 1995). However, it is unknown what is leading to the accumulation of perlecan in AD.

Although, at present, factors triggering Aβ deposit formation are unknown, inflammatory mechanisms have been implicated in AD and in altered metabolism of Aβ (Rogers et al., 1996; Tocco et al., 1997). Furthermore,

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Abbreviations used: Aβ, β-amyloid peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; bFGF, basic fibroblast growth factor; GFAP, glial fibrillary acidic protein; HSPG, heparan sulfate proteoglycan; IL-1, interleukin-1; PBS, phosphate-buffered saline.

there are several clinical and epidemiological reports indicating beneficial effects of antiinflammatory drugs in AD (for review, see McGeer and McGeer, 1995). Regardless of what is the initial event leading to AD pathology, the CNS responds with the establishment of a local inflammatory reaction with microglial activation and production of cytokines such as interleukin-1 (IL-1) (Griffin et al., 1989), which, in turn, may stimulate the production of neurotrophic factors or extracellular matrix components by astrocytes (Giulian and Lachman, 1985). The interaction between these factors in AD may contribute to the establishment of a "vicious cycle" (Fillit and Leveugle, 1995; Merrill and Benveniste, 1996) in which one event precipitates and aggravates the next.

During an inflammation reaction in the brain a series of cellular changes and release of cytokines occur. In response to trauma, for example, glial cells change their morphology, increase their number, and establish new interactions with tissue components located in the injured area. The goal of these reactive glial changes is the removal of cellular debris and edema from the wound site, reestablishment of the blood–brain barrier, formation of an astrocytic scar, and induction of neuronal regeneration. In addition, glial cells participate in the regulation of extracellular matrix components and inflammatory proteins (Reier and Houle, 1988). Initially, microglial cell changes occur in response to neuronal injury, showing cell proliferation, migration to the injury site, and phenotypic and functional changes, as, for example, appearance of phagocytic activity and expression of major histocompatibility complex (MHC) class II antigens. As well, after injury, there is liberation of cytokines and growth factors such as basic fibroblast growth factor (bFGF) (Logan et al., 1992) and local increases in levels of Aβ (Otsuka et al., 1991; Cotman et al., 1996) and extracellular matrix component proteins (Laywell et al., 1992; Brodkey et al., 1995). Among the inflammatory cytokines locally produced after brain injury is IL-1 (Giulian et al., 1986; Nieto-Sampedro and Berman, 1987). It has been demonstrated that IL-1 is one of the inflammatory cytokines that modulate the reactive astrogliosis to mechanical injury (Balasingam et al., 1994; Rostworowski et al., 1997) and that IL-1 is also responsible for induction of intercellular adhesion molecule-1 (Shibayama et al., 1996). Little is known about the regulation of perlecan after brain injury. However, a recent report shows an increase in perlecan immunoreactivity in hippocampus, following intracerebroventricular kainate injections (Shee et al., 1998).

In light of all these previous studies, we further investigated perlecan regulation after brain injury. We analyzed perlecan mRNA and immunoreactive material following a brain needle stab wound. Because an increased production of IL-1 after brain injury has been reported, it seems likely that this cytokine may regulate perlecan synthesis after stab wound injury. To test this hypothesis, we investigated IL-1α regulation of mouse perlecan mRNA expression in vivo. The hippocampus was selected as the region of study based on the representative AD pathology that it exhibits. We analyzed, as well, perlecan regulation in the striatum, a typical brain area where fibrillar deposits of Aβ are not found (Wisniewski et al., 1989; Gearing et al., 1993). Moreover, as a positive control, we studied the response of bFGF mRNA to IL-1α treatment in vivo in both brain areas. We decided to use bFGF as the positive control because of its local elevation after cortical injury (Logan et al., 1992), its requirement of HSPGs to bind and to activate its receptor (Yayon et al., 1991; Ornitz et al., 1992), and, finally, its increased deposition in association with plaques and tangles in AD brain (Gómez-Pinilla et al., 1990; Stopa et al., 1990).

Because glial cells are locally increased after injury and they are responsible for the synthesis of IL-1α, we decided to analyze whether glial cells are able as well to produce perlecan protein. With this goal, we characterized hippocampal glial cultures by performing double-labeled immunocytochemistry to perlecan, with immunocytochemical markers for astrocytes and microglia. Finally, to investigate if the regional specificity in the regulation of perlecan was conserved in vitro, we analyzed perlecan mRNA regulation by IL-1α in both hippocampal and striatal glial cells in culture.

MATERIALS AND METHODS

Animals and in vivo procedures

Three-month-old male C57BL/6 mice (Harlan, Indianapolis, IN, U.S.A.) were used. Animals were anesthetized with 287.5 mg/kg 2,2,2-tribromoethanol (Avertin; stock of 12.5 mg/ml; Aldrich Chemicals Co., Milwaukee, WI, U.S.A.), injected intraperitoneally. Animals were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, U.S.A.), and were used either to perform brain stab wound or for intraventricular injections.

Stab wound. A 1-μl Hamilton syringe was placed in the right side of the brain through the cortex and the hippocampus, perpendicular to the pial surface of the cortex, and was left in place for 5 min. Coordinates were 2.2 mm caudal to bregma, 2–3 mm lateral to the midline suture, and 2–3 mm ventral to the surface of the brain. Animals were killed 3 days after the stab wound, and brains were processed for immunocytochemistry and in situ hybridization.

Intraventricular injections. IL-1α (100 units; specific activity, $>1 \times 10^7$ units/mg; Genzyme, Cambridge, MA, U.S.A.) or vehicle [phosphate-buffered saline (PBS), pH 7.4] in a final volume of 0.5 μl was injected into the right lateral ventricle using a 1-μl Hamilton syringe. This dose was chosen based on results obtained after the performance of a dose–response curve for IL-1α (data not shown). Coordinates were 1.5 mm rostral to bregma, 1 mm lateral to the midline suture, and 2 mm to the surface of the brain. The needle was kept in place for 5 min after the injection and withdrawn slowly to prevent backflow of the solution injected along the needle track. Animals were killed 6, 8, and/or 24 h after the injection ($n = 3–5$ for each group). Brains were quickly removed, and the hippocampus and/or the striatum were dissected, immediately frozen in dry ice, and stored at −80°C. Cytoplasmic total RNA was isolated, and a nuclease protection assay (Blum, 1989) was performed to quantify perlecan mRNA.

Quantitative solution hybridization nuclease protection assay

Unlabeled sense and high-specific-activity ($\sim 1 \times 10^9$ cpm/μg) ^{32}P-labeled antisense RNAs were transcribed according to the manufacturer's recommendation. A standard curve with increasing amounts [0–10 μl of 100 fg/μl (+)-strand] of sense RNA was used for quantification. The standard and known amounts of cytoplasmic RNA were hybridized with ~200 pg of antisense ^{32}P-labeled RNA probe. Samples were heat-denatured at 85°C for 5 min and hybridized overnight at 45°C. After hybridization, samples were treated with a nuclease, phenol/chloroform-extracted, precipitated, resuspended in 1× TE buffer, and electrophoresed on a nondenaturing 5% acrylamide gel. Gels were dried and quantitated by phosphorimage analysis. Results were determined by linear regression analysis from the standard curve and presented as attomoles of mRNA per microgram of total RNA.

Isolation of cDNA clones

Mouse perlecan cDNA clone was generously provided by Dr. J. R. Hassell (Noonan et al., 1991). A 322-bp *Eco*RI/*Pst*I fragment from BPG7 was subcloned into vector Bluescript II $SK^{+/-}$. A 414-bp fragment corresponding to nucleotides 525–1,004 was also subcloned into vector Bluescript/SK^+ from the bFGF cDNA clone, generously provided by Dr. S. Shimasaki (Shimasaki et al., 1988).

Primary hippocampal and striatal glial cultures and in vitro treatment

Postnatal day 0–3 C57BL/6 mice were decapitated, the hippocampus was dissected, and meninges were removed. The tissue was trypsinized and treated with DNase for 15 min at 37°C. This was followed by a series of washes and centrifugations in minimum essential medium/Ham's F-12 supplemented with 10% fetal bovine serum. The resulting homogenate (cell suspension) was filtered through a sterile nylon screen (pore size, 37 μm). Cells were plated at 1×10^6 cells per 60-mm-diameter or ~300,000 cells per 35-mm-diameter dish. Cultures were incubated at 37°C in an atmosphere of 8% CO_2 and 92% air.

Treatment. Cells at 20–23 days in vitro were placed into 0.5% serum for 24–48 h and then were treated with IL-1α (500 units/ml) or vehicle (water) for 0, 6, 8, and 24 h (n = 3–5 for each group). RNA was isolated and quantified by a nuclease protection assay for perlecan mRNA.

Immunocytochemistry

Tissue. Animals were killed, and brains were quickly removed and fresh-frozen with OCT (Tissue Tek, Torrance, CA, U.S.A.) embedding medium immersed in a dry ice–chilled acetone bath. Coronal sections were cut 15 μm thick in a cryostat and mounted in Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA, U.S.A.). Sections were fixed with 5% acetic acid/95% alcohol for 15 min at 4°C and washed with PBS. Tissue was then incubated in blocking buffer (0.3% Triton X-100 and 5% goat serum in PBS) for 1 h, followed by an overnight incubation with primary anti-perlecan antibody (rat monoclonal antibody) that reacts specifically with HSPG core protein (perlecan) and does not present cross-reactivity to other basement membrane proteins (laminin, collagen IV, and ectactin/nidogen) or fibronectin (species cross-reactivity, human, bovine, and mouse; 5 μg/ml; Upstate Biotechnology, Lake Placid, NY, U.S.A.). The following day, after PBS washes, sections were incubated in blocking buffer containing the secondary antibody anti-rat IgG-fluorescein (1:200; Vector Laboratories, Burlingame, CA, U.S.A.) for 2 h at room temperature; sections were then washed in PBS and coverslipped in Permafluor (Lipshaw, Pittsburgh).

Hippocampal glial cultures. Petri dishes 35 mm in diameter were fixed with 5% acetic acid/95% alcohol for 30 min at room temperature, washed in PBS, and then incubated in blocking buffer (0.3% Triton X-100 and 5% rabbit or goat serum in PBS) for 1 h. Double-labeled immunofluorescence was performed, combining antibodies to glial fibrillary acidic protein (GFAP) or Mac-1 with perlecan antibody. Cells were incubated overnight with primary antibodies for perlecan: (a) rat monoclonal anti-HSPG core protein (5 μg/ml; Upstate Biotechnology) or (b) anti-perlecan antibody made in the goat [1:100; a generous gift of Dr. R. Kisilevsky, Kingston, Ontario, Canada (for more detailed information, see Narindrasorasak et al., 1991)], anti-GFAP (mouse monoclonal anti-GFAP; 1:50; Boehringer Mannheim, Indianapolis), or anti-microglia (rat monoclonal anti-Mac-1; 1:50; Boehringer Mannheim), in blocking buffer, at 4°C. Cultures were washed with PBS and incubated in the corresponding combination of secondary antibodies for 2 h at room temperature. GFAP and microglia were visualized using anti-mouse IgG-fluorescein (1:200; Vector Laboratories) and anti-rat IgG-fluorescein (1:200; Vector Laboratories), respectively. Perlecan was visualized by incubation with either biotinylated anti-goat IgG or anti-rat IgG-fluorescein (1:200; Vector Laboratories). After PBS washes, cultures were incubated with streptavidin conjugated to rhodamine (1:500; Molecular Probes, Eugene, OR, U.S.A.) for 1 h at room temperature, washed in PBS, and coverslipped in Permafluor (Lipshaw).

In situ hybridization

Animals were killed by decapitation, and brains were quickly removed, embedded in OCT, and frozen in ice-cold acetone. Tissues were stored at −80°C until used. Sections 15 μm thick were fixed in 4% paraformaldehyde for 10 min. Sections were incubated with prehybridization buffer [50% formamide, 0.6 *M* NaCl, 10 m*M* Tris-HCl (pH 7.5), 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 1 m*M* EDTA (pH 8), 0.5 mg/ml salmon sperm DNA, 500 μg/ml yeast total RNA, and 50 μg/ml yeast transfer RNA] for 3 h at room temperature, followed by an overnight incubation with hybridization buffer [50% formamide, 0.6 *M* NaCl, 10 m*M* Tris-HCl (pH 7.5), 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 1 m*M* EDTA (pH 8), 0.1 mg/ml salmon sperm DNA, 500 μg/ml yeast total RNA, 50 μg/ml yeast transfer RNA, and 10% dextran sulfate] containing 10 ng/ml heat-denatured ^{35}S-labeled perlecan riboprobe (specific activity, $\sim 1 \times 10^9$ cpm/μg) at 50°C in a humidified chamber. After hybridization, sections were washed with different concentrations of saline–sodium citrate (SSC) and then digested with RNase [0.1 mg/ml in 0.5 *M* NaCl, 10 m*M* Tris (pH 7.5), and 1 m*M* EDTA] for 30 min at room temperature. The sections were exposed to Hyperfilm-β_{max} for ~2 weeks, before being coated with liquid photographic emulsion (Kodak NTB-2) for ~1 month.

Statistical analysis

Significant differences between IL-1α-treated animals and control groups were analyzed using Student's *t* test or ANOVA followed by Fisher's protected least significant difference post hoc analysis. The level of significance was set at $p < 0.05$, $p < 0.01$, or $p < 0.001$.

All animal experiments were conducted in accord with *Guidelines for the Care and Use of Experimental Animals*, using protocols approved by the Institutional Animal Care and

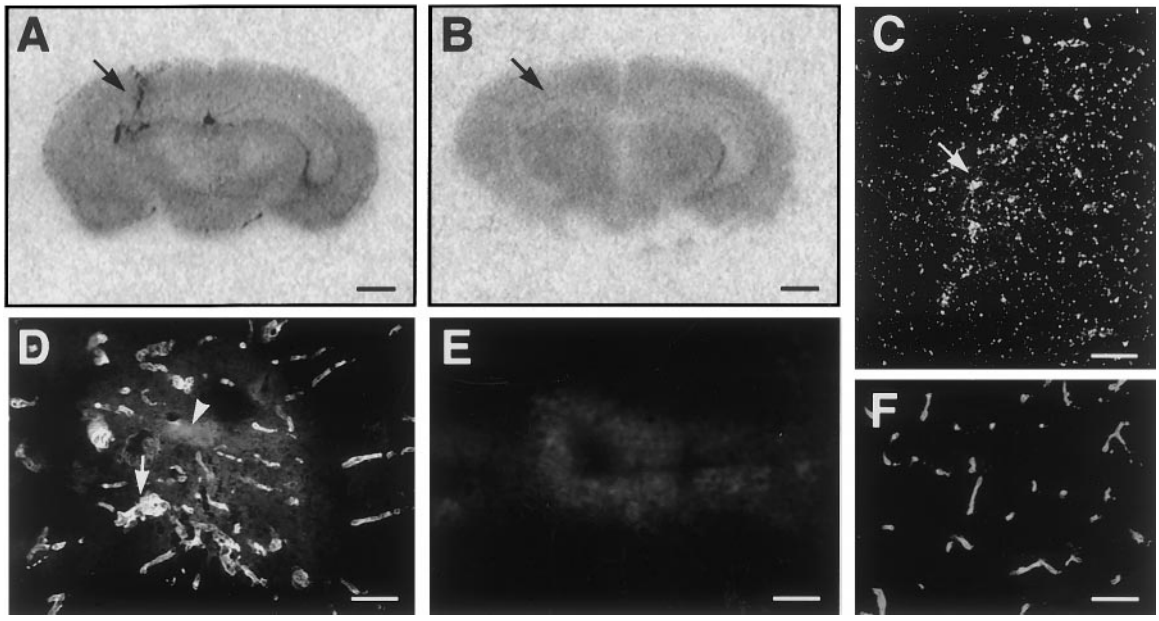

FIG. 1. Perlecan mRNA and protein after brain needle stab wound. **A:** X-ray film autoradiograph shows the hybridization signal in mouse brain that had received a needle stab wound 3 days before the animal was killed. Brains were collected and processed for in situ hybridization, and then sections were exposed to an x-ray film for 2 weeks. The hybridization signal is localized throughout the needle track (arrow). **B:** Consecutive brain coronal section that was hybridized with perlecan sense riboprobe as the control. No hybridization signal is detected surrounding the needle track (arrow). **C:** Dark-field photomicrograph of mouse brain section at the needle track site shows the local increase in perlecan mRNA hybridization signal, revealed by the accumulation of silver grains (arrow), in contrast to the nonspecific signal depicted at the periphery. Tissue was exposed to liquid photomicrographic emulsion for 1 month after the hybridization. **D:** Perlecan immunoreactivity in the mouse brain after injury depicts a perivascular (arrow) and an extracellular (arrowhead) pattern. **E:** Brain section from the same animal, treated with no primary antibody. **F:** Immunostaining for perlecan in the area contralateral to the stab wound site shows a typical vascular pattern of perlecan immunoreactivity. Bar = 1 mm for A and B and 50 μm for C–F.

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RESULTS

Regulation of perlecan mRNA and protein in mouse brain after stab wound injury

We used a needle stab wound to the mouse brain to evaluate perlecan mRNA expression levels and immunoreactivity after injury. Three days after the stab injury, animals were killed, and brains were processed for in situ hybridization or immunocytochemistry. Perlecan mRNA expression was increased along the needle track in both cortex and hippocampus (Fig. 1A), with a net increase in the hybridization signal compared with the background and to the sense-strand negative control (Fig. 1B). At the microscopic level (Fig. 1C), an increase in the amount of silver grains was observed, corresponding to the elevation in level of perlecan mRNA above the background levels at the site of the stab wound. However, it was not possible to determine the cellular source of the hybridization signal.

Perlecan immunoreactivity showed a typical labeling of blood vessels in the contralateral side of the lesion (Fig. 1F). On the injured side of the brain, perlecan exhibited an increase in the immunoreactivity pattern in vessels near the stab wound injury site (Fig. 1D, arrow), associated with additional extracellular immunoreactive material (Fig. 1D, arrowhead). Low levels of nonspecific background binding in the absence of primary antibody are shown in Fig. 1E.

Altogether, we were able to detect an increase in both mRNA levels and immunoreactivity of perlecan after stab injury to the brain. We were not able to determine the cellular origin of the perlecan immunoreactive material in vivo owing to technical difficulties matching the optimal fixations for the antibodies required for double-labeling for perlecan and glial or neural cells in tissue sections.

Regulation of perlecan mRNA by IL-1α in vivo

IL-1 is a cytokine released by reactive glia in response to brain injury and may be involved in injury-induced up-regulation of perlecan expression. Therefore, to test this idea we injected IL-1α into the lateral ventricle and measured its effect on perlecan expression. In addition, because dense-core senile plaques are present in the hippocampus but rarely found in the striatum in AD, both brain areas were collected after the intraventricular injection of IL-1α to determine whether there is a brain region-specific regulation of perlecan. Animals were killed at 6, 8, and 24 h after IL-1α injection, and the right hippocampus and striatum were dissected and analyzed. Perlecan mRNA was quantified by nuclease protection

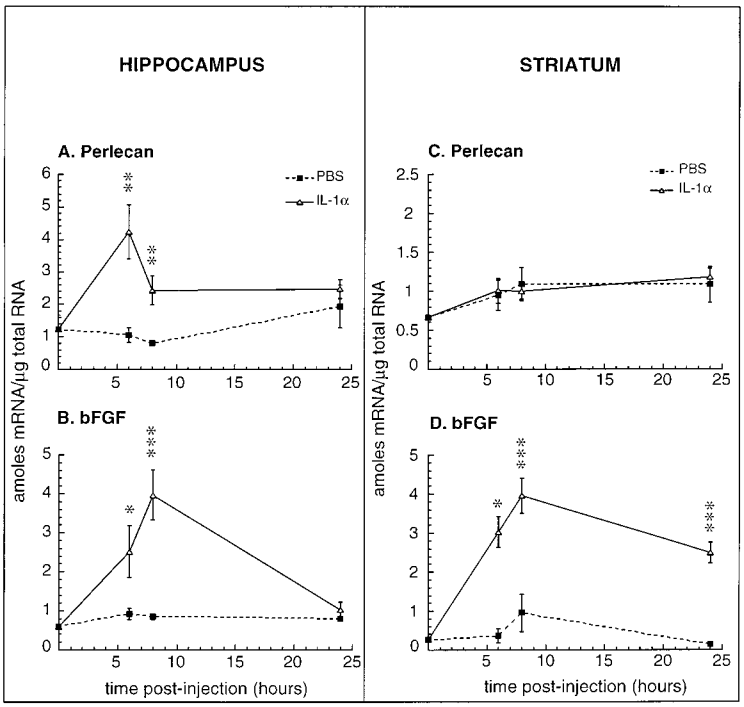

FIG. 2. Quantification of hippocampal and striatal perlecan and bFGF mRNA expression after intraventricular injection of IL-1α. IL-1α was injected into the right lateral ventricle of 3-month-old mice at a dosage of 100 units per animal in a final volume of 0.5 μl. Control animals received PBS injection. Three time points were examined (6, 8, and 24 h after the injection). The value corresponding to zero-time indicates the mRNA levels in noninjected animals. Data are mean ± SEM (bars) values (n = 5), expressed as attomoles of perlecan (**A** and **C**) or bFGF (**B** and **D**) mRNA with respect to the total amount of RNA measured. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, as compared with PBS-injected groups by Student's *t* or ANOVA test.

assay. In the hippocampus, perlecan synthesis showed a significant increase at 6 (almost threefold; $p < 0.01$) and 8 h (twofold; $p < 0.01$) after IL-1α injection (Fig. 2A). At 24 h, perlecan mRNA returned to control values. It is interesting that in the striatum, no increase in perlecan mRNA level was observed at any of the times analyzed (Fig. 2C), suggesting a possible link between the brain area-specific regulation of perlecan mRNA by IL-1α shown here and the selective distribution of the plaques found in AD brains.

Regulation of bFGF mRNA in vivo

Because it is well documented that bFGF mRNA levels increase after IL-1α treatment, we quantified bFGF mRNA in the same animals used to study perlecan mRNA regulation to serve as a positive control for the region-specific regulation of perlecan mRNA. In the hippocampus, bFGF mRNA levels increased at 6 (almost twofold; $p < 0.05$) and 8 h (3.7-fold; $p < 0.001$) after IL-1α injection (Fig. 2B). After 24 h, bFGF mRNA levels did not show any statistically significant difference. In the striatum, bFGF mRNA showed the same pattern of response following IL-1α injection as compared with the hippocampus; however, it was found to sustain a significant up-regulation after 24 h ($p < 0.001$; Fig. 2D). In conclusion, these data show that intraventricular injection of IL-1α is able to up-regulate both perlecan and bFGF mRNAs in the hippocampus, whereas in the striatum IL-1α only was able to up-regulate bFGF expression but not perlecan.

Characterization of perlecan expression and regulation by IL-1α in mixed primary glial cell cultures from hippocampus and striatum

When the brain is injured, glial cells become activated at the site surrounding the lesion. To initiate the removal of cellular debris and the repair of the damaged tissue, cytokines and extracellular matrix proteins are released from these activated cells. To determine whether perlecan may be involved in this glial response to injury, we turned to an in vitro system first to assess whether perlecan is expressed in astrocytes and/or microglia and second to determine whether its expression could be directly regulated by IL-1α. Thus, we set up mixed hippocampal primary glial cultures and performed double-labeled immunofluorescence using antibodies against perlecan core protein, a mouse-specific antibody to Mac-1 antigen to label microglia and macrophages, and an antibody to GFAP, an astrocyte marker. The pattern of perlecan immunoreactivity in vitro showed both a cytoplasmic (Fig. 3A and C) and a fibril-like (Fig. 3E) morphology, often coincidental with cell-to-cell appositions. Small dotted extracellular deposits of perlecan immunoreactivity were also found (Fig. 3C, arrow). Colocalization of perlecan immunoreactivity (Fig. 3A and C) in Mac-1 immunoreactive microglial cells (Fig. 3B) and GFAP immunoreactive astrocytes (Fig. 3D) indicates that both cell types can produce perlecan.

Next, we determined whether perlecan expression in glia is regulated by IL-1α. It has been demonstrated that glia isolated from different brain regions have functional differences (Le Roux and Reh, 1994). Therefore, to determine whether the selective brain region regulation

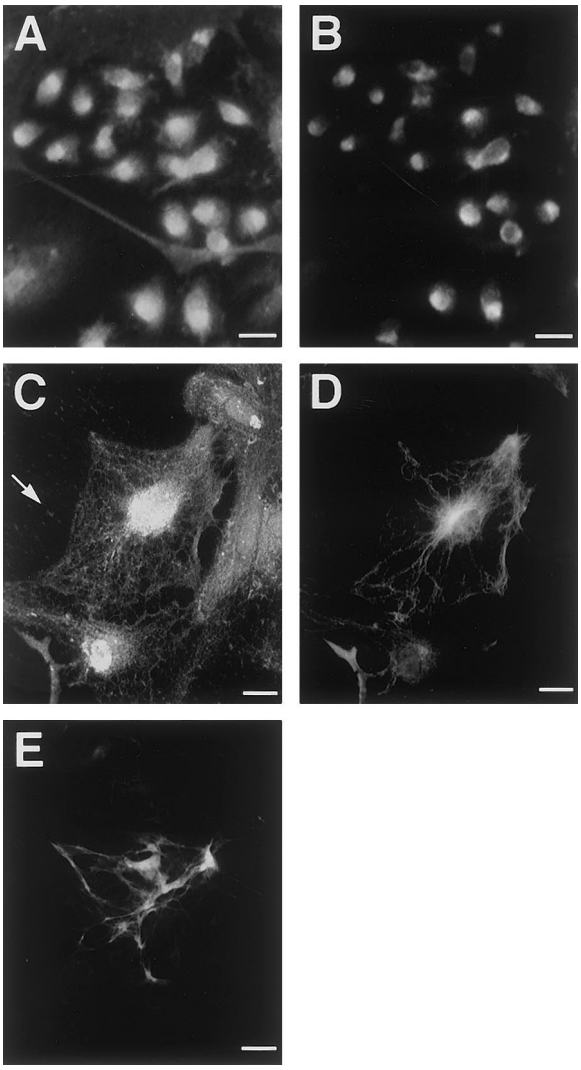

FIG. 3. Perlecan immunoreactivity in hippocampal glial cell cultures. Glial cells at 23–25 days in vitro were double-labeled for perlecan, Mac-1, or GFAP protein. **A** and **B:** Colocalization of perlecan immunoreactivity (A) with Mac-1 immunoreactive microglial cells (B). **C** and **D:** Colocalization of perlecan immunoreactivity (C) with GFAP immunoreactive astroglial cells (D). Microglial cells showed an ameboid-like morphology, without processes. Extracellular deposits of perlecan were also observed (see C, arrow). **E:** Fiber-like morphology of perlecan immunoreactivity, coincident with cell–cell appositions, was observed in the glial cell cultures. Bar = 10 μm for A and B and 20 μm for C–E.

of perlecan by IL-1α observed in vivo could be due to such differences, we challenged mixed glial cultures from hippocampus and striatum with IL-1α. Hippocampal glial cells were treated with IL-1α for 6, 8, and 24 h. After 6 h, we found a significant induction (eightfold; $p < 0.001$) of perlecan mRNA, and we detected a maximal increase at 8 h (10-fold; $p < 0.001$). After 24 h, perlecan mRNA levels remained significantly increased compared with control values ($p < 0.001$; Fig. 4A). It is interesting that in primary striatal glial cultures, we did not detect changes at any time point with respect to the control group (Fig. 4B).

In conclusion, there was a robust and consistent up-regulation by IL-1α in hippocampal perlecan mRNA in vitro, whereas no changes were observed in striatal perlecan mRNA levels. This suggests that the differential response depending on the brain area studied detected by in vivo examination is conserved by isolated glial cells from corresponding brain areas.

To determine whether a parallel increase in the levels of cellular perlecan protein could be detected in hippocampal glia in response to IL-1α treatment, immunocytochemical staining for perlecan, microglia, and astrocytes was performed after 24 h of treatment. No increase in perlecan immunoreactivity was observed in either microglia or astrocytes (data not shown). It is possible that the protein increase takes place sooner or later than the time point examined or that the perlecan protein is released into the media and thus becomes undetectable by immunochemical means.

DISCUSSION

Our results showed an up-regulation of perlecan mRNA and protein in the mouse CNS after a needle stab wound. We also showed the regulation of perlecan mRNA in vivo by the inflammatory cytokine IL-1α to be brain region-specific, with increased perlecan mRNA in hippocampus but no changes in the striatum. In contrast, we found that bFGF mRNA did not show differential regulation according to brain area after IL-1α treatment in vivo, being up-regulated in both hippocampus and striatum. Finally, we demonstrated the ability of IL-1α to

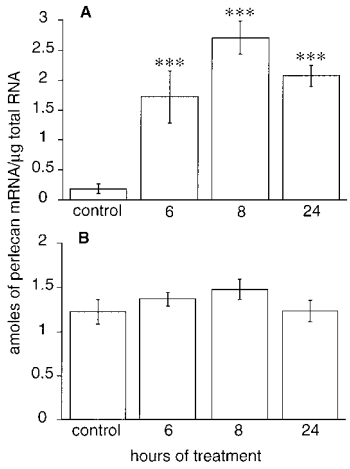

FIG. 4. Quantitative analysis of perlecan mRNA expression after IL-1α treatment in primary hippocampal and striatal glial cell cultures. Hippocampal (**A**) and striatal (**B**) glial cell cultures at 21–23 days in vitro were treated with IL-1α. RNA was collected at 0, 6, 8, and 24 h after treatment. $t = 0$ was used as the control in both experiments. Data are mean ± SEM (bars) values, expressed as attomoles of perlecan mRNA with respect to the total amount of RNA measured. ***$p < 0.001$, compared with the control values, by ANOVA test.

stimulate perlecan mRNA in vitro in hippocampal but not in striatal glial cells. Furthermore, we identified by double-labeled immunocytochemistry the origin of perlecan immunoreactive material to both astroglial and microglial cells in vitro.

To investigate how perlecan, an HSPG, responds to brain injury, we performed a needle stab wound to the brain. Perlecan immunoreactivity in mouse brain showed a vascular distribution in control tissue, as well as the appearance of extracellular deposits and increase in the diameter of blood vessels following the injury. This pattern of staining has been described before in different types of tissue by using different perlecan antibodies (Perlmutter et al., 1990). Perlecan mRNA was increased after injury, as assessed by in situ hybridization. Although, for technical reasons, we were not able to assess directly the cell types responsible for this induction, we speculate based on our in vitro findings that the induction of perlecan occurs in both reactive astrocytes and microglia. In summary, we showed that perlecan is up-regulated after local brain injury, thus indicating that it also participates in the brain's reaction to traumatic injury.

The pathological findings in AD are in many respects comparable to those after brain injury. In fact, there is clinical evidence for the presence of neurofibrillary tangles and diffuse plaques in humans, provoked by repetitive brain trauma, as happens in dementia pugilistica (Roberts et al., 1990). Likewise, epidemiological studies have shown that a previous history of head trauma represents a risk factor for the development of AD (Mortimer et al., 1991). It has been reported that a rapid accumulation of APP and Aβ occurs not only in contusion-type head injury, but also in the needle stab model (Otsuka et al., 1991). What is currently not understood is what are the key steps that lead to a pathological cascade with neuronal degeneration as exhibited in AD instead of a "normal" scar formation with healing. What is the common factor leading to AD pathology regardless of the initial triggering factor? As is discussed by Cotman et al. (1996), a classical brain response to injury is normally limited in time, whereas in AD there is the additional factor of the transformation of Aβ molecule to a β-sheet conformation and its deposition into fibrils. These molecules have the ability to initiate and maintain an inflammatory reaction that is initially acute but becomes chronic and lasts indefinitely. Thus, in AD, a local inflammatory response is constantly present, with a growing list of inflammatory-associated markers (Rogers et al., 1996) that are believed to play an active role in the AD pathogenesis. Interactions between Aβ and some of these inflammatory proteins have already been studied (Cribbs et al., 1997), as well as interactions between the amyloid component and microglial cells, with the demonstration that specific Aβ domains are able to stimulate neuronal killing by microglia (Giulian et al., 1996). There is evidence of cytokine production by microglia in AD brain (Meda et al., 1995; Mrak et al., 1995), including IL-1, which could play a determinant role in senile plaque formation. In this scenario, the increased production of IL-1 by microglial cells could, in turn, interact with other components of senile plaques such as perlecan, helping to perpetuate and aggravate the formation of plaques.

Snow et al. (1994*a*) have demonstrated that infusion of perlecan in the rat brain is able to precipitate plaque formation. Together with this finding is the additional observation that perlecan binds to Aβ and initiates fibril formation (Narindrasorasak et al., 1991; Buée et al., 1993*a*,*b*; Castillo et al., 1997). It is tempting to hypothesize that perlecan participates in producing senile plaques after its stimulation by IL-1 in an inflammatory milieu. An abnormal increase of perlecan would be a consequence of inflammatory events implicated in Aβ deposition as well as an important cause of them. Our results show an up-regulation of perlecan by IL-1α in the hippocampus, one of the brain regions that exhibits typical AD pathology. Moreover, the same type of results have been obtained in cortex (data not shown), another area presenting senile plaques. In contrast, IL-1α does not regulate perlecan in the striatum, suggesting that area-specific regulation of perlecan by IL-1α could be a crucial factor participating in the pattern of distribution of senile plaques in AD. It is important that in vitro regulation of perlecan by IL-1α in primary glial cell cultures from hippocampus and striatum supports our in vivo results indicating that this differential regulation is observed in isolated glial cells. Further investigation will be necessary to determine the exact mechanism and the cell or cells responsible in vivo.

Perlecan and bFGF are two molecules closely associated owing to their interaction at the bFGF receptor level. In fact, bFGF binds with high affinity to heparan sulfate-related molecules, and the fibroblast growth factor family is known as "heparin binding growth factors." This close relationship, both spatially and functionally, is very important for bFGF stability and receptor binding (Klagsbrun and Baird, 1991; Aviezer et al., 1994). To present a positive control for our perlecan mRNA data in vivo, we analyzed in parallel the regulation of bFGF by IL-1α. bFGF mRNA was increased by IL-1α in both hippocampus and striatum. Although IL-1 regulation of bFGF has already been reported (Rivera et al., 1994; Ho and Blum, 1997), this is the first time that perlecan and bFGF regulation by IL-1α is shown in parallel. In contrast to perlecan, we present here that bFGF is not differentially regulated by IL-1α depending on the brain area, indicating that although there is a close relationship between these two molecules, they can be independently regulated by the same cytokine in the same brain area. Given that there are other HSPGs expressed in the CNS besides perlecan, possibly in different brain regions, different HSPGs are used to modulate bFGF receptor binding, which therefore could explain the lack of coregulation of these two molecules in some brain areas. Alternatively, this lack of coregulation may serve to modulate bFGF action in a brain region-selective pattern.

Perlecan is a widely distributed HSPG, found in both normal and AD brains (Couchman and Ljubimov, 1989;

Perlmutter et al., 1990; Murdoch et al., 1994; Snow et al., 1994*a*,*b*). Nevertheless, the specific cellular source of perlecan in the mammalian brain has been little investigated. Recent studies have shown, by immunofluorescence and/or western blotting, the presence of perlecan protein in microglia/macrophages both in vitro and in vivo in rat and human tissue (Su et al., 1992; Miller et al., 1997). We now demonstrate, in agreement with those previous studies, that mouse microglial cells are immunoreactive for perlecan as revealed by double-labeled immunofluorescence in vitro. Moreover, astrocytes appear to express perlecan in vitro. These data support the hypothesis that inflammatory cells and their products such as IL-1α could play a key function in senile plaque genesis. Possibly, the stimulation of microglial cells by Aβ, followed by increased production of IL-1α and finally activation of perlecan synthesis, could stimulate Aβ fibril formation in an autocrine manner. Supporting this idea is the presence of IL-1α receptors in both microglia and astrocytes (for review, see Otero and Merrill, 1994). However, this is not the only possible sequence in the pathogeny of plaque formation. For instance, the presence of perlecan in diffuse plaques, which have not yet presented an inflammatory reaction and thus do not present activated microglial cells (Snow et al., 1990*a*,*b*), as well as the induction of perlecan gene expression before amyloid formation in experimental murine AA amyloidogenesis (Ailles et al., 1993), could indicate that other factors are also implicated in perlecan deposition. For example, toxic molecules released locally and other types of cells, such as neurons or endothelial cells, could also be responsible for perlecan synthesis.

In summary, the data presented here have implications regarding the pathogenesis of senile plaques, as well as the involvement of the immune system and perlecan in AD. Moreover, these studies support the use of antiinflammatory drugs, drugs manipulating perlecan metabolism, or antagonism of perlecan binding to Aβ via the administration of low-molecular-weight heparin (Leveugle et al., 1998) in the aim to halt the, until now, inexorable evolution of AD.

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