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Immunocytochemical and in situ hybridization studies of the heparan sulfate proteoglycan, glypican, in nervous tissue

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SUMMARY

Using immunocytochemistry and in situ hybridization histochemistry, we have investigated in embryonic and postnatal rat nervous tissue the localization and cellular sites of synthesis of glypican, a glycosylphosphatidylinositol-anchored heparan sulfate proteoglycan. Glypican immunoreactivity is present in the marginal layer (prospective white matter) and in the dorsal root entry zone of E13-16 spinal cord, as well as in the optic nerve and retina at this stage, but does not appear at significant levels in brain until approximately E19. The proteoglycan shows a wide distribution in grey matter and axonal projections of postnatal brain, including the hippocampal formation, the parallel fibers of cerebellar granule cells, and in the medulla and brainstem. Northern analysis demonstrated high levels of glypican mRNA in brain and skeletal muscle, and in rat PC12 pheochromocytoma cells. In situ hybridization histochemistry showed that glypican mRNA was especially prominent in cerebellar granule cells, large motor neurons in the brainstem, and CA3 pyramidal cells of the hippocampus. Our immunocytochemical and in situ hybridization results indicate that glypican is predominantly a neuronal membrane proteoglycan in the late embryonic and postnatal rat central nervous system.

Key words: glypican, heparan sulfate, proteoglycan, nervous tissue

INTRODUCTION

Heparan sulfate proteoglycans are ubiquitous components of plasma membranes and are involved in a number of biological functions including: as extracellular matrix receptors in cell-cell and cell-substratum interactions, in the organization of epithelia, in mediating the actions of basic fibroblast growth factor, and as co-receptors for extracellular matrix components such as fibronectin and the interstitial collagens (for reviews, see Bernfield et al., 1992; David, 1993). The syndecan family of transmembrane heparan sulfate proteoglycans, of which four members have been identified up to now, has been studied most intensively.

We have previously biochemically characterized (Klinger et al., 1985; Ripellino and Margolis, 1989) and cloned (Karthikeyan et al., 1992) a heparan sulfate proteoglycan of brain. Its primary structure revealed that this brain proteoglycan is the rat homolog of glypican, a glycosylphosphatidylinositol-anchored heparan sulfate proteoglycan that was first identified in human lung fibroblasts (David et al., 1990). To determine its localization and cellular sites of synthesis in nervous tissue, we have used antibodies to a synthetic glypican peptide for immunocytochemical studies and have examined the distribution of glypican mRNA by in situ hybridization histochemistry. Our results demonstrate a relatively wide distribution of glypican in the late embryonic and postnatal rat central nervous system. It is especially abundant on axons and is synthesized by neurons, including discrete groups of large motor neurons in the brainstem, cerebellar granule cells, and CA3 pyramidal cells of the hippocampus.

MATERIALS AND METHODS

Preparation of antibodies to rat glypican and immunocytochemistry

A peptide of 17 amino acids, corresponding to residues 346-361 of rat glypican (numbering beginning at initiation methionine; Karthikeyan et al., 1992) to which an N-terminal cysteine was added, was synthesized (Applied Biosystems model 430A peptide synthesizer), purified by reverse-phase HPLC, and its identity confirmed by amino acid analysis. The peptide (CPKVNPHGSGPEEKRHR), which had no significant similarity with other reported protein sequences, was coupled through the cysteine residue to keyhole limpet hemocyanin (KLH) using *m*-maleimidobenzoic acid *N*-hydroxysuccinimide ester (MBS) as the coupling reagent (Liu et al., 1979). A 5 mg sample of peptide was dissolved in 400 µl of 50 mM PBS (pH 7.5), made 20 mM in dithiothreitol (DTT), and incubated for 1 hour at 37°C. Excess DTT was removed from the reduced peptide by gel filtration on a Sephadex G-10 column (0.9 cm × 15 cm) eluted with 50 mM PBS (pH 6) containing 0.02% sodium azide, and fractions were pooled based on absorbance at 280 nm.

KLH (5 mg) was diluted to 350 µl with PBS (pH 7.2, final concen-

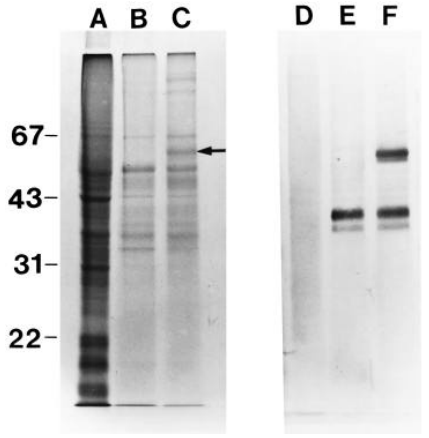


Fig. 1. Characterization of anti-glypican antibodies. Samples (20 µg) of total rat brain proteins (lanes A and D) or 5 µg of a partially purified preparation of heparan sulfate proteoglycan from rat brain (Klinger et al., 1985; lanes B,C,E and F) were electrophoresed on a 10% SDS-polyacrylamide minigel and the proteins were stained with Coomassie Blue (lanes A-C), or transferred to nitrocellulose and stained with affinity-purified antibody to glypican (lanes D-F). The heparan sulfate proteoglycans in lanes C and F were digested with heparitinase (1.5 milli international units/50 µg protein). The arrow (lane C) indicates the major 55 kDa glypican core protein that appears after heparitinase treatment.

tration of 25 mM) and reacted with 0.7 mg MBS dissolved in dimethylformamide and added slowly with stirring. After stirring for 30 minutes at room temperature, the KLH-MB reaction product was passed through a column of Sephadex G-25 (0.9 cm × 5 cm) equilibrated with 50 mM PBS (pH 6) to remove excess MBS. Absorbance was monitored at 250 nm and the KLH-MB eluted as a turbid fraction immediately after the void volume. The KLH-MB was then mixed with 5 mg of reduced peptide (both in 50 mM PBS, total volume of ~6 ml), adjusted to pH 7-7.5, and the reaction was stirred for 3 hours at room temperature.

The KLH-peptide conjugate at a concentration of ~0.8 mg/ml in saline was used for immunization of rabbits. A 160 µl sample in complete Freund's adjuvant was initially injected into the popliteal lymph nodes and 40 µl intradermally, followed at 2 and 4 weeks by 100 µl intradermally in incomplete Freund's adjuvant, and subsequent 25-50 µg subcutaneous booster injections in incomplete Freund's adjuvant at monthly intervals. IgG fractions were prepared by ammonium sulfate precipitation of serum, and the antibodies were affinity-purified after coupling the peptide (3 mg) to 3 ml of Affi-Gel 10 (Bio-Rad) in 0.1 M bicarbonate buffer (pH 8.5) for 18 hours at 4°C according to the manufacturer's instructions. IgG (100 mg) was mixed for 18 hours at 4°C with 3 ml settled volume of affinity matrix in a total volume of 12 ml 50 mM PBS (pH 7.2). The beads were washed with PBS several times by centrifugation to remove unbound protein, poured into a column, and washed further with PBS before elution successively with 0.1 M glycine buffer (pH 2.5), 50 mM PBS, 50 mM phosphate buffer (pH 7.2) containing 0.5 M NaCl, 50 mM PBS, 10 mM PBS (pH 8), and 50 mM diethylamine (pH 11.5). All except the 0.1 M glycine buffer contained 0.1% CHAPS and 0.02% sodium azide. Fractions were immediately neutralized, pooled based on absorbance at 280 nm, dialyzed against 50 mM PBS containing 0.02% sodium azide, and concentrated by pressure (Diaflo PM 30 membrane, Amicon) and Centricon-30 (Amicon) ultrafiltration. The pH 2.5 and 11.5 eluates both contained antibodies reactive with the synthetic peptide as determined using a dot binding assay (Rauch et al., 1992), with the glypican core protein on immunoblots, and with brain sections (see below), although the affinity-purified antibodies eluted at pH 2.5 were generally more sensitive when used for immunocytochemistry, and those eluted at pH 11.5 were more

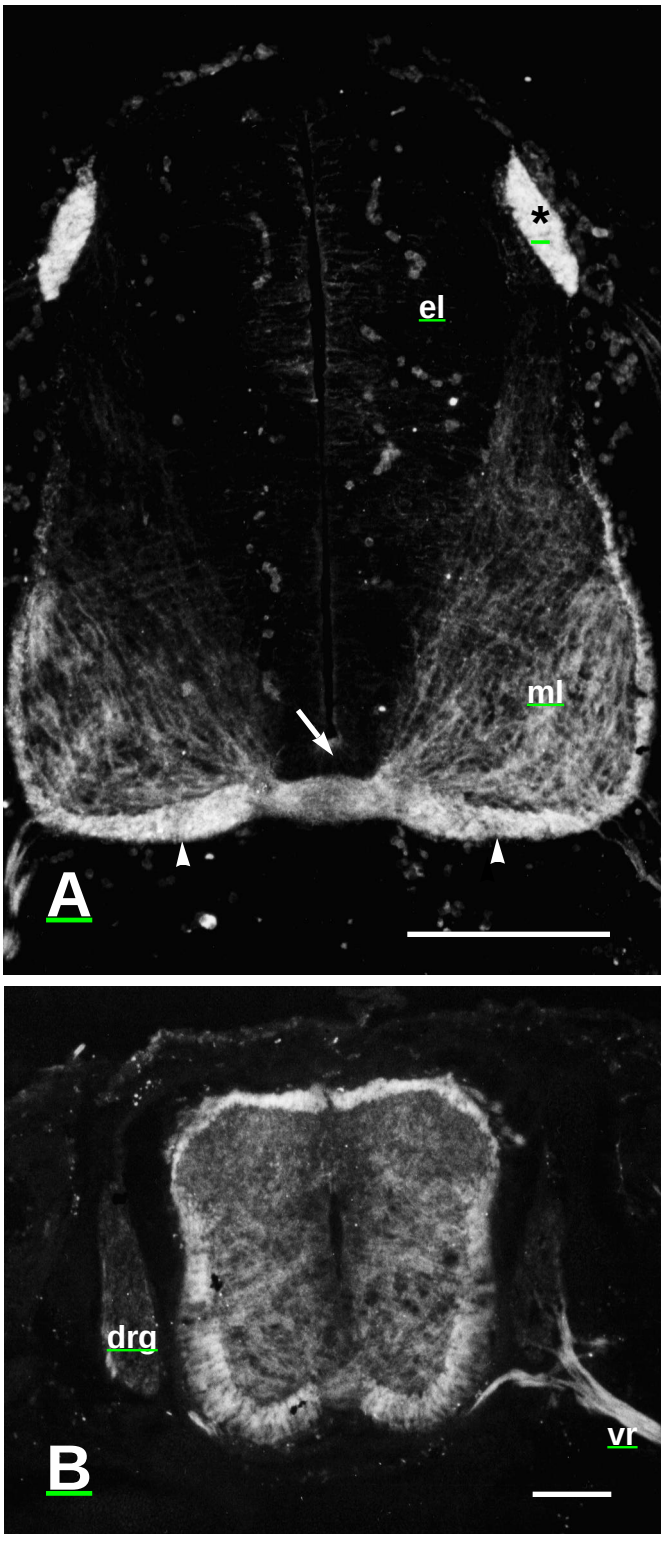


Fig. 2. Immunofluorescence localization of glypican in embryonic spinal cord. Glypican immunoreactivity is seen in the ventral marginal layer (arrowheads), the dorsal root entry zone (asterisk), and on commissural axons of the mantle layer (ml) of E13 spinal cord (A), whereas the floor plate (arrow) and the ependymal layer (el) are unstained. By E16 (B) the entire marginal layer and the ventral roots (vr) and dorsal root ganglia (drg) are stained. Bars, 200 µm.

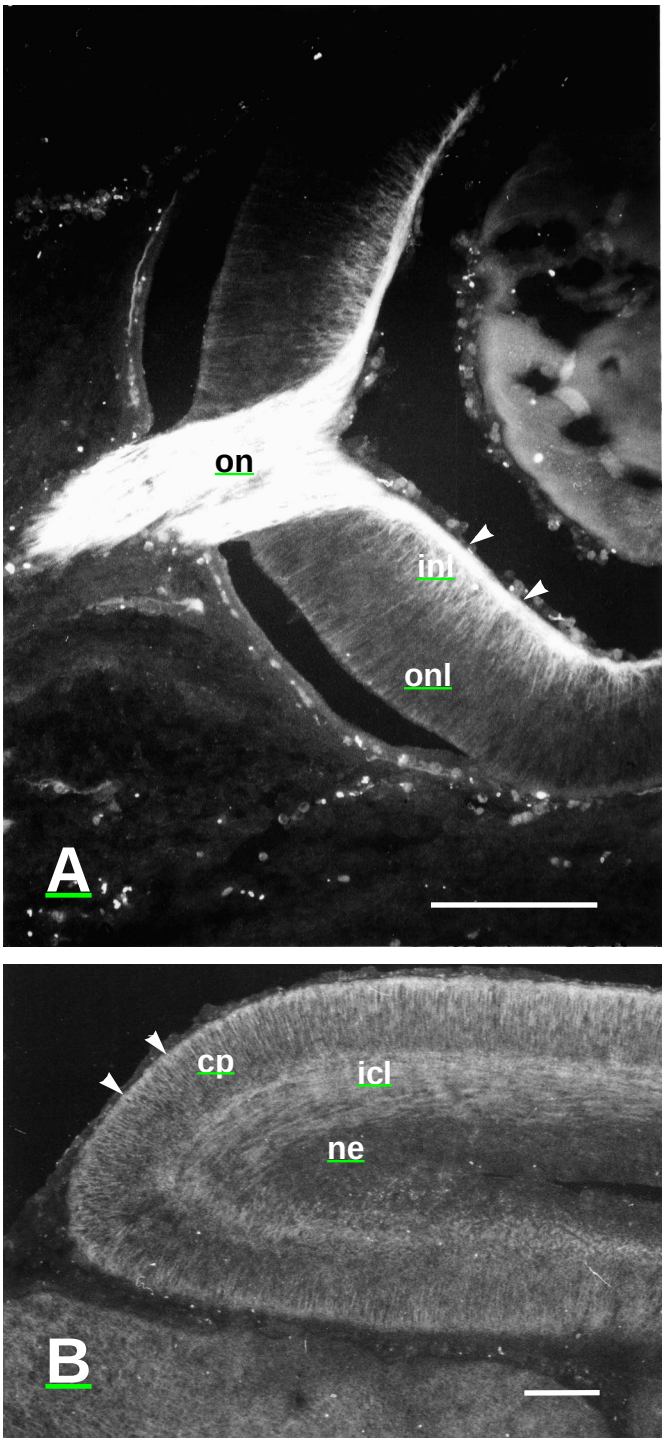


Fig. 3. Immunofluorescence localization of glypican in the embryonic eye and brain. (A) Staining is present in the optic nerve (on), the nerve fiber layer of the optic cup (arrowheads) and, more weakly, in the inner nuclear layer (inl) at E16, whereas the outer nuclear layer (onl) of the retina is unstained. (B) Glypican immunoreactivity is seen in cortical layer 1 (arrowheads) and the intermediate cortical layer (icl) of E19 cerebral cortex, but not in the cortical plate (cp) and neuroepithelium (ne). Bars, 200 μm.

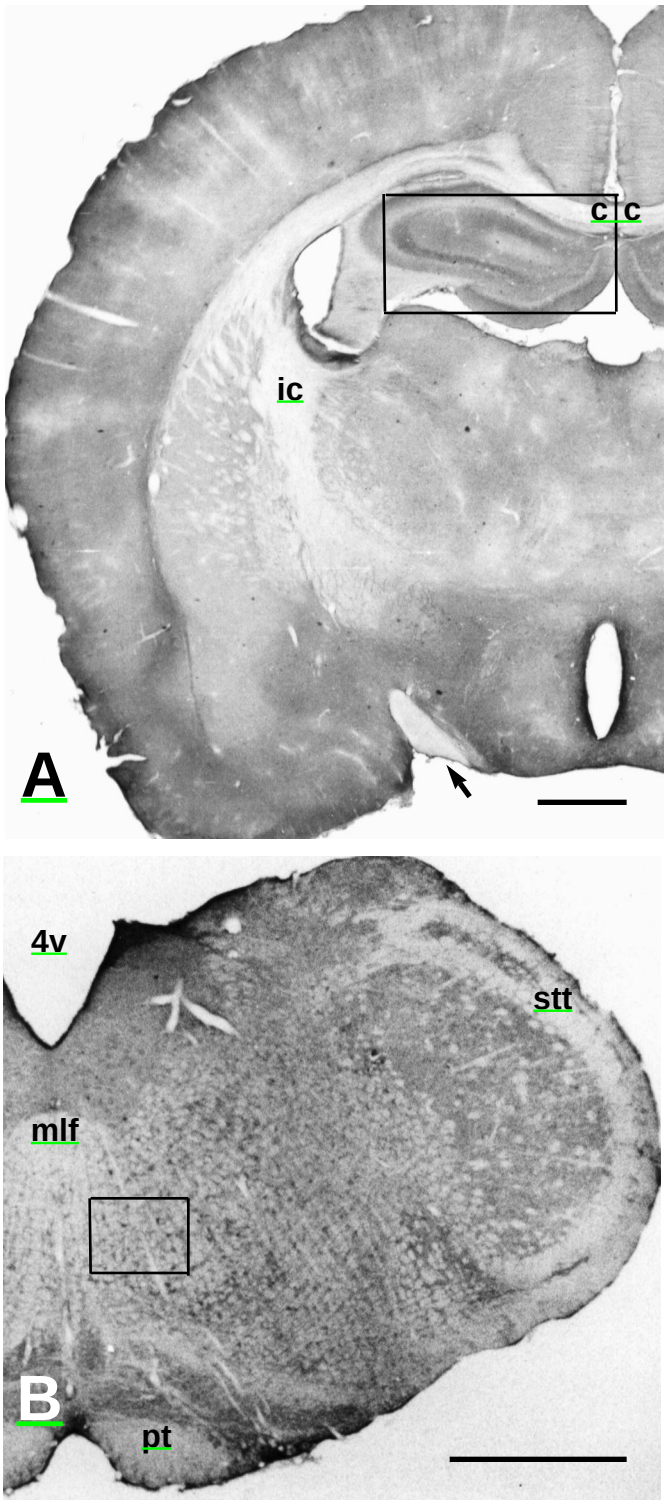


Fig. 4. Immunoperoxidase staining of glypican on neuronal fibers and cell bodies in the adult cerebrum and brainstem. White matter tracts including the corpus callosum (cc), internal capsule (ic), and optic tract (arrow) in the cerebrum (A); and the pyramidal tract (pt), the medial longitudinal fasciculus (mlf), and the spinal trigeminal tract (stt) of the medulla (B) are unstained. 4v, 4th ventricle. Boxes indicate areas shown at higher magnification in Fig. 5. Bars, 1 mm.

immunocytochemistry, and those eluted at pH 11.5 were more sensitive for staining of immunoblots.

Immunocytochemistry of Vibratome sections of perfusion-fixed rat brain was performed as described previously (Rauch et al., 1991), using the affinity-purified anti-glypican antibodies and peroxidase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA). Localization of glypican in rat embryos was performed by immunofluorescence with rhodamine-conjugated anti-mouse IgG (Jackson ImmunoResearch) using cryostat sections prepared as described below.

In situ hybridization histochemistry

Rat embryos were fixed for 4-6 hours at room temperature in 4% paraformaldehyde/0.1 M PBS, washed twice for 5 minutes in PBS, and cryoprotected by gentle shaking overnight at 4°C in 15% sucrose/0.1 M PBS before freezing on powdered dry ice, whereas postnatal brain was frozen directly after dissection. Sections (15-20 μm) were cut using a Hacker-Bright cryostat, thaw-mounted on poly-L-lysine-coated slides, and quickly dried under a stream of cool air. The tissue was fixed for 5 minutes in 3% paraformaldehyde in 0.1 M PBS (pH 7.4), and rinsed twice for 1 minute in PBS and twice for 1 minute in 2× SSC. All solutions were made with diethyl pyrocarbonate-treated water. Sections were then treated for 3 minutes at 37°C with proteinase K (1 μg/ml in 0.1 M Tris-HCl, pH 8.0, containing 50 mM EDTA), rinsed twice with water, and acetylated for 10 minutes with 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0. After rinsing for 1 minute each in 2× SSC and 0.1 M PBS, sections were incubated for 30 minutes in glycine/PBS (2 mg/ml, pH 7.0), rinsed for 1 minute in 2× SSC, dehydrated through a series of graded ethanols, and air dried.

A *Hin*dIII-*Xho*I restriction fragment comprising the 838 nucleotides at the 5′ end of a rat glypican clone (Karthikeyan et al., 1992) was subcloned into pGEM7Zf by directional cloning. The plasmid was linearized with *Cla*I and transcribed into digoxigenin-labeled antisense RNA with T7 RNA polymerase (Promega) using the GENIUS 4 RNA labeling kit (Boehringer Mannheim). A sense probe was prepared by deletion of the *Hin*dIII-*Cla*I fragment followed by blunt-end ligation, linearization with *Xho*I, and transcription with SP6 RNA polymerase. The resulting 297-base probes, which had no significant identity with other reported nucleotide sequences, were used for in situ hybridization histochemistry, and the antisense probe was also used with northern blots.

Sections were covered with digoxigenin-labeled riboprobe, which was diluted to a concentration of 0.2-1 ng/ml in 30 μl of hybridization solution (40% formamide, 10% dextran sulfate, 1× Denhardt's solution, 4× SSC, 10 mM dithiothreitol, 1 mg/ml yeast RNA, 1 mg/ml sheared salmon sperm DNA) and denatured for 10 minutes at 65°C. Hybridization was carried out overnight at 52°C in an atmosphere of 40% formamide, after which sections were washed for 5 minutes at 52°C with 50% formamide/2× SSC followed by a further 20 minute wash using fresh solution. Slides were rinsed twice for 1 minute at room temperature with 2× SSC, treated for 30 minutes at 37°C with RNase A (20 μg/ml 2× SSC), and rinsed twice for 1 minute in 2× SSC. After incubation for 5 minutes at 52°C in 50% formamide/2× SSC, sections were treated for 2 hours at room temperature with 2% blocking reagent (Boehringer Mannheim no. 1096176) in 0.1 M sodium maleate buffer (pH 7.5) containing 0.15 M NaCl.

The blocking solution was removed and sections were incubated for 1 hour at room temperature with alkaline phosphatase-conjugated sheep anti-digoxigenin Fab fragments (Boehringer Mannhein) diluted 1:500 in 2% blocking solution containing 0.3% Triton X-100. Slides

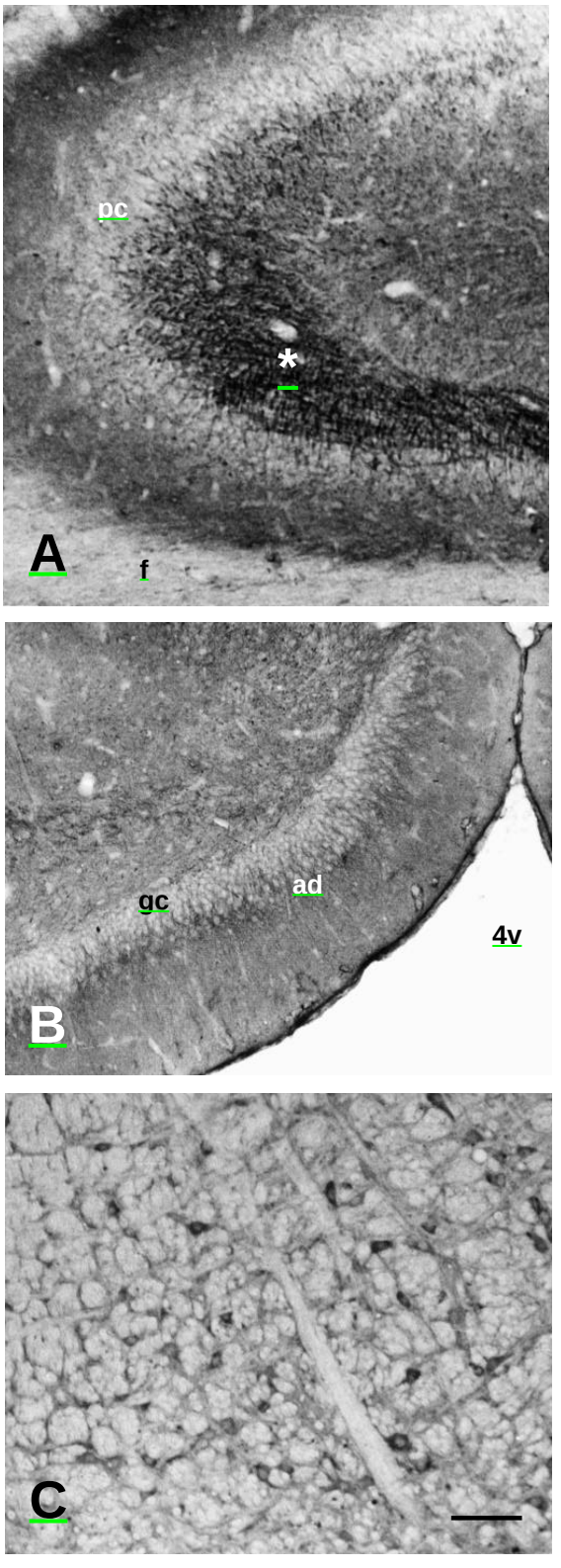


Fig. 5. Glypican immunoreactivity in the hippocampal formation and medulla. A higher magnification of the boxed area in Fig. 4A shows staining of glypican on neuronal processes of hippocampal area CA3 (A), and in the dentate gyrus (B). Glypican immunoreactivity in the gigantocellular reticular nucleus of the medulla is seen in C. In the hippocampus (A), the most intense staining is seen in collaterals of CA3 cells (asterisk), whereas the adjacent pyramidal cell bodies (pc) and fimbria (f) are unstained. In the dentate gyrus (B), staining is seen in the granule cell apical dendrites (ad) below unstained granule cell bodies (gc). Bar, 100 μm.

were washed for 10 minutes in 0.1 M TBS, pH 7.5, followed by 30 minutes in 0.1 M Tris-HCl (pH 9.5) containing 0.1 M NaCl and 50 mM $MgCl_2$. Sections were covered with chromogen solution (338 μg/ml of nitro blue tetrazolium, 175 μl/ml of 5-bromo-4-chloro-3-indolyl phosphate, and 240 μg/ml of levamisole in 0.1 M Tris-HCl, pH 9.5, containing 0.1 M NaCl and 50 mM $MgCl_2$) and incubated in the dark at room temperature in a humidified chamber until color development was complete, after which slides were washed twice for 5 minutes in 10 mM Tris-HCl (pH 8.1) containing 1 mM EDTA. The sections were then dehydrated through a series of graded ethanols, cleared in xylene, coverslipped, and sealed in Permount (Fisher Scientific Co., Pittsburgh, PA).

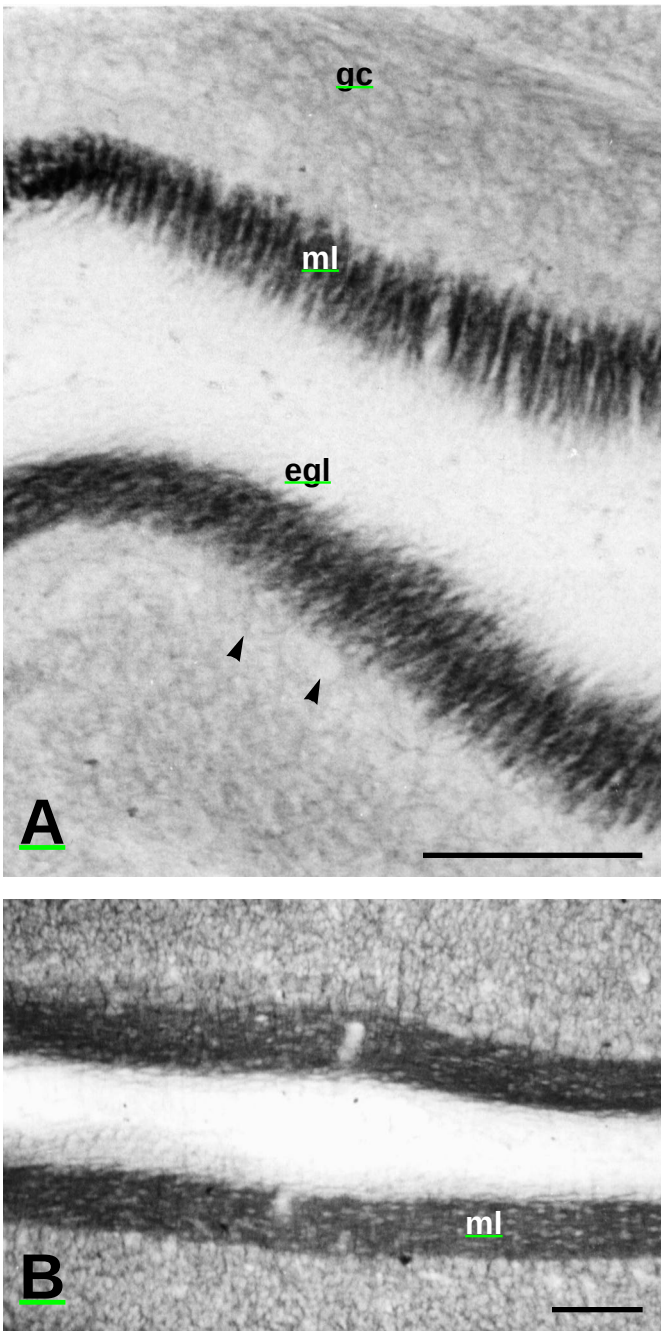


Fig. 6. Immunoperoxidase staining of glypican in cerebellum. (A) Strong staining is seen in the molecular layer (ml) and weaker, diffuse staining in the granule cell layer (gc) in two apposed folia in a sagittal section of 9-day postnatal cerebellum. Note that the external granule cell layer (egl) and the Purkinje cells (arrowheads) are unstained. In a coronal section (B), staining in the molecular layer can be clearly identifed as being on the parallel fibers. Bars, 100 μm.

RESULTS

Immunocytochemistry

Antibodies raised to a glypican peptide sequence reacted on immunoblots of a partially purified preparation of rat brain heparan sulfate proteoglycans (Klinger et al., 1985) with the 55 kDa glypican core glycoprotein, which appeared only after heparitinase treatment, as well as with a major 41 kDa band and a minor 39 kDa band, which were also present in the undigested proteoglycans (Fig. 1). These bands of lower molecular mass (Fig. 1, lanes E and F), which were not clearly identifiable on Coomassie Blue-stained gels of the proteoglycan preparation, are presumably degradation products derived from regions of the glypican core protein that do not contain heparan sulfate chains. The antibodies did not react with any components present on immunoblots of total rat brain proteins that were not digested with heparitinase (Fig. 1D), and reactivity with tissue sections was inhibited by incubation of the antibodies with an excess of the glypican peptide used for immunization (data not shown).

At E13 (Fig. 2A), glypican immunoreactivity is seen in the fibers of the ventral marginal layer (prospective white matter) and the dorsal root entry zone, and in the commissural axons coursing through the mantle layer, but is largely absent from the ependymal layer (prospective grey matter), whereas by E16 (Fig. 2B) the entire marginal layer is stained, as are the ventral roots at both stages. No staining is seen in glial structures such as the roof plate or floor plate, or in the surrounding mesenchyme and adjoining tissue. At E16 there is also very little

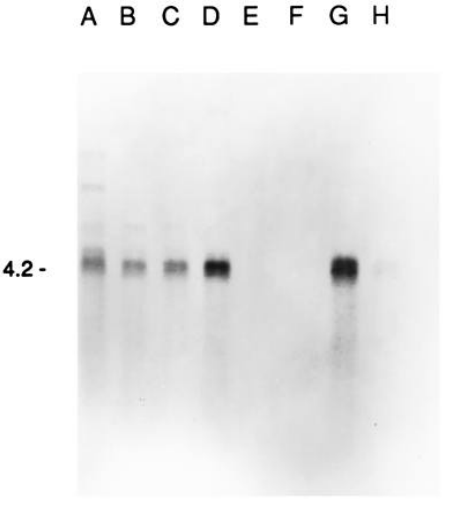


Fig. 7. Glypican expression in rat tissues (4.2 kb). Northern blot of mRNA from 4-day (A) and adult (B) whole brain, adult cerebellum (C), PC12 pheochromocytoma cells (D), liver (E), kidney (F), skeletal muscle (G), and lung (H), electrophoresed on a 1% agarose gel containing 6% formaldehyde and 4 μg of mRNA/lane. The blot was probed with a digoxigenin-labeled antisense RNA transcript and stained with alkaline phosphatase-conjugated anti-digoxigenin Fab (Roche et al., 1992).

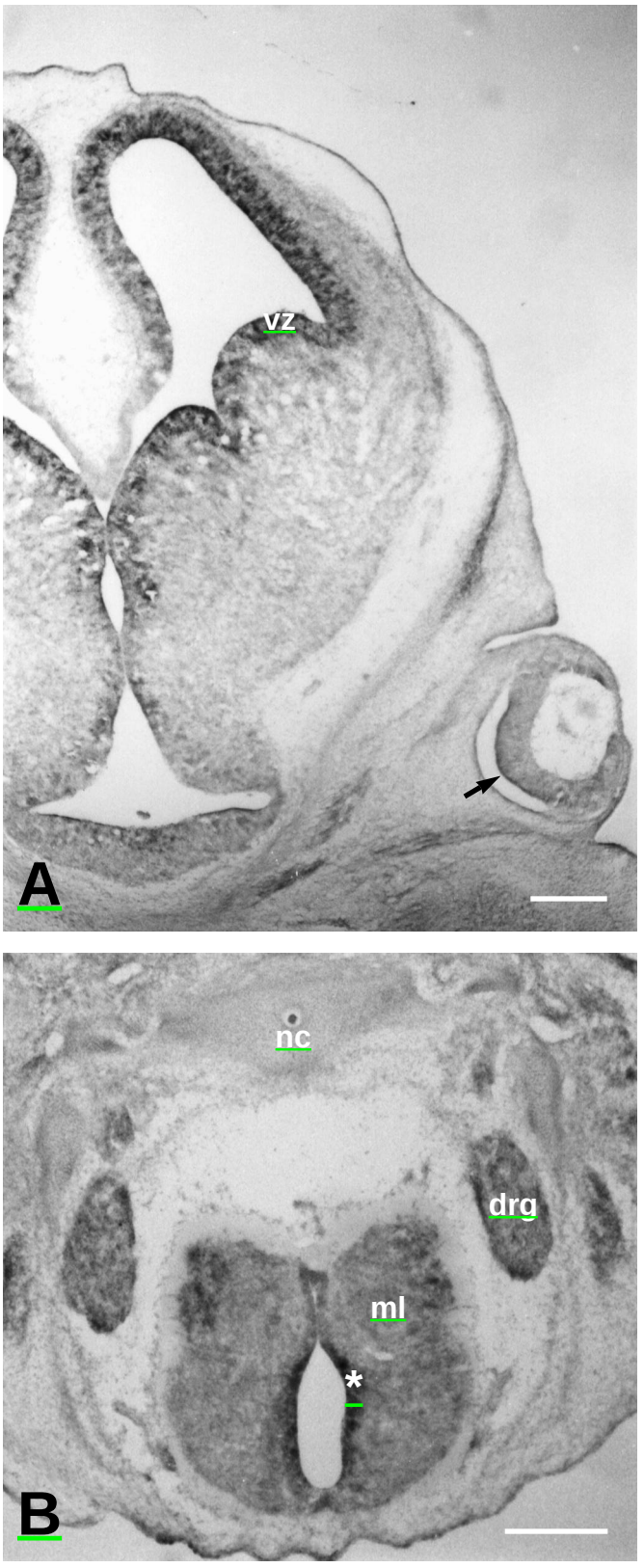


Fig. 8. In situ hybridization histochemistry of glypican at E15. Glypican mRNA is present in: (A) the ventricular zone (vz) and retina (arrow); and (B) in the ependymal layer (asterisk) and mantle layers (ml) of the spinal cord, and in the dorsal root ganglia (drg) and notochord (nc). Bars, 300 μm.

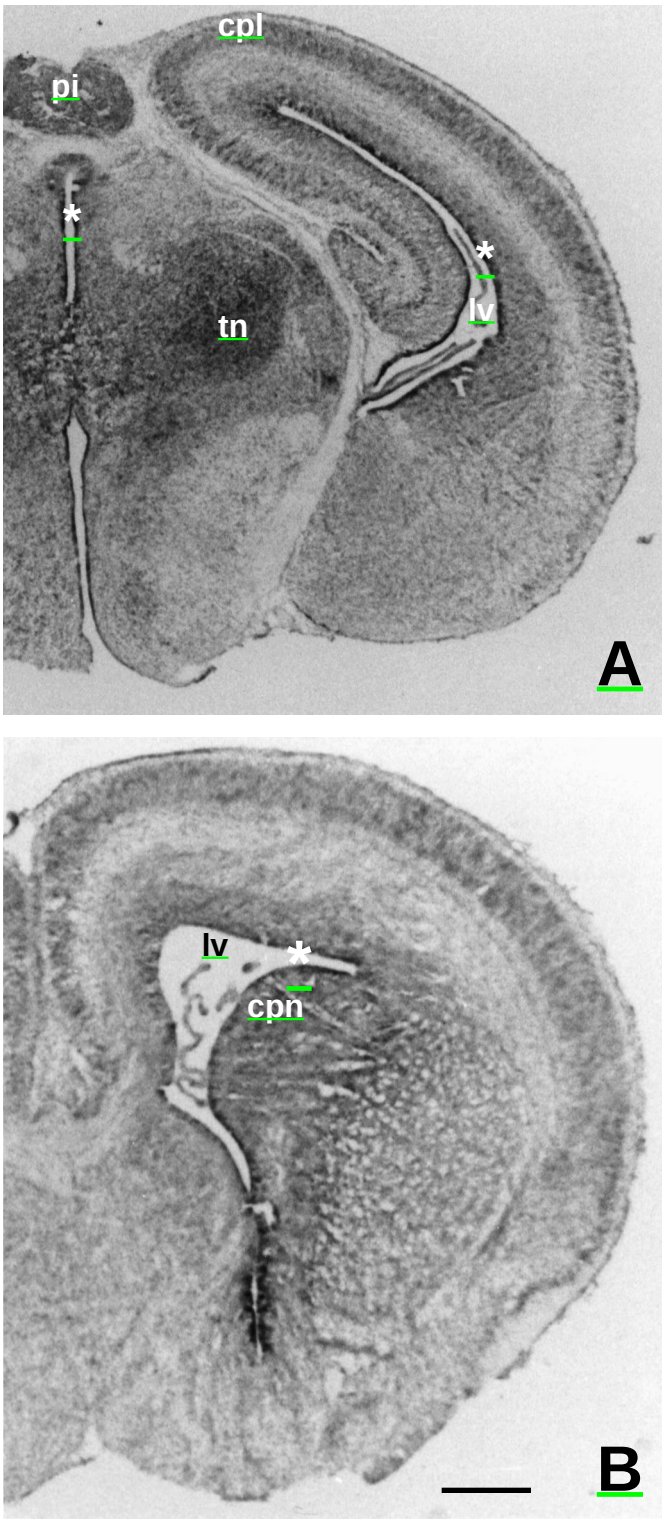


Fig. 9. In situ hybridization histochemistry of glypican at E19. Glypican mRNA in E19 cerebrum is seen in the ventricular zone (asterisk) surrounding the 3rd ventricle and lateral ventricle (lv), in the thalamic nuclei (tn), and the caudate putamen. Neuroepithelium (cpn) and other areas with significant density, such as the pineal (pi) and the optical plate (cpl), had the same appearance in sections hybridized with a control sense probe. The section shown in A is posterior to that of B. Bar, 500 μm.

specific staining in the head or brain other than the optic nerve and nerve fiber layer of the retina (Fig. 3A), but by E19 cerebral cortical layer 1 and the intermediate cortical layer also show glypican immunoreactivity (Fig. 3B).

In mature rat brain, anti-glypican antibodies produced relatively widespread staining of grey matter and axonal projections in the cerebrum and medulla, whereas myelinated fiber tracts (e.g. the corpus callosum, internal capsule, optic tract, pyramidal tract, the medial longitudinal fasciculus and the spinal trigeminal tract) were generally unstained (Fig. 4). There is strong staining of CA3 axon collaterals (Fig. 5A) and apical dendrites of granule cells (Fig. 5B) in the hippocampal formation, and of neuronal processes in the gigantocellular reticular nucleus of the medulla (Fig. 5C). The probable association of glypican with specific hippocampal connections is suggested by our in situ hybridization studies, which demonstrate that glypican synthesis in this region is apparently limited to the CA3 pyramidal cell bodies (see below).

In early postnatal cerebellum, staining was intense in the molecular layer but diffuse and much weaker in the granule cell layer, whereas Purkinje cell bodies and the external granule cell layer were not labeled (Fig. 6A). Coronal sections of cerebellum (Fig. 6B) showed that the stained fibers in the molecular layer ran perpendicular to those seen in the sagittal section of Fig. 6A, demonstrating that the glypican immunoreactivity seen in the molecular layer is present on the parallel fibers of the granule cells, rather than on Bergmann glial processes.

Northern analysis

A major 4.2 kb glypican message was seen on northern blots of mRNA from 4-day and adult whole brain, adult cerebellum, PC12 pheochromocytoma cells and muscle (Fig. 7). Additional faint bands were visible at 4.9, 5.7, 7.7 and 9.8 kb in 4-day brain, but were barely detectable in adult brain. No message was seen in liver or kidney, and only very faintly in lung, even though glypican was initially identified in human lung fibroblasts (David et al., 1990). It is, however, likely that glypican is expressed in these rat tissues, though at a considerably lower level than in muscle or nervous tissue. Glypican message was also detected in rat C6 glioma and human U-373 MG glioblastoma cells (data not shown), consistent with a recent report demonstrating the presence of glypican mRNA in a large number of human tumor cell lines, including both glioma and neuroblastoma cells (Lories et al., 1992).

In situ hybridization histochemistry

In situ hybridization histochemistry of embryonic central nervous tissue and of early postnatal and adult rat brain demonstrated, as expected, a more limited distribution of mRNA in neuronal cell bodies as compared to the widespread immunocytochemical staining of neuronal processes. In embryos at E15, glypican mRNA is seen in the ventricular zone of the brain, where active cell proliferation is occurring, and also in

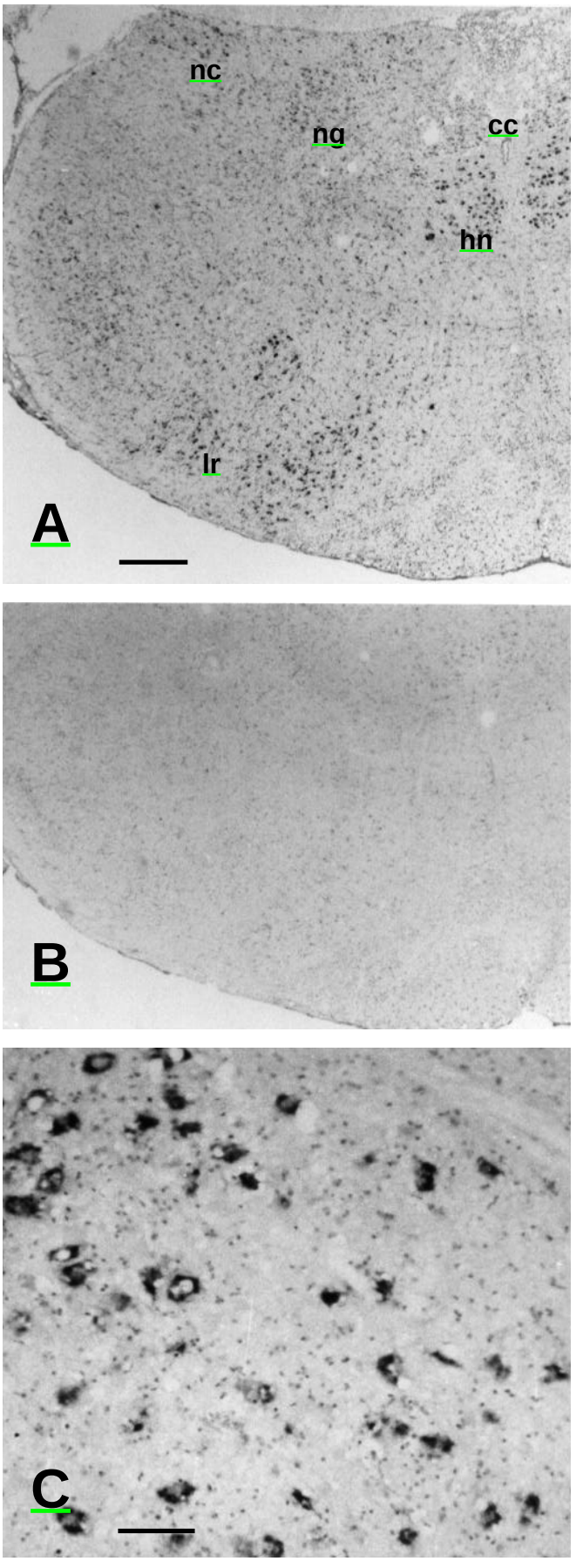
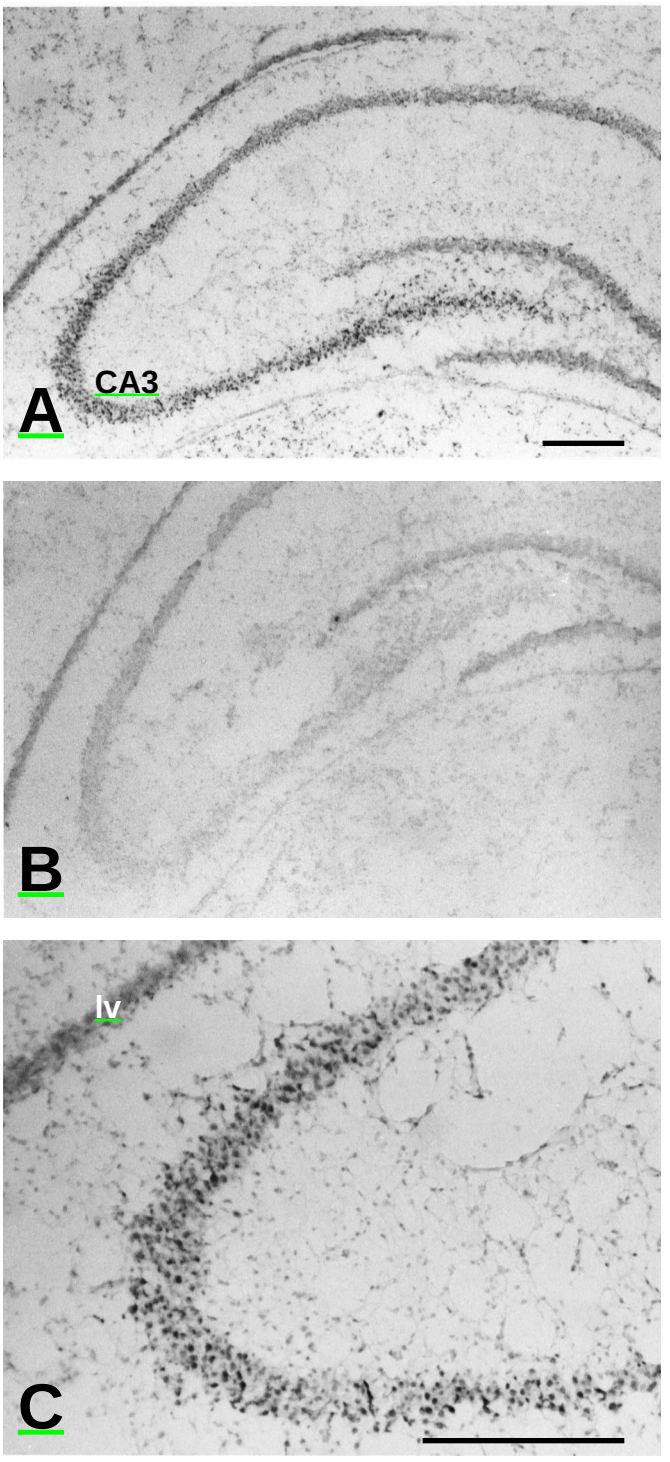


Fig. 10. In situ hybridization histochemistry of glypican in a hemisection of adult rat brainstem. (A) The central canal (cc) and staining of motor nuclei (nucleus gracilis, ng; nucleus cuneatus, nc; hypoglossal nucleus, hn; lateral reticular nucleus, lr). A control section from the same area hybridized with a sense probe is shown in B; and C shows a higher magnification of staining in large motor neurons. Bars: 500 μm (A and B) and 100 μm (C).

the retina (Fig. 8A). In the spinal cord at the same embryonic stage, the highest level of glypican mRNA is apparent in the ependymal layer, and it is also seen in the surrounding mantle layer as well as in the dorsal root ganglia and in the notochord (Fig. 8B). By E19, glypican mRNA in the cerebral hemispheres is seen in the ventricular and subventricular zones, in the thalamic nuclei, and the neuroepithelium of the caudate putamen (Fig. 9).

In postnatal brain, glypican mRNA was most prominent in the CA3 pyramidal cells of the hippocampus and in motor nerve nuclei in the brainstem and spinal cord (Figs 10 and 11). Glypican mRNA was also apparent in both the external and internal granule cell layers of early postnatal cerebellum (Fig. 12), consistent with its immunocytochemical localization on parallel fibers (Fig. 6), which are the granule cell axons. In all cases, hybridization with a sense probe did not give the staining patterns noted above.



DISCUSSION

Our immunocytochemical and in situ hybridization results indicate that glypican is present primarily on neuronal processes, although we cannot exclude its synthesis by glial cells. There is also a distinct developmental pattern in the appearance of glypican in different regions of the central nervous system. It is initially found in the ventral marginal layer and in the dorsal root entry zone of E13 spinal cord, by E16 in the entire marginal layer of the spinal cord and in retina, but not until approximately E19 is there significant immunoreactivity or mRNA in the brain. This developmental pattern of expression is generally the opposite of that described for cerebroglycan (MSPG M13), another neuronal glycosylphosphatidylinositol-anchored heparan sulfate proteoglycan with a 58.6 kDa core protein whose primary structure has recently been reported (Stipp et al., 1994). Northern analysis and in situ hybridization histochemistry demonstrated that cerebroglycan, which has 37% amino acid identity with glypican, is expressed only in embryonic and very early postnatal but not in adult rat brain, disappearing after cell migration and axon outgrowth have ceased. These two structurally related heparan sulfate proteoglycans may therefore have complementary functions in nervous tissue.

Most of the available information concerning the functional roles of central nervous tissue proteoglycans has concerned the chondroitin sulfate proteoglycans (Margolis and Margolis, 1993). With the exception of the NG2 proteoglycan (Nishiyama et al., 1991), which may function as a cell surface receptor for collagen VI (Nishiyama and Stallcup, 1993), and the transmembrane form of phosphacan (Maurel et al., 1994), both of which are present in brain at relatively low concentrations, the chondroitin sulfate proteoglycans of nervous tissue whose primary structures have been determined are secreted molecules rather than integral membrane proteins. These include neurocan (Rauch et al., 1992) and brevican (Yamada et al., 1994), two extracellular matrix proteoglycans that contain hyaluronic acid binding regions as well as epidermal growth factor-like, lectin-like, and complement regulatory protein-like domains, and phosphacan (Maurel et al., 1994), which represents an extracellular variant of a receptor-type protein tyrosine phosphatase. Neurocan and phosphacan bind specifically, reversibly, and with high affinity (K_d ~1 nM) to

Fig. 11. In situ hybridization histochemistry of 8-day rat cerebrum showing glypican mRNA in CA3 pyramidal cells of the hippocampus (A). A similar section hybridized with a sense probe (B) shows nonspecific background density in adjacent areas of the hippocampus, dentate gyrus and lateral ventricle. The different appearance of the lateral ventricle (lv) above the specifically stained CA3 cells is more clearly seen at higher magnification (C). Bars, 300 μm.

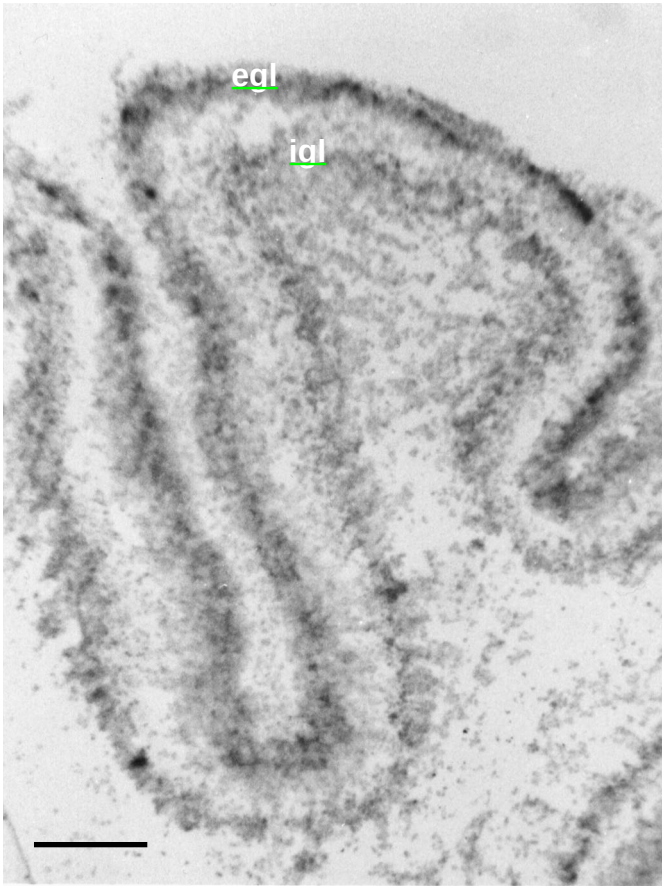


Fig. 12. In situ hybridization histochemistry of glypican in 6-day rat cerebellum. Glypican mRNA is evident in the external (egl) and internal (igl) granule cell layers. Bar, 200 μm.

neural cell adhesion molecules and the extracellular matrix protein tenascin, and inhibit cell adhesion and neurite outgrowth (Grumet et al., 1993, 1994; Friedlander et al., 1994). Phosphacan may also modulate signal transduction across the plasma membrane by competing for ligands of the transmembrane phosphatase.

Less information is available to implicate central nervous system heparan sulfate proteoglycans in specific functional processes (for a review, see Margolis and Margolis, 1993). However, it has been demonstrated that in murine neural precursor cells and cell lines derived from them, the developmentally regulated change from a preferential binding of basic fibroblast growth factor (FGF) by embryonic day 9 neuroepithelial cells to binding of acidic FGF at embryonic day 11 is due to a concomitant switch in both the temporal expression of the different growth factors and the binding specificity of a heparan sulfate proteoglycan having a 45 kDa core protein (Nurcombe et al., 1993). This heparan sulfate proteoglycan colocalizes with basic FGF in embryonic mouse brain (Ford et al., 1994). Syndecan-3 from neonatal rat brain has also been shown to bind basic FGF (Chernousov and Carey, 1993) and the heparin binding growth-associated molecule (HB-GAM/pleiotrophin; Raulo et al., 1994), whereas there was no binding to a number of other potential growth factor and extracellular matrix ligands.

In a previous characterization of PC12 cell proteoglycans (Gowda et al., 1989), we demonstrated that these cells synthesize a single major heparan sulfate proteoglycan with an average molecular mass of 110 kDa (determined by gel filtration) and a core protein with an apparent molecular mass of 65 kDa, based on SDS-PAGE. This proteoglycan, which we can now tentatively identify as glypican, was released from the cell surface into the culture medium, and was accompanied by a very minor heparan sulfate proteoglycan of ~300 kDa. The strong expression of glypican in rat PC12 pheochromocytoma cells (Fig. 7), which are of adrenal medullary origin and undergo neuronal differentiation in response to nerve growth factor and other agents, suggests that this may be a useful model system for exploring the neurobiological functions of glypican. However, there was no obvious difference in glypican message levels between undifferentiated PC12 cells and process-bearing cells resulting from treatment with nerve growth factor (M. Flad and R.K. Margolis, unpublished results).

We earlier called attention to the 23% identity and 47% similarity of glypican and the 67 kDa protein coded by the OCI-5 cDNA clone (Karthikeyan et al., 1992), which corresponds to a developmentally regulated transcript in rat intestine (Filmus et al., 1988). The OCI-5 protein, which contains a spacing of cysteine residues and potential glycosaminoglycan attachment sites very similar to those in glypican, may be involved in cytoskeletal organization or in cell attachment. Evidence has also been presented indicating that heparan sulfate proteoglycans are involved in the guidance of a subset of pioneer axons in cultured cockroach embryos (Wang and Denburg, 1992). Further study will obviously be required to evaluate the possible role of glypican in specific neurobiological processes such as interactions of neurons with glia or extracellular matrix molecules, and in mediating the effects of growth factors.

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Note added in proof

After submission of this manuscript, Litwack et al. (1994) reported in situ hybridization studies of glypican expression in adult rat nervous tissue, which gave results generally similar to ours.