The amino acid sequence of rat N-syndecan core protein was deduced from the cloned cDNA sequence. The sequence predicts a core protein of 442 amino acids with six structural domains: an NH$_2$-terminal signal peptide, a membrane distal glycosaminoglycan attachment domain, a mucin homology domain, a membrane proximal glycosaminoglycan attachment domain, a single transmembrane domain, and a noncatalytic COOH-terminal cytoplasmic domain. Transfection of human 293 cells resulted in the expression of N-syndecan that was modified by heparan sulfate chain addition. Heparitinase digestion of the expressed proteoglycan produced a core protein that migrated on SDS-polyacrylamide gels at an apparent molecular weight of 120,000, identical to N-syndecan synthesized by neonatal rat brain or Schwann cells. Rat genomic DNA coding for N-syndecan was isolated by hybridization screening. The rat N-syndecan gene is comprised of five exons. Each exon corresponds to a specific core protein structural domain, with the exception of the fifth exon, which contains the coding information for both the transmembrane and cytoplasmic domains as well as the 3′-untranslated region of the mRNA. The first intron is large, with a length of 22 kilobases. The expression of N-syndecan was investigated in late embryonic, neonatal, and adult rats by immunoblotting and Northern blotting analysis. Among the tissues and developmental stages studied, high levels of N-syndecan expression were restricted to the early postnatal nervous system. N-syndecan was expressed in all regions of the nervous system, including cortex, midbrain, spinal cord, and peripheral nerve. Immunohistochemical staining revealed high levels of N-syndecan expression in all brain regions and fiber tract areas.

The syndecans are a gene family of transmembrane cell surface proteoglycans (reviewed in Refs. 1 and 2). Syndecans play an important role in tissue morphogenesis and differentiation by virtue of their ability to bind a number of extracellular adhesive proteins and growth factors. Four different syndecan core proteins that are the products of different genes are synthesized by mammalian cells. Syndecan synthesis is highly regulated (3–7) and is dependent on both cell type and developmental state (8). Syndecan core proteins are characterized by highly conserved transmembrane and noncatalytic COOH-terminal cytoplasmic domains but structurally distinct extracellular domains. This modular structural design suggests that individual mammalian syndecans have evolved to carry out specific functions within the tissues where they are expressed, probably related to the binding of specific extracellular ligands, but that these may be linked to common intracellular activities.

We reported the cloning of a partial cDNA sequence from neonatal rat Schwann cells that coded for the syndecan core protein, which we called N-syndecan (9). This proteoglycan is also expressed in the central nervous system. A highly homologous cDNA was cloned from chick embryo limb buds (10, 11). The chick proteoglycan, which appears to be the homologue of rat N-syndecan, was named syndecan-3.

N-syndecan purified from neonatal rat brain has been used to study the ligand binding properties of the proteoglycan. In contrast to what has been reported for syndecan-1, brain N-syndecan binds poorly in solid-phase assays to most extracellular matrix proteins, including fibronectin, laminin, and collagen I, III, IV, and V. The proteoglycan does bind with high affinity ($K_D = 5 \times 10^{-12}$ M) to basic fibroblast growth factor (bFGF)$^1$(12). N-syndecan has been proposed to be an endogenous heparin-like cofactor for bFGF in the developing brain. bFGF has been postulated to have a number of effects during development of the central nervous system, e.g. regulation of oligodendrocyte terminal differentiation (13, 14). N-syndecan also binds to heparin binding growth-associated molecule (HB-GAM)$^1$(15), an extracellular matrix-associated adhesive protein that is expressed in the developing brain (16), and may function as a receptor for HB-GAM in neonatal brain tissue. Recently, we reported the purification of a novel, peripheral nerve-specific, extracellular matrix protein, p200, that binds N-syndecan (17). p200 is expressed in peripheral nerves during early postnatal development. Purified p200 promotes adhesion and spreading of Schwann cells.

A determination of the biological function of N-syndecan would be facilitated by knowledge of the primary structure of the core protein and its expression in recombinant form, as well as information on the patterns of expression of N-syndecan in vivo. In this study, we report the complete amino acid sequence and expression of rat N-syndecan, the cloning and characterization of the rat N-syndecan gene, and an analysis of the tissue- and development-specific expression of N-syndecan.

**MATERIALS AND METHODS**

cDNA Cloning—The cloning and sequencing of a partial cDNA coding for rat N-syndecan was reported previously (9). This cDNA contained the coding sequence for the transmembrane and cytoplasmic...
domains of the core protein and a portion of the ectodomain but lacked a translational start site and signal peptide. Additional 5’ cDNA se-
quen ce was obtained by sequence-specific reverse transcriptase cata-
lyzed primer extension using total RNA isolated from 2-day-old rat
brain as template. RNA was isolated by using UltraSpect® RNAzol (Bio-
tec Laboratories, TX) as described previously (9). Antisense
primers based on the known N-syndecan cDNA sequence were used as
primers for cDNA synthesis by avian myeloblastosis virus reverse trans-
scriptase (Promega Corp.). After tailing the 3’ end of the resulting
cDNA products with terminal deoxynucleotidyl transferase and dCTP,
the specific products were amplified by two rounds of PCR using oli-
go-guanosine as the sense primer and the nested sequence-specific
antisense primers. The PCR products were gel purified and ligated into
plasmid pCRII (Invitrogen). Plasmids were isolated after transformation
into bacteria. DNA sequences were analyzed using the dye termi-
nator cycle sequencing method (Perkin Elmer) and an Applied Biosys-
tems 373A DNA sequencer.

Isolation and Characterization of N-syndecan Genomic DNA—A rat
Schwann cell N-syndecan cDNA probe was used to screen an EMBL3
rat liver genomic library (Clonetech). A phage clone was isolated (JJH1)
that by DNA sequence analysis was found to contain the coding infor-
mation for exons 3, 4, and 5 and part of intron II. Rescreening the
library with JJH1 as a probe resulted in the isolation of an overlapping
genomic clone (NG11) that extended the sequence up-
stream by approximately 10 kb but did not contain exon 1. Another
round of screening with NG11 as a probe resulted in the isolation of an
overlapping genomic clone (GNG26) that extended the
sequence upstream by approximately 8 kb but did not contain exon 1.
Repeated screening of the EMBL3 library with GNG26 did not yield
clon es that hybridized to cDNA sequence corresponding to exon 1.
The remaining sequence was obtained by screening a Lambda FIX II rat
kidney genomic library (Stratagene) with a 9-kb SauI restriction frag-
ment from the 5’ end of clone GNG26. This yielded clone GNG31 that
overlapped clone GNG26 at its 3’ end and extended upstream to contain
exon 1. The N-syndecan genomic clones were mapped by restriction
enzyme digestion and Southern hybridization using defined segments
of the N-syndecan cDNA as probes. Restriction fragments of interest
that included all exon sequences and intron/exon splice junctions were
subcloned into pGEMTZ (Promega) for DNA sequence analysis.

cDNA Expression—Attempts to isolate cDNAs containing the
entire protein coding sequence or to generate such cDNAs by reverse trans-
scriptase-linked PCR amplification with rat brain or Schwann cell RNA
as template or by overlapping PCR with the corresponding partial
cDNAs as templates were all unsuccessful. This may be due to the
excessively high G+ C content of the the 5’ end portion of the N-syndecan
cDNA sequence (see “Results”). An expression construct that contained cDNA coding
information for the mature N-syndecan core protein was constructed by
ligating a cDNA containing the coding sequence for the mature
N-syndecan core protein to the rat syndecan-1 signal peptide (6). This
cDNA was subcloned into an expression plasmid that uses the consti-
tutively active cytomegalovirus promoter and was used to transfect
human fibrosarcoma cells as described previously (18). Expression of N-syndecan
was detected by immunoblot analysis, using affinity-purified polyclonal
anti-rat N-syndecan antibodies, as described previously (9).

In Vivo Expression of N-syndecan—The panel of tissues indicated in
“Results” were excised from day 18 rat embryos, postnatal day 2, and
adult (more than 3 months of age) rats. Brain cortex and spinal cord
tissues were also obtained from rats ranging in age from embryonic
day 15 to adult. The tissues were homogenized in ice-cold phosphate-buff-
ered saline (PBS, 0.05 M sodium phosphate, 0.15 M sodium chloride, pH
7.5) plus 0.5 mM 2-mercaptoethanol and 25 μM 2-mercaptoethanol. The
homogenates were centrifuged at 17,000 x g for 45 min.
The PBS-soluble material was removed, and the resulting pellets
were extracted with 1% Triton X-100 in PBS and centrifuged as above.
The Triton X-100 extracted material was removed, and the pellets were
dissolved in electrophoretic sample buffer with 2% SDS. Aliquots of the
extracts were subjected to SDS gel electrophoresis on polyacrylamide
gels and electrophoretically transferred (4–6 h, 70 V) to Immobilon P
membranes (Millipore, Bedford, MA). The blots were stained with
affinity-purified anti-N-syndecan antibodies. Bound antibodies were de-
tected by enhanced chemiluminescence (Amersham Life Science, Inc.).
In some experiments, N-syndecan was treated with heparitinase
(Seikagaku America, Rockville, MD) as described previously (9, 12).
Preparation and characterization of affinity-purified antibodies di-
rected against bacterially expressed N-syndecan have been described
(9). Anti-peptide antibodies directed against a synthetic peptide corre-
sponding to the COOH-terminal seven amino acids (KQEEFYA) of the
N-syndecan core protein cytoplasmic domain were also prepared. The
peptide was covalently coupled to keyhole limpet hemocyanin (Pierce)
and injected into rabbits along with synthetic adjuvant (RIBI Immuno-
chemicals, Hamilton, MT). The antibodies were affinity-purified on a
column containing immobilized bacterially expressed core protein as
described previously (9).

Northern Blot Analysis—Total RNA was isolated from rat tissues
using UltraSpect® RNAzol, fractionated on 1.5% agarose-formaldehyde
gels (20 μg/ lane), transferred to nylon membranes (Schleicher and
Schuell), and immobilized by UV cross-linking. The membranes were
hybridized to a 1.6-kb 32P-labeled N-syndecan cDNA probe from the
3’ untranslated region. After hybridization, the membranes were
washed twice with 0.1 X SSC, 0.1% SDS at 65 °C. Hybridization signals
were visualized by autoradiography using DuPont Reflection film with in-
tensifying screens at ~ 70 °C. Hybridization signals were quantitated by
scanning the autoradiograms with a Molecular Dynamics laser densi-
tometer and normalized to the quantity of 28 S rRNA loaded on the gel,
determined by scanning photographs of ethidium bromide-stained gels.

Immunohistochemistry of Parafilm-embedded Brain Tissue—
Paraformaldehyde-fixed and paraffin-embedded brain and spinal cord
tissue obtained from postnatal rats were immunostained as described
previously (19). Following dewaxing, the sections were immersed in
0.3% H2O2 to block endogenous peroxidase activity and then treated
with 1 mg/ml hyaluronidase (Sigma) for 30 min at room temperature.
The tissues were blocked by incubation for 1 h with 10% goat serum and then incubated overnight with affinity-purified anti-N-syndecan
antibodies. After rinsing, the bound antibodies were detected by the
perox-
diase-antiperoxidase method using 3,3’-diaminobenzidine (Sigma) as
substrate.

RESULTS

Cloning and Sequence Analysis of Rat N-syndecan cDNA—
Previously, we reported the cloning of a partial rat N-syndecan
cDNA that was truncated at the 5’ end. Additional cDNA sequence
was obtained by carrying out primer extension and PCR amplification
as described under “Materials and Methods.” The resulting product extended the cDNA sequence by
approximately 380 bp in the 5’ direction and provided the
remaining protein coding sequence. The cDNA and deduced
core protein sequences are shown in Fig. 1.

The deduced amino acid sequence predicts a polypeptide of
442 amino acids. The proposed initiation methionine is pre-
ceded by a purine (G) at position –3 and is, thus, in a “strong”
initiation context (20). Several important structural features can be predicted by perusal of the deduced amino acid se-
quence. The linear sequence can be divided into six structural
domains (Fig. 2A). The sequence immediately downstream of the
initiation codon contains an uninterrupted stretch of hy-
drophobic amino acids. This is consistent with the membrane
topology of syndecans (type I transmembrane proteins) and
identifies this region as a putative signal peptide. Applying
proposed rules for predicting signal peptide cleavage sites (21),
the cleavage is predicted to occur on the COOH-terminal side of
either Ala-39 or Gly-44. The latter site would place the cleavage
precisely at the end of exon 1 (see below). The nucleotide
sequence of the 5’-untranslated region and putative signal
peptide is extremely rich in G and C residues (79% of the total
nucleotides). There are strings of uninterrupted G or C se-
quen ces of 22, 19, and 18 bases, plus one in which 19 of 20 bases
are G or C. This region is expected to form rather stable
secondary structures, which may account for the difficulty in
isolating this part of the cDNA (9).

The potential glycosaminoglycan acceptor sites, identified by
the consensus sequence Ser-Gly, occur in two clusters. One
cluster is in a region of approximately 50 amino acids imme-
diately following the signal peptide that contains the reported
syndecan glycosaminoglycan attachment sequences, including three in
tandem. The second cluster of three potential glycosaminogly-
can attachment sites is in a domain of approximately 90 amino
acids located adjacent to the transmembrane domain.

The membrane distal and membrane proximal glycosaminoglycan
attachment domains are separated by a domain of approxi-
amino acids that is rich in proline and threonine residues and shows significant sequence homology to mucin-like proteins. These domains, which comprise the core protein ectodomain, are followed by a stretch of 24 hydrophobic residues that constitutes the transmembrane domain and a COOH-terminal domain of 34 amino acids that form the noncatalytic cytoplasmic domain. The structural features of the latter two domains, including their high degree of amino acid sequence homology with other members of the syndecan family of core proteins, have been described previously (9, 18, 22).

**Structure of the Rat N-syndecan Gene**—Rat N-syndecan genomic sequences were isolated by hybridization screening of lambda phage rat genomic libraries as described under “Materials and Methods.” Four overlapping genomic clones that contained the coding information for N-syndecan were isolated. A composite map of the rat N-syndecan gene derived by analysis of these clones is shown in Fig. 2B. The gene consists of five exons and has a total length of approximately 30 kb. Each of the five exons corresponds to identifiable domains of the core protein and mRNA (Fig. 2C). Exon 1 encodes the 5'-untranslated mRNA sequence plus the protein signal peptide; exon 2 encodes the membrane distal glycosaminoglycan attachment domain; exon 3 encodes the proline- and threonine-rich spacer domain with homology to mucin-like sequences; exon 4 encodes the membrane proximal glycosaminoglycan attachment domain; and exon 5 encodes the transmembrane and cytoplasmic domains of the polypeptide plus the 3'-untranslated region of the mRNA. The first intron is rather large, with an estimated length of 22 kb. The other introns range in length from 0.6 to 2.5 kb. The sequences at the exon splice sites are typical of what has been found in other mammalian genes and are shown in Fig. 3. With respect to overall organization and exon and domain structure, the rat N-syndecan gene shows striking similarity to the mouse syndecan-1 gene (23, 24).

**Expression of N-syndecan cDNA**—The N-syndecan expression vector described under “Materials and Methods” was used to transfect human 293 cells, which lack endogenous immunoreactive N-syndecan. Immunoblot analysis of transfected cultures with affinity-purified anti-N-syndecan antibodies revealed the synthesis of a high molecular weight immunoreactive smear, which is characteristic of proteoglycans, that comigrated with native N-syndecan synthesized by rat Schwann cells or rat brain (Fig. 4). After digestion with heparitinase, the immunoreactive protein produced by the transfected cells was shifted to an apparent molecular weight of approximately 120,000. The heparitinase-digested product of
the transfected cells comigrated with heparitinase-digested N-syndecan extracted from neonatal rat brain or Schwann cells (Fig. 4). These results demonstrate that the cloned cDNA contains the information necessary to encode mature N-syndecan core protein that is modified by addition of heparan sulfate chains in a manner similar to the native proteoglycan.

![Fig. 3. Intr/exon splice site sequences in the rat N-syndecan gene.](image)

The coding strand genomic sequence is shown along with the corresponding amino acid sequence. The lengths of the four introns are indicated. Critical splice donor and acceptor sequences are indicated in boldface.

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**N-Syndecan Expression and Genomic Organization**

| Exon 1 | Exon 2 |
|--------|--------|
| GCC GCC GIG GIGA | TCTGTTACCCCTCCCAG GCT GAA |
| Ala Ala Gly | Intron 1 22 kb | Ala Gln |

| Exon 2 | Exon 3 |
|--------|--------|
| TCT GCT G | TCTAA | TCCCACCCCCCAG AC TGC GAG |
| Ser Gly Tyr | Intron II 1.4 kb | Phe Glu |

| Exon 3 | Exon 4 |
|--------|--------|
| GPG GCT G | GATAA | CCCAACCCTCCAG ACC CCA ATC |
| Val Ala Gln | Intron III 2.5 kb | Thr Pro Thr |

| Exon 4 | Exon 5 |
|--------|--------|
| CPC GTA G | GATAA | TGTCCTTTCTCCCCAG CT CTC ATC |
| Leu Val Ala | Intron IV 0.6 kb | Val Ile |

FIG. 3. Intr/exon splice site sequences in the rat N-syndecan gene. The coding strand genomic sequence is shown along with the corresponding amino acid sequence. The lengths of the four introns are indicated. Critical splice donor and acceptor sequences are indicated in boldface.

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**Fig. 4. Immunoblot analysis of rat N-syndecan expressed by cDNA transfection of cultured cells.** Human 293 cells were stably transfected with with rat N-syndecan cDNA expression vector (lanes 1 and 2); N-syndecan was detected by immunoblot analysis on 6% SDS-polyacrylamide gels and stained with affinity-purified anti-rat N-syndecan antibodies. Extracts of newborn rat brain (lanes 3 and 4) and rat Schwann cells (lanes 5 and 6) were also analyzed. Some samples were incubated with heparitinase (+) before immunoblot analysis. Numbers to the right indicate position of migration of molecular weight markers (in thousands).

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**Fig. 5. Expression of N-syndecan in rat tissues.** A, extracts of the indicated tissues were prepared from 4-day-old rats and subjected to immunoblot analysis on a 7.5% SDS-polyacrylamide gel and detected with anti-N-syndecan antibodies as described under “Materials and Methods.” The amount of protein loaded per lane was 20 μg. Numbers to the left indicate the position of migration of molecular weight standards (in thousands). Sciatic n., sciatic nerve; sk. muscle, skeletal muscle. B, total RNA was isolated from the indicated tissues of 4-day-old rats and subjected to Northern blot analysis with an N-syndecan cDNA probe as described under “Materials and Methods.” The top panel shows the autoradiogram of the hybridization signals. N-syn, N-syndecan. The bottom panel shows the 28S rRNA bands after ethidium bromide staining. The arrow in the upper panel indicates the position of migration of the N-syndecan mRNA.

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The transfected cells comigrated with heparitinase-digested N-syndecan extracted from neonatal rat brain or Schwann cells (Fig. 4). These results demonstrate that the cloned cDNA contains the information necessary to encode mature N-syndecan core protein that is modified by addition of heparan sulfate chains in a manner similar to the native proteoglycan.

*N-Syndecan Expression in Vivo—Regulated expression of cell surface proteoglycans provides a mechanism for restricting the biological activity of these molecules (8). Knowledge of N-syndecan expression in vivo would provide important information related to its function. Immunoblot analysis with affinity-purified anti-N-syndecan antibodies was used to measure the steady-state levels of N-syndecan in a variety of tissues obtained from late embryonic, early postnatal, and adult rats. As shown in Fig. 5A, N-syndecan was detected in extracts of peripheral and central nervous system tissue of early postnatal rats. N-syndecan was found in all major subdivisions of the central nervous system, including cortex, midbrain, and spinal cord. N-syndecan was not detected by immunoblot analysis in the other neonatal rat tissues examined, including eye, tongue, lung, liver, kidney, stomach, and skeletal muscle (Fig. 5A), as well as spleen, heart, and ovary (data not shown). Immunoblot analysis failed to detect N-syndecan in extracts of adult rat tissues (data not shown), including brain and spinal cord (see below). N-syndecan was not detected in tissue extracts of late embryonic rats (data not shown), with the exception of the nervous system (see below).

The distribution of N-syndecan mRNA in rat tissues was determined by Northern blot analysis. The mRNA was detected in peripheral nerve, brain, and spinal cord of early postnatal rats but not in eye, tongue, lung, liver, or kidney (Fig. 5B). Thus, the distribution of N-syndecan mRNA correlated with the pattern of expression of the proteoglycan.

The developmental time course of N-syndecan expression was measured in rat brain and spinal cord tissue. As shown in Fig. 6A, N-syndecan was present at low levels in late embryonic (E18) rat brain and spinal cord extracts. N-syndecan levels increased during the early postnatal period and reached a maximum on postnatal day 7. Thereafter, the N-syndecan levels declined to very low levels in adult tissues. The temporal pattern of N-syndecan expression was reflected in the levels of N-syndecan mRNA in brain and spinal cord tissue, as measured by Northern blot analysis (Fig. 6B). The highest levels of expression were observed in the early postnatal period, reaching a maximum at 7 days postnatal, with very low levels of expression in late embryonic and adult tissue. An almost identical temporal pattern of expression in the brain of HB-GAM, an N-syndecan ligand, has been reported previously (16).
Evidence for Shedding in Vivo—The deduced protein sequence of N-syndecan predicts a transmembrane polypeptide. Studies with cultured cells have shown that soluble forms of syndecans can be generated by proteolytic cleavage of the ectodomains at a site very close to the membrane spanning domain, a process referred to as membrane shedding (8, 25). This generates products that contain all of the glycosaminoglycan chains but not the transmembrane or cytoplasmic domains and are only slightly smaller than the full-length proteoglycans. To assess the membrane association of N-syndecan in vivo, neonatal rat brain tissue was fractionated into soluble and particulate fractions by homogenization in PBS without detergent, followed by centrifugation. The particulate fraction was extracted with buffer containing 1% Triton X-100 (TX-100), and the Triton-insoluble material was solubilized in 2% SDS. Equivalent aliquots (by volume) of the PBS extract, Triton X-100 extract, and SDS soluble material were subjected to immunoblot analysis and stained with antibodies directed against the extracellular domain (Anti-ecto) of the N-syndecan core protein. After visualization of the bound antibodies by enhanced chemiluminescence, the membrane was stripped and restained with anti-peptide antibodies directed against the COOH-terminal cytoplasmic domain (Anti-cyto) of the core protein. Numbers to the left indicate the position of migration of molecular weight standards (in thousands).

FIG. 7. Differential extraction of N-syndecan from neonatal rat brain tissue. Brain tissue from 4-day-old rats was homogenized in PBS and centrifuged as described under “Materials and Methods.” The particulate fraction was extracted with 1% Triton X-100 (TX-100), and the resulting insoluble material was solubilized in 2% SDS. Equivalent aliquots (by volume) of the PBS extract, Triton X-100 extract, and SDS soluble material were subjected to immunoblot analysis and stained with antibodies directed against the extracellular domain (Anti-ecto) of the N-syndecan core protein. After visualization of the bound antibodies by enhanced chemiluminescence, the membrane was stripped and restained with anti-peptide antibodies directed against the COOH-terminal cytoplasmic domain (Anti-cyto) of the core protein. Numbers to the left indicate the position of migration of molecular weight standards (in thousands).

DISCUSSION

The sequence of the rat N-syndecan core protein deduced from the cDNA sequence reveals several features of the core protein structure that are similar to other syndecans, as well as some distinct features. These similarities and differences most likely reflect common functional roles, such as extracellular ligand binding, that are carried out within the context of cell type-specific differences in function. It is presumably the latter that dictates the need for multiple forms of syndecans in mammalian cells. Syndecans show the greatest amino acid sequence homology in the transmembrane and cytoplasmic domains. The details and possible functional consequences of the high degree of structural conservation in these domains have been dis-
cussed previously (9, 18, 22, 26). In contrast, there is a rela-
tively low level of sequence homology among the ectodomains. On the basis of sequence homology, the syndecans can be di-
vided into syndecan-1/N-syndecan and syndecan-2/syndecan-4 subfamilies. Although the amino acid sequences of the ectodo-
mainsofN-syndecanandsyndecan-1arenotstrikinglysimilar,
the two core proteins appear to reflect a common structural
organization, with membrane proximal and membrane distal
glycosaminoglycan attachment domains separated by a pro-
tile-rich spacer domain. This similarity in overall structural
organization is reflected in the exon structure of the corre-
sponding genes (23, 24). In both genes, each protein domain is
encoded by a separate exon, with the exception of exon 5, which
encodes both the transmembrane and cytoplasmic domains as
well as the relatively long 3′-untranslated sequences. Also con-
served is the unusual length of the first intron. These striking
similarities in gene organization and protein structure provide
strong support for the suggestion that the syndecans arose by
gene duplication during mammalian evolution.

The deduced amino acid sequence of N-syndecan predicts a
polypeptide with a relative molecular mass of approximately 50
kDa. This is considerably lower than the observed mass of
native heparitinase treated N-syndecan of 120 kDa. This dis-
crepancy could result from a combination of anomalous migra-
tion of the polypeptide on SDS-polyacrylamide gels, which is a
hallmark of syndecan core proteins, as well as the presence of
an unidentified posttranslational modification. Interpretation
of the migration on SDS gels is also complicated by the fact that
N-syndecan core protein forms SDS-resistant dimers and tet-
ramers (18). Deglycosylation of native N-syndecan with hep-
aritinase, nitrous acid, or trifluoromethanesulfonic acid all
yield a product with a relative molecular mass of approxi-
mately 120 kDa. Digestion with chondroitinase has no effect on
N-syndecan electrophoretic mobility (9, 12). The band that is
observed following digestion of N-syndecan with these reagents
is diffuse, however, suggesting the possible presence of addi-
tional modifications. The presence of the mucin homology do-
main in the N-syndecan core protein suggests the possibility of
mucin-type oligosaccharides, but no additional data to support
this conclusion have been obtained. Digestion of N-syndecan
with neuraminidase has no effect on the core protein migration
(data not shown). The cDNA sequence reported here contains
sufficient coding information to direct the synthesis of a heparan sulfate proteoglycan that is indistinguishable in overall size and sensitivity to heparitinase from native N-syndecan extracted from neonatal rat brain tissue or Schwann cells.

Immunoblot analysis of proteoglycan steady-state levels and Northern blot analysis of mRNA levels in rat tissues revealed that the highest levels of N-syndecan expression were restricted to cells of the central and peripheral nervous system of neonatal rats. These results do not rule out lower levels of synthesis by other tissues. We have found, for example, that rat heart and arterial tissue contain N-syndecan mRNA. In the heart, N-syndecan mRNA increases dramatically at birth and, in contrast to what was observed in the nervous system, persists in adult animals.\(^2\) In spite of this, however, we were unable to detect N-syndecan in heart tissue.

The cell types in the neonatal brain that are responsible for high levels of N-syndecan expression are not known. Temporally, the period of highest expression correlates well with the period of oligodendrocyte differentiation, which in rats begins at the time of birth and persists for the first three weeks of postnatal life (13). Interestingly, these cells express FGF receptors. Administration of exogenous bFGF in culture results in cell proliferation and inhibition of terminal differentiation (13). These effects are prevented when the cells are grown in the presence of sodium chloride, and the chloride inhibition can be reversed by exogenous heparin (14). Brain N-syndecan binds bFGF with high affinity (12) and is, thus, a good candidate for the endogenous heparan sulfate molecules that function as co-receptors for bFGF activation.

Additional functions for N-syndecan in the developing nervous system are suggested by other findings. Brain N-syndecan binds with high affinity (\(K_D = 5 \times 10^{-10}\) M) to HB-GAM (15), an 18-kDa secreted protein expressed in early postnatal brain and other tissues. Affinity chromatography experiments have shown that N-syndecan is the major protein in brain with HB-GAM binding activity. Although the exact function of HB-GAM is not known, it has been shown to have neurite outgrowth activity in vitro (27). The high affinity and selectivity of N-syndecan binding to HB-GAM and the striking correspondence of their temporal patterns of expression in brain strongly suggest that these two proteins together carry out an important adhesion-dependent activity during early postnatal development of the brain.

Another interesting question is the physiological function of membrane shedding of N-syndecan. Evidence was presented for a large amount of N-syndecan in brain tissue that was not attached to membranes, consistent with the shedding of syndecans that has been observed with cultured cells. Membrane shedding would provide a mechanism for terminating functional activity that is dependent on attachment to the plasma membrane. This could include cell-cell adhesion activity or binding events that were dependent on cytoskeletal attachment, or generated intracellular signals through the cytoplasmic domain. There is evidence that syndecan cytoplasmic domains can interact with actin filaments (28, 29), and syndecan expression has been shown to alter the morphology and cytoskeletal organization of epithelial cells (30) and Schwann cells (31).

N-syndecan that is released by shedding could also become incorporated into the brain extracellular matrix. Although biochemically and ultrastructurally distinct from collagen-rich fibrous extracellular matrices present in peripheral tissue, there is increasing evidence for the existence of an extracellular matrix in the brain. In adult brain tissue, a prominent component of this matrix appears to be large chondroitin sulfate proteoglycans (32). Association with an extracellular matrix is suggested by the identification of a PBS- and Triton X-100-insoluble pool of N-syndecan in brain tissue. Matrix-associated N-syndecan could provide sites for cell adhesion or pathways for cell migration for cells that express N-syndecan binding proteins on their surface. Alternatively, it could provide a reservoir of heparin-binding growth factors.

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\(^2\) V. K. Asundi, B. F. Keister, R. C. Stahl, and D. J. Cavey, submitted for publication.