**Isolation of a Neuronal Cell Surface Receptor of Heparin Binding Growth-associated Molecule (HB-GAM)**

**IDENTIFICATION AS N-SYNDECAN (SYNDECAN-3)**

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HB-GAM (heparin binding growth-associated molecule; pleiotrophin) is a secretory, extracellular matrix-associated protein that is strongly expressed in developing nervous tissues and belongs to a novel family of differentiation/growth factors. It promotes axonal growth from perinatal rat brain neurons and is suggested to be mitogenic for some cell types and to display cell-transforming activity. Since the receptors of HB-GAM in cells are unknown, we have started isolation of putative cell surface receptors from brain neurons and from perinatal rat brain. For this purpose, recombinant HB-GAM was produced with the aid of a baculovirus vector and used as an affinity matrix in receptor isolation. A detergent-solubilized component from cultured brain neurons and from brain was identified that binds specifically to HB-GAM and migrates on sodium dodecyl sulfate-polyacrylamide gel electrophoresis as a broad smear with an apparent molecular mass of about 200 kDa. This cell surface component was found to contain heparan sulfate chains, which are bound to a core protein with an apparent molecular mass of 120 kDa. Gel electrophoretic characteristics, immunochemical analysis, and partial peptide sequencing revealed that the cell surface component isolated as an HB-GAM receptor is N-syndecan (syndecan-3). In a solid phase binding assay, N-syndecan was found to bind to HB-GAM in a similar manner as to basic fibroblast growth factor (Kₚ = 0.6 nM).

Immunofluorescence microscopy indicated that in brain neurons, N-syndecan occurs at the surface of the cell soma and of the neurites that grow along HB-GAM-coated substrates. Anti-N-syndecan antibodies added to culture media had an inhibitory effect on HB-GAM-induced neurite outgrowth. We suggest that N-syndecan mediates the neurite outgrowth-promoting signal from HB-GAM to the cytoskeleton of growing neurites.

HB-GAM§ (p18) was recently isolated from brain as a neurite outgrowth-promoting protein, the expression of which in tissue peaks during the rapid perinatal development of rat brain (1). The same protein was also isolated from uterus as a mitogen for NIH 3T3 cells (2). Molecular cloning of full-length cDNAs encoding HB-GAM (3) and the corresponding mitogenic protein, designated as pleiotrophin (4), has revealed the same sequence, confirming that the proteins defined as HB-GAM and pleiotrophin are identical. The HB-GAM sequence has been recently also found in a number of other studies in which proteins involved in cell and tissue development have been searched (5–8).

The amino acid sequence of HB-GAM is more than 90% conserved from man to chicken (3, 4, 6, 8), and it is about 50% homologous with the sequence of the mouse MK (mid-gestation kidney protein) (9–10) and of the MK-type chicken protein (RHBB, retinoic acid-inducible heparin-binding protein) (11), which are suggested to mediate retinoic acid-induced cell differentiation. These proteins thus form a novel family of heparin binding factors that play a role in cell and tissue development.

Neurite outgrowth-promoting activity in vitro has been demonstrated for the HB-GAM and MK proteins in a number of studies (1, 4–6, 8). This effect appears to be due to substrate-bound proteins (1, 12, 13). Correspondingly, HB-GAM forms extracellular tracts along growing neurites in tissue, suggesting that it functions as a cell matrix-associated protein that plays a role in the formation of neuronal connections (14). Variable results have been reported regarding the mitogenic activities of the protein family (2, 4, 6, 8, 12, 13), which may be explained by a recent finding that only very specific cell types at a certain stage of development respond by cell proliferation to the MK/HB-GAM-type proteins (13). Interestingly, a cell-transforming activity has been recently demonstrated for HB-GAM (15–17).

The types of interactions with cells that have been demonstrated for HB-GAM and other members of the protein family suggest interactions with cell surfaces. A heparin-inhibitable binding of HB-GAM to a 140-kDa cell surface component of NIH 3T3 cells has been shown (18), and heparitinase treatment of brain neurons has been shown to strongly reduce HB-GAM-induced neurite outgrowth (14). However, cell surface receptors of HB-GAM have not been defined. We have, therefore, started purification of putative HB-GAM receptors. Since the neurite outgrowth-promoting property is the characteristic feature of HB-GAM and other members of the protein family, and HB-GAM is strongly expressed in perinatal rat brain (1, 4), the purification has been started in the first place from early postnatal rat brain and from brain neurons.

We have previously expressed the HB-GAM cDNA with the aid of a baculovirus vector and demonstrated that the recombinant HB-GAM interacts with cells in the same manner as the protein isolated from tissue (12). In the present study, we have produced and purified the baculovirus-derived HB-GAM in high amounts and used the recombinant protein as an affinity matrix in receptor isolation. These experiments indicate a distinct isolation of a neuronal cell surface component, the core...
protein of which has an apparent molecular mass of 120 kDa.

The protein is identified as N-syndecan (19), which is suggested to occur at the surface of growing neurites and to mediate their binding to HB-GAM.

EXPERIMENTAL PROCEDURES

Cells and Cell Culture—Brain cells were dispersed from cerebral hemispheres of 17-day-old rat embryos as described previously (20). These cells have been previously characterized and shown to be mainly (80-90%) neurons (20). Neurite outgrowth assays on HB-GAM-coated substrates were carried out with freshly prepared neuron preparations in a serum-free DMEM medium containing 10 mg/ml bovine serum albumin as described previously (1). Neurite outgrowth in the assays was evaluated by phase contrast microscopy and by staining the cells with anti-neurofilament antibodies (12). Antibodies tested in the neurite outgrowth assays were diazylated against the DMSO-BSA medium.

Cell surface labeling of the neuron preparations was carried out with lactoperoxidase-catalyzed iodination as described previously (21). The cells were metabolically labeled with 50 μCi/ml 125I (Amersham Corp.) for 18 h in the complete serum-containing medium (20).

Receptor Isolation—Recombinant HB-GAM was produced with the aid of an exchange chromatography on a Glazen tip-5 column from the culture medium of the SF9 cells as described previously (12). The HB-GAM affinity column was prepared by coupling 5 mg of the recombinant protein to a 1-ml NHS-activated Hi-TRAP column (Pharmacia, Sweden) according to the manufacturer’s instructions. A control column was prepared in the same way using 5 mg of a boiled column matrix in another lysine-rich heparin-binding protein that was also produced with the baculovirus vector and purified as described previously (22). A matrix blank column was prepared as the affinity columns, but no protein was added during the coupling reaction.

To analyze HB-GAM binding components from cell surface-labeled or metabolically labeled neurons (see above), the cells were extracted with 50 mM octyl glucoside in ice-cold PBS containing 1 mM phenylmethylsulfonyl fluoride and 2 mM sodium azide. The homogenized tissue was centrifuged at 40,000 x g for 0.5 h, and the supernatant was passed through the column that had been equilibrated with 50 mM octyl glucoside. The columns were then washed with 20 column volumes of the extraction buffer and eluted with a linear gradient of NaCl from 150 to 600 mM in the extraction buffer. Fractions of 1 ml were collected and analyzed on SDS-PAGE. The components that bound specifically to HB-GAM and were eluted at about 0.5 x NaCl were pooled according to the SDS-PAGE analysis and recycled through the affinity columns. Further purification of the specific binding component was carried out on a Glazen tip-5 column (Glazen, Germany). The column was equilibrated with 2 ml of PBS containing 50 mM octyl glucoside. The fraction that binds specifically to the HB-GAM affinity column was diluted 10-fold with the equilibration buffer and passed through the column. The column was washed with 2 ml of the equilibration buffer, and the HB-GAM binding component was then eluted with 400 μl of 0.5 x NaCl in PBS containing 50 mM octyl glucoside. The first 200 μl were discarded, and the column was then blown dry with a syringe to collect the putative receptor.

Characterization of the HB-GAM Receptor—Sulfate-labeled fractions from the HB-GAM affinity columns were subjected to gel electrophoresis and autoradiography. Brilliant Blue intensification by silver staining (Bio-Rad). The apparent molecular mass of the core protein of the proteoglycan receptor was estimated after heparitinase digestion and after nitrous acid depolymerization (23).

For Western blotting experiments, the samples were run on 4-15% SDS-PAGE and transferred on nitrocellulose (Amersham) with Tank binding protein (Millipore) in 10 mM CAPS, pH 9.0, for 2 h at 70 V. Binding of different antibodies to the filters was assayed as described previously (1) using alkaline phosphatase-conjugated secondary antibodies for the detection. Affinity-purified polyclonal antibodies against a recombinant core protein structure of N-syndecan were tested as described previously (18). Monoclonal anti-HNK-1 antibodies and anti-CD 44 antibodies (Hermes 3 and 4H1) for the assays were kindly provided by Dr. Froize B. Jungalwala (Eunice Kennedy Shriver Center for Mental Retardation, Waitham, MA) and by Dr. Sirpa Jalkanen (University of Turku, Finland), respectively. Commercial anti-human CD57/HNK-1 antibodies (clone VCI.1; Sigma) and anti-human CD44 antibodies (clone A3DS; Sigma) were also tested in the assays according to the manufacturer’s instructions. Tryptic peptides were prepared from the putative HB-GAM receptor by using sequencing grade trypsin (Boehringer Mannheim) at the approximate enzyme-substrate ratio of 1:50 as recommended by the manufacturer. The peptides were separated by HPLC on a Vydac C18 column that was eluted with a linear gradient of 0-60% acetonitrile in 0.1% trifluoroacetic acid. Amino acid sequencing of the peptides was performed by automated Edman degradation with Applied Biosystems sequencer 477A/120A in the gas phase mode as described previously (25).

Assay—N-Syndecan was purified by immunoaffinity chromatography from neonatal rat brains (27), radioiodinated, and used for solid phase binding assays. The binding assays were carried out as described previously (27) except that the proteins were diluted in water instead of carbonate buffer prior to coating the wells.

To study HB-GAM binding to the cell surface, neuro-enriched cells were prepared from perinatal rat brain (see above) and allowed to adhere to the wells that had been coated with poly-l-lysine followed by 10% fetal calf serum. The cells were kept on the wells for 3 days, during which time the neurons extended extensively neurites in essentially confluent cultures. The cells were then washed with DMEM containing 10 mM octyl glucoside and cooled to 0 °C before the binding assays were performed.

Recombinant HB-GAM (20 μg) was iodinated using the chloramine-T method as described previously (14). The labeled protein was then purified by heparin-Sepharose chromatography as described previously (12), followed by gel filtration on Sephadex G-25. SDS-PAGE followed by autoradiography revealed a 15-kDa band that migrated in the gel electrophoresis as the unlabeled protein. The specific radioactivity of 125I-labeled HB-GAM was 3.5 x 10⁶ cpm/μg. Binding of 125I-labeled HB-GAM to the cells was assayed by incubating the monolayers at different concentrations of the probe for 3 h on ice bath in PBS containing 0.1 mg/ml Carb, 0.1 mg/ml BSA, and 10 mg/ml NaOH for counting of the cell-bound radioactivity. Specific binding of 125I-labeled HB-GAM to the cells was calculated by subtracting the radioactivity due to nonspecific binding of the probe from the total bound radioactivity. Nonspecific binding of 125I-labeled HB-GAM to the cells was determined in the presence of 100-fold excess of unlabeled HB-GAM and was about 40% of the total bound radioactivity in different experiments. Binding assays using HB-GAM iodinated in the presence of Iodobead (Pierce Eurochemie, Od Beijerland, Netherlands) gave similar results as compared with the binding assays in which the probe was prepared with the chloramine-T method. In some experiments, soluble heparin (grade I, Sigma) was added in the assay medium.

Immunofluorescence Microscopy—Brain neurons cultured for 20 h on HB-GAM-coated substrates were studied by indirect immunofluorescence microscopy essentially as described previously (1). The cells were fixed with 4% paraformaldehyde, 0.05% glutaraldehyde, and the background due to excess aldehydes was quenched by 0.12% glycine in PBS containing 10 mg/ml of BSA. In some experiments, the fixed cells were permeabilized with 50% methanol. Binding of the primary antibodies to the cells was detected with rhodamine-conjugated, affinity-purified anti-rabbit IgG (Cappel).

RESULTS AND DISCUSSION

Identification of Neuronal Cell Surface Components That Bind to HB-GAM—To identify cell surface binding sites of HB-GAM, cells dispersed from embryonic rat brain were surface-labeled with lactoperoxidase-catalyzed iodination and extracted in octyl glucoside, and the extracts were passed through

13000 HB-GAM Receptor
HB-GAM affinity columns. The affinity columns were eluted with salt gradients in the presence of detergent, and the eluted fractions were analyzed with SDS-PAGE and autoradiography. Several weakly labeled bands (not shown) were eluted at 0.2–0.3 M NaCl, after which the major surface-labeled component was eluted at about 0.5 M NaCl in 50 mM octyl glucoside (Fig. 1, lane 1). This component migrates as a broad smear at the top of 5–20% gradient gels (Fig. 1, lane 1). No binding of this component was observed to blank columns analyzed in parallel with the HB-GAM affinity columns. Affinity columns were also prepared from recombinant amphoterin (22), which resembles HB-GAM in its binding to heparin and in its high content of lysine residues. Amphoterin affinity columns, however, did not bind the high molecular weight material that binds to HB-GAM. Further elution of the HB-GAM affinity columns with high salt (up to 2 M NaCl) or with heparin (1 mg/ml) did not reveal additional cell surface-labeled components that bind to HB-GAM. These experiments suggested that the high molecular weight component identified binds specifically to HB-GAM.

The strong binding of HB-GAM to heparin (1) and the gel-electrophoretic behavior of the cell surface component that binds to HB-GAM suggest a proteoglycan nature of the putative HB-GAM receptor. We therefore labeled metabolically primary neuron cultures with 35SO4. Extraction and analysis of HB-GAM binding components from metabolically labeled cells also revealed a component that is detached from HB-GAM at about 0.5 M NaCl and gives a smear at the top of 5–20% gels (Fig. 1, lane 2). This experiment suggests that brain neurons synthesize the putative receptor in primary cultures in which neurite extension is rapid at the stage of metabolic labeling. When the labeled fractions were digested with heparitinase, the sulfate label was shifted nearly quantitatively from the top to the front of the gels (Fig. 1, lane 3). No change was observed in the label by treating the fractions with N-glycosidase F (Fig. 1, lane 4) or with heparinases I and II and with chondroitinase ABC (not shown). These experiments indicate that the putative receptor contains heparan sulfate chains.

Interestingly, HB-GAM-induced neurite outgrowth is strongly inhibited by treating the brain neurons with the heparitinase, which hydrolyzes the HB-GAM binding component identified, but not by treating with the heparinases I and II (14), which do not hydrolyze the HB-GAM binding component. This suggests that the HB-GAM binding cell surface component identified has a role in HB-GAM-induced neurite outgrowth.

Isolation of the Putative HB-GAM Receptor from Brain—To get higher amounts of the putative HB-GAM receptor, the isolations were also carried out from early postnatal rat brain, when the HB-GAM expression is very high in tissue (1). Coomassie Blue staining of the gels did not reveal any HB-GAM binding components eluting above 0.3 M NaCl in detergent. However, staining of the gels with alcian blue (to visualize proteoglycans) or by alcian blue followed by silver staining (to visualize proteoglycans and proteins) clearly detected a component that bound to HB-GAM as the component from labeled cells and was detected in the same way as a smear on the top of 5–20% gels (Fig. 1, lane 5). In addition to this component, a low molecular weight component was detected in the same fractions just above the dye front of the gels (Fig. 1, lane 5). However, this component was not detected in surface-labeled brain cells (Fig. 1, lane 1) and was not, therefore, further characterized.

The fractions eluting at about 0.5 M NaCl were pooled according to the gel staining data and concentrated by recycling through the HB-GAM affinity column, which was eluted at one step with 2 M NaCl in 50 mM octyl glucoside. Analysis of the isolated material on 5–20% and 4–15% gradient gels stained with alcian blue followed by silver staining suggested that a highly purified receptor was obtained (Fig. 2, lane 3). Heparitiniase digestion, as well as nitrous acid cleavage, revealed an apparent core protein mass of 120 kDa (Fig. 2, lanes 2 and 4). About 10 μg of the putative receptor protein was obtained per 1 g of wet tissue, which is compatible with the high expression level of HB-GAM in early postnatal rat brain (1).

It is noteworthy that the HB-GAM affinity column isolates the putative receptor in a highly selective manner both from crude extracts of labeled cells and of brain. This indicates that
HB-GAM, despite of its polycationic nature, interacts with the neuron surface in a very specific manner. The HB-GAM-neuron interactions cannot be, therefore, interpreted to be due to non-specific polycationic adhesion but most likely reflect binding of HB-GAM to specific cell surface receptor(s) in the neurons.

Identification of the HB-GAM Receptor as N-Syndecan—The putative HB-GAM receptor is apparently a transmembrane component, since its elution requires detergent. It is extensively glycosylated as revealed by the gel electrophoretic properties of the intact and the heparinase-treated component. The protein moiety of the putative receptor has an apparent molecular mass of 120 kDa. Since these properties are essentially the same as those very recently described (19) for the rat N-syndecan (syndecan-3), we tested whether affinity-purified anti-core protein antibodies to N-syndecan (19) bind to the putative receptor. Western blotting revealed that both the intact and the deglycosylated receptor bind the anti-N-syndecan antibodies (Fig. 2, lanes 5 and 6). Specificity of this binding is suggested by the finding that the antibodies used bind specifically to N-syndecan in Western blotting of crude extracts of brain (19). Furthermore, some other antibodies that bind to proteoglycans, like anti-HNK-1 antibodies and various anti-CD44 antibodies, did not give any signal in Western blotting of the putative receptor (not shown).

Tryptic peptides were prepared from the putative receptor isolated by HB-GAM affinity chromatography (see above) and from the receptor that was further purified by Qiagen (see “Experimental Procedures”). Similar peptide maps were obtained in HPLC from both preparations. The sequences of two major peptides from the affinity-purified receptor (Fig. 2, lane 3) were read as XASVTYQKPD and XARPGLGLXDNAID (the positions where identification of the amino acid was not clear are marked by X). These sequences match accurately with the deduced amino acid sequence of the rat N-syndecan (19) and are preceded by a lysine residue in the N-syndecan sequence, as expected for tryptic fragments. Peptide sequencing thus confirms that the component isolated as the HB-GAM receptor is N-syndecan or a very closely related component. The peptide sequences are specific for N-syndecan (19, 26) and thus discern the isolated receptor from other forms of transmembrane proteoglycans that are currently known.

Binding of HB-GAM to N-Syndecan—The above results raise the question of whether HB-GAM binds the proteoglycan isolated from brain as N-syndecan (27). The possible HB-GAM-N-syndecan binding was studied using an assay in which binding of N-syndecan to an immobilized ligand is measured. Recent studies have shown that N-syndecan binds in this assay strongly (K_D = 0.5 nM) to bFGF in a manner that depends on the heparan sulfate chains of the proteoglycan (27). However, several other heparin-binding proteins, including a variety of growth factors and extracellular matrix proteins, do not bind N-syndecan in the assay (27).

N-Syndecan was found to bind strongly to recombinant HB-GAM (Fig. 3; K_D = 0.6 nM). The binding of N-syndecan to HB-GAM was inhibited by soluble heparin (Fig. 3A), consistent with binding mediated by heparan sulfate chains. Interestingly, bFGF in solution effectively competed for the HB-GAM binding (Fig. 3A), suggesting that HB-GAM and bFGF bind to a similar carbohydrate structure in N-syndecan.

It has been previously shown that the interactions of heparin-type carbohydrate chains with proteins can be highly specific (28). Since heparan sulfates contain a variety of carbohydrate structures, further studies are required to clarify the carbohydrate sequence of the brain syndecan that binds HB-GAM. The role of the protein moiety of N-syndecan in HB-GAM binding also warrants further studies.

Binding of HB-GAM to the Cell Surface—Previous studies have shown that about 1 nm HB-GAM induces a half-maximal response in neurite outgrowth assays with brain neurons (14), which would be consistent with N-syndecan-type binding. Since HB-GAM, however, enhances neurite outgrowth as a substrate-bound protein, it is difficult to evaluate its affinity to the cell surface from neurite outgrowth experiments. Binding of iodinated HB-GAM to monolayers of brain cells was therefore studied. These studies indicated an apparent K_D of 0.8 nm to the cell surface with 2 x 10^4 binding sites/cell (Fig. 4). Addition of soluble heparin to the assay medium (tested at 0.5 nm HB-GAM as in Fig. 4) inhibited HB-GAM binding to the cells almost completely. Thus, 92 and 97% inhibition of HB-GAM binding was observed at the heparin concentrations 0.25 and 2.5 µg/ml, respectively. Interactions of HB-GAM with the neuron surface are thus consistent with the interactions observed with N-syndecan.

Localization of N-Syndecan at the Surface of Brain Neurons That Extend Neurites along HB-GAM-coated Substrates—The finding that N-syndecan may function as an HB-GAM receptor raises the question of whether N-syndecan occurs at the surface of neurons that are extending neurites along the HB-GAM-containing matrix. We therefore prepared neuron-enriched cell suspensions from embryonic rat brain and induced these to extend neurites using HB-GAM-coated substrates. Under these conditions, over 80% of the process-containing cells are neurofilament-positive neurons (12). Immunostaining of such HB-GAM-induced brain neurons revealed that virtually all (more than 90%) cells clearly bind affinity-purified anti-core protein antibodies against N-syndecan (Fig. 4). The immunostaining was not enhanced by permeabilization of the cells, suggesting that N-syndecan occurs at the surface of the neurons that are extending neurites along the HB-GAM-coated substrate. This finding agrees with the experiments in which N-syndecan was
isolated from surface-labeled neurons by the HB-GAM affinity chromatography (Fig. 1, lane B).

In HB-GAM-induced neurons, N-syndecan was stained both in the cell soma and in the neurites. In the cell soma, N-syndecan frequently formed patches at the plasma membrane (Fig. 5, panel C). Interestingly, a prominent immunostaining was observed at the surface of growing neurites. The varicosities and the growth cones, including the filopodia that make contacts with the HB-GAM-containing substrate, were clearly discerned by immunostaining with the anti-N-syndecan antibodies (Fig. 5, panels A and B).

Effect of Anti-N-syndecan Antibodies on HB-GAM-induced Neurite Outgrowth—Binding of the anti-N-syndecan antibodies to the surface of the neurites that grow along HB-GAM-coated substrates raises the question of whether the antibodies are able to perturb HB-GAM-neuron interactions. Counting of adherent cells on HB-GAM-coated substrates did not reveal any effect of the antibodies (see the text of Fig. 6). However, phase contrast microscopy showed that neurons extended shorter neurites in 20-h assays in the presence of anti-N-syndecan antibodies as compared with cultures without antibodies or with cultures containing nonimmune IgG. Counting of neurofilament-positive neurites revealed that 70 μg/ml anti-N-syndecan antibodies in the assay medium inhibited neurite growth more than 50%, whereas nonimmune rabbit IgG did not have a significant effect (Fig. 6, bars 1–3). The lowest concentration of anti-N-syndecan antibodies that inhibited HB-GAM-induced neurite outgrowth was found to be 14 μg/ml. The inhibitory effect of the anti-N-syndecan antibodies depends of the substrate used, since neurite outgrowth on poly-I-lysine was not inhibited by the antibodies (Fig. 6, bars 4–5).

Since HB-GAM also binds to the sulfated carbohydrate epitope HNK-1 (29), and antibodies against this epitope stain the neuron surface, anti-HNK-1 antibodies were also tested in the assays. These antibodies, however, did not have any inhibitory effect on HB-GAM-induced neurite outgrowth when tested in parallel with the anti-N-syndecan antibodies (not shown).

Concluding Remarks—Transmembrane proteoglycans have recently gained growing interest in the regulation of cell interactions during development. Syndecans (26, 30, 31) are suggested to function as cell surface receptors of extracellular matrix molecules, mediating the effects of matrix components to the cytoskeleton, and to act as co-receptors of heparin binding growth factors, like the basic fibroblast growth factor. Other examples of interesting cell surface proteoglycans are β-glycan, which is one receptor of the transforming growth factor β (32, 33), and the CD44 molecules, which are involved in lymphocyte homing (34). From these considerations, it is of interest that the detergent-soluble component of brain and of brain neurons that binds specifically to HB-GAM is a cell surface proteoglycan.

N-Syndecan (syndecan-3) was very recently cloned based on homology with other syndecans (19, 35). The function of N-syndecan is currently unknown, although it has been shown to bind bFGF with high affinity and has been proposed to function as a bFGF "co-receptor" (27). Based on homology with other syndecans and its mode of distribution, N-syndecan was suggested to play a role in the interactions of cells with extracellular molecules (19). In a previous study, however, N-syndecan was found to bind poorly or not at all to the matrix adhesive proteins fibronectin and laminin (27). The present results provide strong evidence suggesting that HB-GAM is one of the extracellular molecules with which N-syndecan interacts.

Besides the distinct binding of N-syndecan to HB-GAM, there are also other clues suggesting that N-syndecan has a role in HB-GAM-mediated neuronal development. Biochemical studies have previously shown that both HB-GAM (1) and N-syndecan (19) are strongly expressed in brain as compared with other tissues of the perinatal and newborn rat. Interestingly, the expression of N-syndecan (19) peaks during the rapid perinatal development of rat brain as the expression of HB-GAM (1). Recent immunohistochemical studies suggest that even the expression patterns of HB-GAM and N-syndecan are very similar in developing rat brain. Furthermore, in freshly prepared

2 R. Nolo and H. Rauvala, unpublished results.
N-syndecan antibodies (80 from five random microscopic fields ononic forebrain neurons were cultured on the wells for 20 h in the serum-nant HB-GAM fields used to evaluate neurite growth was
ron interactions.

brain neurons that extend neurites along HB-GAM-coated substrates, anti-N-syndecan antibodies bind to the surface of the neurites and also perturb neurite growth. HB-GAM-induced neurite outgrowth is also inhibited by heparin and by low molecular weight heparin but not by other polyanionic substances, like chondroitin sulfate and polyaspartic acid (14). Treatment of brain neurons with the heparitinase that hydrolyzes N-syndecan would be a co-receptor in HB-GAM-neuron interactions. The present results do not discount the possibility that N-syndecan plays a role in HB-GAM-mediated neurite outgrowth.

Although the extracellular region of the N-syndecan sequence is unique, the cytoplasmic domain contains the conserved sequence of syndecans that is suggested to function in cytoskeletal coupling or in signal transduction (26). Since neurite growth is dependent on cytoskeletal coupling (see Ref. 36 for reviews), N-syndecan at the surface of the neurons could mediate the neurite-promoting signal from HB-GAM to the cytoskeleton. It remains to be shown whether N-syndecan could also act as a receptor of HB-GAM or of some other member of the HB-GAM/MK-type proteins in other cell types in addition to the neurons.

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REFERENCES


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Footnote 3:

E. Raulo and H. Rauvala, unpublished results.

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