Biochemical Engineering of Neural Cell Surfaces by the Synthetic N-Propanoyl-substituted Neuraminic Acid Precursor*

(Received for publication, February 18, 1998, and in revised form, April 20, 1998)

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Sialylation of glycoproteins and glycolipids plays an important role during development, regeneration, and pathogenesis of diseases. During times of intense plasticity within the nervous system, such as development and regeneration, sialylation of neural cells is distinct from the time of its maintenance. In this study, a synthetic precursor of neuraminic acid, N-propanoylmannosamine (N-propanoyl neuraminic acid precursor (P-NAP)), is applied to the culture medium of oligodendrocyte progenitor cells, microglia, astrocytes, and neurons from neonatal rat brains to alter sialylation of glycoconjugates within these cells. P-NAP is metabolized and incorporated as N-propanoyl neuraminic acid into glycoproteins of the cell membrane. P-NAP stimulates the proliferation of astrocytes and microglia but not of oligodendrocyte progenitor in vitro. However, P-NAP increases the number of oligodendrocyte progenitor cells expressing the early oligodendroglial surface marker A2B5 epitope. In the presence of P-NAP, cerebellar neurons (but not astrocytes) in microexplant cultures start to express the oligodendroglial progenitor marker А2В5 epitope, which is normally undetectable on these cells. The controls, which were performed in the absence of any additive or in the presence of the physiological precursor of neuraminic acid, N-acetylmannosamine, did not show any increase in А2В5 expression.

Cell surface components such as terminal neuraminic acids of glycoconjugates orchestrate a variety of biological functions such as proliferation, cell-cell interaction, and migration (for a review, see Ref. 1). One strategy to interfere with cell surface neuraminic acids, i.e. sialylation, is to exploit its natural metabolism. It was shown earlier that synthetic neuraminic acid precursors that do not occur naturally are tolerated and metabolized by different cells (2, 3). New strategies for remodeling cell surfaces emerge from these findings, since they offer a possibility to introduce also (nonphysiological) reactive functional groups on the surface of cells (4). The consequences of such remodeling must be analyzed for the distinct cell types of different organs, particularly in our case the mammalian brain.

Numerous studies on the function of sialic acids in the immune system have shown that they contribute to its regulation (for a review, see Ref. 5). However, the role of sialic acids is complex and diverse. For example, sialic acids facilitate the binding affinities of the host endogenous cell membrane for factors involved in the regulation of the complement system (5–7). The content of sialic acids on the surface of cells also determines the recognition by phagocytes as desialylated erythrocytes, lymphocytes, and thrombocytes are phagocytosed by macrophages (8, 9). Moreover, sialic acids act in concert with selectins during the first steps in the homing of blood cells, thus being involved in cell-cell adhesion. In some systems, the higher the content of sialic acids on cell surface molecules, the lower their binding efficiency to their receptors, as has been shown for the leucocyte function-associated antigen-1 binding to intercellular adhesion molecule-1 (10).

To study the biological functions of N-acetylneuraminic acid, there are, in principle, two possibilities. First, N-acetylneuraminic acid can be removed from the cell surface by the use of neuraminidases. Second, it is also possible to influence specific functions of neuraminic acid by application of synthetic, unnatural N-acetyllamnosamines, which serve as precursors for neuraminic acid. These precursors, such as N-propanoylmannosamine (N-propanoyl-substituted neuraminic acid precursor (P-NAP)†), are metabolized and incorporated as N-propanoyl neuraminic acid into glycoconjugates of cells in vivo and in vitro by simple application to the cell’s exterior (Refs. 2, 3, and 11; this study). In vitro experiments revealed that biological functions such as virus infection and cell proliferation can be influenced by altering the neuraminic acid composition. For example, the application of P-NAP to an epithelial cell line results in the partial replacement of N-acetylneuraminic acid by N-propanoyl neuraminic acid by about 50% (3). This modification inhibits the sialic acid-dependent infection of these cells with primate polyomaviruses (3). Furthermore, human fibroblasts lose their density-dependent inhibition of proliferation when treated with P-NAP (12).

In our studies, we concentrate on the individual cell types of the mammalian central nervous system. We applied the unnatural sialic acid precursor P-NAP to cell cultures enriched for three glial cell types and to cerebellar microexplant cultures of the developing rat brain. These microexplants contain neurons, astrocytes, microglia, and additionally progenitors of myelinating oligodendrocytes. Oligodendrocyte progenitor cells are proliferative and migratory, features that have been proposed to play also an important role in the regeneration of the adult nervous system when naturally occurring cell death or myelin damage requires compensation (for a review, see Ref. 13). Cerebellar microexplant cultures serve as a model system to study

* This work was supported by the Deutsche Forschungsgemeinschaft as a part of the priority program “Functions of Glial Cells” (Schn 349/4–1), by the Bundesministerium für Bildung und Forschung, the Sonnenfeld Stiftung, and the Fonds der Chemischen Industrie. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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† The abbreviations used are: P-NAP, N-propanoyl neuraminic acid precursor; A-NAP, N-acetylneuraminic acid precursor; PBS, phosphate-buffered saline; GFAP, glial fibrillary acidic protein; BrdUrd, 5-bromo-2’-deoxyuridine; HPLC, high pressure liquid chromatography.
the outgrowth pattern of oligodendrocyte progenitor cells, astrocytes, and neurons as well as neurite fasciculation (14, 15). They were maintained in the presence of synthetic neuraminic acid precursors to study some of these aspects. We also used enriched cell cultures to compare proliferative effects of P-NAP on the three glial cell types.

We show that P-NAP is metabolized by glial cells in culture and is expressed as N-propanoyl neuraminic acid in glycoproteins of the cell membrane. P-NAP induces the proliferation of astrocytes and microglia but not of oligodendrocyte progenitor cells. Instead, we provide evidence that P-NAP-treatment of oligodendrocyte progenitor cells and cerebellar neurons induces the expression of the A2B5 epitope. P-NAP increases the number of A2B5-positive early oligodendrocytes. The A2B5 antibody has been described as reacting with gangliosides of neural tissues (16–18). It has been regarded as a specific marker for a subset of rat oligodendrocyte progenitor cells (for a review, see Ref. 19). However, some rat neurons in culture have also been shown to carry the A2B5 epitope (20). Although cerebellar neurons do not express the A2B5 epitope under the culture conditions of our experiments, they start to express this epitope on their neurites after application of P-NAP.

**EXPERIMENTAL PROCEDURES**

**Cell Cultures**

Preparation of Mixed Gliial Cell Cultures and Cell Cultures Enriched in Astrocytes, Oligodendroglia Progenitor Cells, and Microglia, Respectively—Mixed glial cell cultures from whole brains of neonatal Wistar rats were prepared according to the method of McCarthy and de Vellis (21). It is well established that neurons do not survive under these culture conditions. After 7–14 days in culture, microglia and oligodendroglia progenitor cells were sequentially shaken off the confluent astrocyte monolayers and plated onto glass coverslips coated with poly-L-lysine (10 μg/ml in water). Remaining astrocytes were detached from the culture flasks after 5 min of trypsinization according to standard procedures. Oligodendroglia, mainly comprising oligodendrocyte progenitor cells, were plated at a density of 5 × 10⁴, microglia were plated at a density of 2.5 × 10⁵ (low density) or 1 × 10⁵, astrocytes at a density of 1 × 10⁵ cells/coverslip (11 mm diameter). Oligodendroglia progenitor cells were maintained in Sato medium (supplemented with 1% horse serum (22)). Microglia and astrocytes were maintained in basal Eagle’s medium supplemented with 10% horse serum. Enriched cell cultures were maintained for 1–3 days in the presence or absence of unnatural or naturally occurring neuraminic acid precursors (see below).

PC12 Cells—PC12 cells were grown in plastic dishes, 35 mm in diameter, coated with collagen I (20 μg/ml, Sigma) using RPMI 1640 medium supplemented with 10% horse serum. Differentiation and neurite outgrowth was stimulated with 100 ng/ml nerve growth factor (Boehringer-Mannheim) (23).

Preparation of Cerebellar Microexplants—Cerebellum of 6–7-day-old Wistar rats were prepared as described earlier (15). In brief, cerebelli were freed from the meninges, choroid plexus, and deep cerebellar nuclei. The remaining cerebellar cortex was then forced through a 70-μm-mesh screen. Remaining astrocytes were detached from the culture flasks after 5 min of trypsinization according to standard procedures. Oligodendroglia, mainly comprising oligodendrocyte progenitor cells, were plated at a density of 5 × 10⁴, microglia were plated at a density of 2.5 × 10⁵ (low density) or 1 × 10⁵, astrocytes at a density of 1 × 10⁵ cells/coverslip (11 mm diameter). Oligodendrocyte progenitor cells were maintained in Sato medium (supplemented with 1% horse serum (22)). Microglia and astrocytes were maintained in basal Eagle’s medium supplemented with 10% horse serum. Enriched cell cultures were maintained for 1–3 days in the presence or absence of unnatural or naturally occurring neuraminic acid precursors (see below).

**Neuraminic Acid Precursors**

The physiological precursor for neuraminic acid is N-acetylmannosamine (N-acetylneuraminic acid precursor (A-NAP)). A-NAP was purchased from Sigma. The synthetic derivative of neuraminic acid precursor P-NAP is not commercially available and was synthesized as described by Keppler et al. (24).

**Immunocytochemistry**

All staining procedures were performed as described by Schmidt et al. (24). PC12 cells, primary cultures of oligodendrocyte progenitor cells, astrocytes, microglia, or cerebellar microexplant cultures were immunohistochemically stained with mouse monoclonal antibodies directed against A2B5, O4, or O7 antigen, respectively, and/or with polyclonal antibodies directed against the neural cell adhesion molecule L1 (25). Staining was done on fixed cells. Immunocytochemistry with polyclonal antibodies against glial fibrillary acidic protein (GFAP, Dako, Hamburg, Germany) and with myelin basic protein was performed with paraformaldehyde-fixed cells. If cells were not fixed with 4% paraformaldehyde before immunocytochemistry, they were fixed prior to embedding with Mowiol (Hoechst, Frankfurt, Germany). A2B5, O4, and myelin basic protein antibodies were purchased from Boehringer Mannheim, Darmstadt, Germany. Neuraminidase antibody and O7 antibodies were a kind gift of Dr. I. Sommer (University of Glasgow, Glasgow, UK). Polyclonal L1 antibodies have been described by Park et al. (26). Dichlorotriazinyl amino fluorescein- or Cy3-conjugated secondary antibodies were purchased from Dianova (Hamburg, Germany). For A2B5 and O4 immunocytochemistry, chain-specific Cy3-conjugated secondary antibodies were used. Glycolipids dissolved in methanol were added to the wells of 96-well microtiter plates (50 μl/well; Falcon 3912) and adsorbed to the plastic by evaporating the methanol at room temperature. Glycoproteins of crude membranes solubilized in buffer containing 150 mM NaCl, 10 mM Tris, 1 mM CaCl₂, 1 mM MgCl₂, 1% Triton, and a protease inhibitor mixture (P 8340, Sigma) at pH 7.4 were also added to the wells of 96-well microtiter plates (50 μl/well) and adsorbed to the plastic by overnight incubation at 4 °C. Wells were blocked with PBS containing 1% bovine serum albumin (200 μl/well) for 1 h at room temperature. After wells were washed three times with PBS, they were incubated for 1 h with monoclonal antibodies to neural cell adhesion molecule (5B8; Hybridoma Bank) or A2B5 epitope (0.2 μg/ml, 100 μl/well) in PBS containing 1% bovine serum albumin. Plates were again washed three times with PBS and incubated for 1 h with horseradish peroxidase-conjugated anti-mouse antibodies in PBS containing 1% bovine serum albumin; bound secondary antibodies were visualized by incubation with H₂O₂ and 2,2-azino-di-3-ethylbenzthiazolinesulfonate-6 (Sigma) as horseradish peroxidase substrates, according to the manufacturer’s instructions. The color reaction was terminated by the addition of 0.6% aqueous SDS, and the optical density of the reaction products was determined at 405 nm in an enzyme-linked immunosorbent assay reader.

**Proliferation Assay**

The proliferation of cells in single cell cultures was analyzed using the 5-bromo-2′-deoxyuridine (BrdUrd) labeling and detection kit III from Boehringer Mannheim. Labeling was performed according to the supplier’s instructions in 24-well plates. Single cells were allowed to settle for 4 h before the addition of any neuraminic acid precursor or growth factors. The platelet-derived growth factor (Boehringer Mannheim) and basic fibroblast growth factor (British Bio-Technology Limited, Oxford, UK) were both used at a concentration of 10 ng/ml as a positive control for the proliferation of oligodendrocyte progenitor cell cultures (27, 28). Murine macrophage colony-stimulating factor (R & D Systems, Minneapolis, MN) turned out to be a potent stimulator of rat microglia proliferation at a concentration of 30 ng/ml (this study) and was therefore used as a positive control for these cultures. Astrocytes and microglia were incubated for 2–3 days with BrdUrd and oligodendrocyte progenitor cells for 1–2 days, depending on the age of the primary cultures from which these cells were shaken off. In each experiment (with double values) the background, omitting BrdUrd, was subtracted.

**Quantification of N-Acetylmuramic Acid and N-Propanoyl Muramic Acid in Mixed Glial Cultures**

Mixed glia cultures were maintained for 3 days in the presence of 5 mM A-NAP or P-NAP before cells were scratched off and pelleted. Cell pellets (10⁶ cells) were lysed by hypotonic shock in distilled water and repeated freezing and thawing (two times). The crude membrane fractions were pelleted by centrifugation at 30,000 × g for 20 min (4 °C). The pellet extracts were lyophilized. Glycolipids were extracted using methanol/chloroform (1:2, 1:1, 2:1 (v/v)) for 30 min each and centrifugation at 10,000 × g (30 min, 4 °C). Glycolipid-containing methanol/chloroform supernatants were collected and lyophilized. Dried samples were hydrolyzed for 2 h with 200 μl of 2 M acetic acid. The pH values of the hydrolysates were adjusted to 4, and further purification was carried out on a cation exchanger (AG-50W-X12, H⁺ form; Bio-Rad, München, Germany).
Germany). Glycoprotein-containing pellets were hydrolyzed and purified as described for glycolipids. Purified eluted neuraminic acids were fluorescence-labeled according to Hara et al. (29). Labeled neuraminic acids were chromatographed using a reversed phase C18 column (Lichrosorb C18, 5 μm, 250 × 4.6 mm; Knauer, Berlin, Germany) with a fluorescence detector (Ginkotek; excitation wavelength, 377 nm; emission wavelength, 448 nm). Eluent A contained distilled water, while eluent B contained acetonitrile/methanol (60:40, v/v). The flow rate was 1 ml/min, and separations were carried out using a gradient that first ran for 20 min in the isocratic mode with 10% B; then B was raised to 25% within 25 min and finally to 50% within another 15 min. Eluted neuraminic acids were identified by matrix-assisted laser desorption-ionization time of flight mass spectrometry and quantified using defined standards as performed and described in Keppler et al. (3).

RESULTS

Cerebellar Microexplants Treated with P-NAP Show Early A2B5-positive Oligodendrocyte Progenitor Cells That Migrated from the Explant Cores—Cerebellar microexplants from neonatal brains mostly consist of small cerebellar neurons, astrocytes, and oligodendrocytes (14, 15). They send out radially oriented, partly fasciculated neurites (14, 15), which we also detected under our culture conditions (Fig. 1, A, D, and F; open arrows). Cells of different morphologies left the explant cores within 5 days in culture; most of them exhibited small and round cell bodies (Fig. 1, A and D, arrowheads). Due to their morphology, these cells were regarded as small cerebellar neurons. When the explants were treated with 5 mM P-NAP (Fig. 1D), there was no significant difference in the outgrowth pattern of these neurons when compared with the control in the absence of neuraminic acid precursors (Fig. 1A).

It is well established that the proliferative and migratory active pool of oligodendrocyte progenitor cells differentiates into cells of the oligodendrocyte lineage and astrocytes, depending on the culture conditions (19, 30). Progressive developmental stages of oligodendrocytes are distinguishable by their sequential expression of antigenic markers. One of the first identifiable markers of a subset of very early progenitors is the A2B5 epitope, which is followed by O4 expression before differ-

**FIG. 1.** Cerebellar microexplants from 6-day-old rats were maintained for 5 days in the absence (A–C) or presence of P-NAP (D–F). Explants were immunostained with antibodies against GFAP (B and E) and A2B5 (C and F). A and D show phase contrast micrographs. Note the A2B5-positive cells with numerous processes and the punctate neurite-like staining in F. Some of the cells in F are also seen in E because of fluorescence intensity overlap. They were not GFAP-positive. The arrowheads (A and D) point toward small cerebellar neurones, open arrows (A, D, and F) point to fasciculated neurites, and arrows (F) point to A2B5-positive oligodendrocyte progenitor cells. Bar, 60 μm.
entiation occurs into myelin-forming oligodendrocytes (for a review, see Ref. 19). When cerebellar explants were maintained in the presence of P-NAP for 5 days and immunostained with the A2B5 antibody, oligodendrocyte progenitor cells with typically elongated cell bodies and a few processes had migrated out of the explant cores (Figs. 1F and 4C, arrows). These A2B5-positive cells were exclusively found in the presence of P-NAP (Fig. 1F) but not in the presence of physiological A-NAP (Fig. 2) or in the absence of neuraminic acid precursors (Fig. 1C). A2B5-positive cells were already detected after 1 day in culture in the presence of 5 mM P-NAP and predominantly exhibited a bipolar morphology (not shown). Under control conditions, in the absence of P-NAP, A2B5-positive cells appeared after 3 days in culture with a weaker A2B5 staining (not shown) and were no longer detected after 5 days (Fig. 1C).

The A2B5-positive oligodendrocyte progenitor cells had migrated a significant distance from the rim of the explant cores (Fig. 1F, arrows, and Fig. 2). The distances migrated by these A2B5-positive cells that had migrated from the explant cores were dose-dependent and became maximal with 5 mM P-NAP (Fig. 2). Proliferation assays were not performed in these cultures due to the low overall number of cells outside the explant cores (but see below).

When microexplant cultures were stained with O4 antibodies, more mature oligodendrocyte progenitor cells were seen in the presence and in the absence of P-NAP (Fig. 3). There was also no effect of P-NAP on the distances of O4-positive oligodendrocyte progenitor cells that had migrated from the rims of the explants (Fig. 3). Immunostaining with antibodies against later mature developmental stages of the oligodendroglial lineage such as O7 or myelin basic protein antibodies did not show significant numbers of positive cells (not shown). The area covered by GFAP-positive astrocytes was also not affected by P-NAP as compared with the control (Fig. 1, B and E).

P-NAP Leads to a de Novo Expression of the A2B5 Epitope in Neurons of Cerebellar Microexplant Cultures—Neurites extending from rat cerebellar microexplants normally do not express the A2B5 epitope. In the presence of P-NAP (Fig. 1, open arrows), neurites become strongly A2B5-positive (for comparison see Fig. 1, C and F). A2B5-positive neurites were already seen after 1 day in culture (not shown). The cell bodies of the morphologically identified small cerebellar neurons (Fig. 1, arrowheads) were not stained by A2B5 antibodies (Fig. 1, compare D and F). In the presence of P-NAP, A2B5 expression on neurites coincided with the expression of the neuronal marker L1 (Fig. 4, compare B and C, open arrows). This confirms that these A2B5-positive processes are of neuronal origin. A2B5 expression on the neurites of the small cerebellar neurons was prominent at a concentration of 5 mM P-NAP as seen in Figs. 1F and 4C but was not seen with lower concentrations of P-NAP (0.05 and 0.5 mM; data not shown). A2B5-positive oligodendro-
cyte progenitor cells and their processes were not positive for the neuronal marker L1 as shown for the cells in Fig. 4, B and C (arrows). Neurites of nerve growth factor-differentiated PC12 cells did not express the A2B5 epitope in the presence of P-NAP after 5 days in culture (not shown). GFAP-positive astrocytes of the microexplant cultures are also negative for the A2B5 epitope (Fig. 1, compare E and F).

Fig. 4. P-NAP induces the expression of the A2B5 epitope in cerebellar neurons. Cerebellar microexplants were maintained for 5 days in the presence of 5 mM P-NAP. A, phase contrast micrograph. B, immunostaining with antibodies against the neural cell adhesion molecule L1 demarcating fasciculated neurites (open arrows). C, immunostaining with antibodies against A2B5. Note the cells with elongated cell bodies (arrows) and numerous processes and the neurites being positive for A2B5 in C (open arrow). Bar, 60 μm.

P-NAP Leads to a Time-restricted Accumulation of Early A2B5-positive Oligodendrocyte Progenitor Cells in Vitro—Cell cultures enriched in oligodendrocyte progenitor cells were maintained for up to 5 days in the presence of P-NAP or A-NAP both at a concentration of 5 mM. For all further experiments, this concentration was used because it showed the most prominent effects on the presence of A2B5-positive oligodendrocyte progenitor cells outside the microexplant cultures (Fig. 2). After treatment with P-NAP, the number of A2B5-expressing oligodendrocyte progenitor cells increased in comparison with A-NAP-treated cultures and with control cultures in the absence of neuraminic acid precursors after 24 and 48 h (Fig. 5A). The morphology of A2B5-positive oligodendrocyte progenitor cells was the same under all conditions. We observed a decrease in the total number of A2B5-positive cells from 24 h to 5 days in culture (Fig. 5A) and a continuous increase in O4-positive oligodendrocyte progenitor cells under all conditions (Fig. 5B) implicating a progressive differentiation of the cells. Although the number of A2B5-positive cells increased in the presence of P-NAP, the number of O4-positive cells was not significantly different at each time point selected.

P-NAP Differentially Influences the Proliferation of Glial Cell Types—We performed proliferation assays using the incorporation of BrdUrd as a measure to answer the following questions: 1) is the increase in the number of A2B5-positive cells in the presence of P-NAP due to an increase in the proliferation rate of oligodendrocyte progenitor cells? and 2) are other glial cell types of the mammalian brain such as astrocytes or microglia also influenced by synthetic P-NAP?

Cell cultures enriched in oligodendrocyte progenitor cells, astrocytes, or microglia were maintained in 5 mM P-NAP or A-NAP for 3 days. Proliferation of microglia was specifically stimulated by P-NAP and not by A-NAP, whereas proliferation of oligodendrocyte progenitor cells was not significantly altered by any neuraminic acid precursor (Fig. 6). Astrocyte cultures were stimulated by both A-NAP and P-NAP (Fig. 6). The specific proliferative effect of P-NAP for microglia was also seen when cells were plated at low density (2.5 × 10⁴; data not shown).

P-NAP Is Incorporated into Glycoproteins of Mixed Glial Cell
Cultures—Mixed glial cultures were maintained for 3 days in the presence of P-NAP or A-NAP, both at 5 mM in the culture medium. To prove that glial cells can synthesize N-propanoyl neuraminic acid from the appropriate precursor (P-NAP), all sialic acid components of membrane glycoproteins or glycolipids present in mixed glial cultures were isolated and quantified. After HPLC purification of hydrolyzed glycoconjugates, 180 pmol of neuraminic acids/10^7 cells could be isolated from glycoprotein fractions of both P-NAP- and A-NAP-treated cultures (Fig. 7). When mixed glial cultures were maintained in the presence of A-NAP, these 180 pmol of neuraminic acids were represented only as N-acetylanuraminic acid, whereas in the presence of P-NAP, 42 pmol (or 23%) of all neuraminic acids were represented by N-propanoyl neuraminic acid (Fig. 7). In a second series of experiments, the incorporation of N-propanoyl neuraminic acid into glycolipids was analyzed. No N-propanoyl neuraminic acid could be detected in any glycolipid fraction (data not shown). This shows that P-NAP was metabolized and incorporated only in glycoproteins under our culture conditions.

To exclude the possibility that mixed glial cultures express the A2B5 epitope on glycoproteins after growing in the presence of P-NAP, we performed dot-blot and enzyme-linked immunosorbent assays. We found A2B5 immunoreactivity only in the glycolipid fraction of cells grown in the presence of P-NAP and not in any glycoprotein fraction (data not shown).

**DISCUSSION**

We have shown that mixed glial cell cultures metabolize synthetic neuraminic acid precursors and express them as novel, modified neuraminic acids on their cell surfaces after we simply added these substances to the culture medium. It is thus possible to biochemically engineer sialylation, i.e. the composition of sialylated glycoproteins. This precursor is not cytotoxic, as we confirmed for neural cells in this study.

We demonstrate a differential role of P-NAP on the proliferation of various cell types of the central nervous system. In contrast to oligodendrocyte progenitor cells, microglia were stimulated to proliferate in the presence of P-NAP. This proliferation was independent of the density of cells used for the assay. A role for cell-cell contact in the P-NAP-dependent process of proliferation seems, therefore, unlikely. Astrocytes were stimulated to proliferate by P-NAP and also by A-NAP, the physiological neuraminic acid precursors. This might be explained by a general shortage of N-acetylanuraminic acid for maximal proliferation of astrocytes under the culture conditions used in our studies.

In our present study, we support previous findings that sialic acids might play a role in oligodendrocyte progenitor cell migration. The potential of oligodendrocyte progenitor cells to migrate represents a form of plasticity within the nervous system, and this not only occurs during development but also during adulthood. Oligodendrocyte progenitor cells can migrate to demyelinated regions when transplanted at a distance (31). It is the A2B5-positive rather than the more mature O4-positive cell type that migrates considerable distances and is able to remyelinate (32).

Oligodendrocyte progenitor cells in culture that were maintained in the presence of P-NAP transiently accumulated or were retained in an A2B5-positive developmental stage. The functional role of the A2B5 epitope, which is described to be part of gangliosides (16–18), has not yet been determined, but the A2B5-positive stage of oligodendrocytes has been shown to correlate with the migratory active phase of these cells in vitro (24) and in vivo (32). The application of the naturally not occurring P-NAP but not of the physiological A-NAP led to an accumulation of potentially migratory active oligodendrocyte progenitor cells in vitro. This was not due to proliferation. It is not clear whether oligodendrocyte progenitor cells, which are not positive for the A2B5 epitope are forced by P-NAP to become A2B5-positive or whether P-NAP forces A2B5-positive cells to remain in this state. If the A2B5 epitope is considered to be a functional marker of cells of the early oligodendrocyte lineage, P-NAP can be considered as a potent regulator of the lineage progression of oligodendrocytes at early stages of their development. In agreement with these findings, P-NAP did not influence the number of the more mature O4-positive cells. Unfortunately, there was no other specific marker available to immunostain oligodendrocyte progenitor cells that would recognize the same early stage of the oligodendroglial lineage as does the A2B5 antibody.

Although we observed a dose-dependent effect of P-NAP on the distances oligodendrocyte progenitor cells migrated out of the cerebellar explants in culture, this does not necessarily mean that single cells have an increased velocity. Another possibility is that with increasing P-NAP concentration more
oligodendrocyte progenitor cells might stay in the A2B5-positive developmental stage, and/or this potentially active migratory phase might be prolonged. Based on our results, we suggest that nontoxic agents such as P-NAP that sustain this A2B5-positive state may support regenerative events in this respect.

It is not very likely that N-propanoyl neuraminic acid itself is recognized by the A2B5 antibody, since astrocytes, PC12 cells and microglia, which also metabolize P-NAP, were A2B5-negative. Furthermore, the finding that A2B5 immunoreactivity is not detectable on glycoproteins of mixed glial cultures grown in the presence of P-NAP is another indication that P-NAP itself is not recognized by the A2B5.

The only cells that are induced to express the A2B5 epitope by P-NAP are those that carry, at a crucial point of their development, this particular epitope, namely oligodendrocyte progenitor cells and subsets of neurons.

We claim that P-NAP treatment of neurons can induce antigen expression, as we have exemplified by the P-NAP-specific induction of the A2B5-antigen on the surface of neurites of cerebellar neurons in vitro. This is in congruence with findings that P-NAP can induce the expression of β1-integrins in some tumor cell lines. Any functional consequence of P-NAP-induced A2B5 expression on neurons is of potential interest and remains to be elucidated.

Acknowledgments—We thank Anke Vogel, Regina Krauß, and Brigitte Hunger for excellent technical assistance and Dr. Susan Lyons for helpful discussion and comments.

REFERENCES
1. Varki, A. (1992) Glycobiology 2, 25–40
2. Kayser, H., Zeitler, R., Kannicht, C., Grunow, D., Nuck, R., and Reutter, W. (1992) J. Biol. Chem. 267, 16934–16938
3. Keppler, O. T., Stehling, P., Herrmann, M., Kayser, H., Grunow, D., Reutter, W., and Pawlitza, M. (1995) J. Biol. Chem. 270, 1308–1314
4. Mahal, L. K., Yarema, K. J., and Bertozzi, C. R. (1997) Science 276, 1125–1128

2 C. Schuler, personal communication.