Poly(*N*-acetyllactosaminyl) Oligosaccharides in Glycoproteins of PC12 Pheochromocytoma Cells and Sympathetic Neurons†

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ABSTRACT: Endo-β-galactosidase treatment of glycopeptides derived from the trypsinate and membranes of PC12 pheochromocytoma cells and cultured sympathetic neurons demonstrated the presence of poly(*N*-acetyllactosaminyl) units on tri- and tetraantennary oligosaccharides, some of which have a core fucose residue and a 2,6-substituted α-linked mannose residue. Nerve growth factor induced differentiation of the PC12 cells led to a small but significant decrease in the proportion of these oligosaccharides. Poly(*N*-acetyllactosaminyl) oligosaccharides were also identified in a major 230 000-Da cell-surface glycoprotein (the nerve growth factor inducible large external, or NILE, glycoprotein) of PC12 cells and appear to account for much or all of the difference in size between this glycoprotein as compared to the immunochemically cross-reactive 205 000-Da species present in postnatal brain. Glycoproteins containing poly(*N*-acetyllactosaminyl) oligosaccharides were selectively labeled by treatment of PC12 cells with endo-β-galactosidase to expose *N*-acetylglucosamine residues, followed by incubation with galactosyltransferase and UDP-[^{14}C]galactose. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and fluorography revealed the presence of a number of distinct PC12 cell glycoproteins that contain these oligosaccharides and have apparent molecular weights in the range of 25 000–250 000. Treatment of PC12 cells with nerve growth factor (NGF) altered the relative labeling of several of the glycoprotein bands, with a time course similar to the effects of NGF on neurite outgrowth. However, most of the NGF effects on glycoprotein labeling are also seen in PC12 cells cultured in suspension, conditions under which neurite extension and certain other NGF effects do not occur, and they can be partially induced by long-term treatment with forskolin (which increases cellular levels of cyclic AMP) but not by epidermal growth factor.

Oligosaccharides of the poly(*N*-acetyllactosamine) series are of considerable current interest since they have been identified in glycoproteins and glycolipids that express a number of developmentally regulated and tumor-associated antigens [for reviews, see Fukuda (1985) and Feizi (1985)]. They are characterized by the presence of up to six or more disaccharide repeating units having the structure (Galβ1–4GlcNAcβ1–3)$_n$, which in the case of glycoproteins are linked to a conventional $(Man)_3(GlcNAc)_2$ complex oligosaccharide core. These carbohydrate chains, which have been referred to as polyglycosyl chains (Järnefelt et al., 1978; Krusius et al., 1978) or as poly(lactosaminoglycans) (Fukuda et al., 1980), were originally detected in erythrocytes and their precursor cells and have more recently been found in a number of other cell types including Chinese hamster ovary cells, thyroid, teratocarcinoma cells, and granulocytes. Susceptibility to several bacterial endo-β-galactosidases is considered diagnostic of the presence of such oligosaccharide chains, although sulfation and branching at internal β-galactosidic linkages is known to confer resistance to enzymatic hydrolysis (Fukuda & Matsumura, 1976; Scudder et al., 1984). In view of the potential importance of these oligosaccharides in various developmental and cell-surface events, we have investigated their prevalence and localization in nervous tissue, where we have demonstrated their presence on a large number of glycoproteins of cultured PC12 pheochromocytoma cells and in rat sympathetic neurons. Poly(*N*-acetyllactosaminyl) oligosaccharides were also identified in a major 230 000-Da nerve growth factor inducible cell-surface (NILE) glycoprotein of PC12 cells (McGuire et al., 1976; Salton et al., 1983a,b; Stallcup et al., 1985) whose structure we have previously examined (Margolis et al., 1983b; Salton et al., 1983b). These oligosaccharides appear to account for much or all of the difference in size between this glycoprotein in PC12 cells as compared with the somewhat smaller but immunochemically cross-reactive species present in brain.

Experimental Procedures

PC12 cells were cultured as described by Greene and Tischler (1976) in RPMI 1640 medium (GIBCO, Grand Island, NY) containing 10% heat-inactivated (30 min, 56 °C) horse serum and 5% fetal calf serum (both from KC Biological, Lenexa, KS). Metabolic labeling was for 72 h in the presence of [6-^{3}H]glucosamine (Amersham, 23 Ci/mmol) or [1-^{14}C]-glucosamine (New England Nuclear, 50 mCi/mmol) at a concentration of 20 μCi/mL. When (NGF) (Mobley et al., 1976) was used, it was added at a concentration of 50 ng/mL, beginning at least 10 days before cell labeling. Epidermal growth factor (EGF), which was kindly provided by Dr. Frederick Maxfield (New York University Medical Center), was used at a concentration (5 ng/ml) that is optimal for eliciting responses in PC12 cells (Huff et al., 1981).

Brain glycoproteins of 30-day-old rats were labeled for 18 h after bilateral intracerebral injection of 7.5 μL of saline solution containing 50 μCi of [6-^{3}H]glucosamine/μL.

PC12 cell membranes, trypsinate, and culture medium were obtained and processed as previously described (Margolis et

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al., 1983a), and the glycopeptides resulting from Pronase treatment of the lipid-free protein residue were fractionated by sequential lectin affinity chromatography (Cummings & Kornfeld, 1982; Margolis et al., 1983a) after prior removal of glycosaminoglycans by precipitation with cetylpyridinium chloride (Margolis et al., 1983a).

The NILE glycoprotein was extracted from PC12 cells and brain and purified by indirect immunoprecipitation, and glycopeptides were prepared and fractionated as described previously (Salton et al., 1983a; Margolis et al., 1983b).

Postnatal day 2 rat superior cervical ganglion neurons were dissociated as described by Salton et al. (1983a) and cultured for 24 h in medium with NGF as described for the PC12 cells and then for 48 h in the presence of cytosine arabinoside (10 μM) to eliminate dividing nonneuronal cells. The cells were cultured for a further 48 h after removal of cytosine arabinoside and labeled for 72 h with [^{3}H]glucosamine under the same conditions used for the PC12 cells. Glycopeptides were prepared from the cell membranes and fractionated by sequential lectin affinity chromatography according to the same methods as described for the PC12 cells.

For endo-β-galactosidase treatment of labeled glycopeptides, samples containing less than 25 μg of oligosaccharide were digested for 16 h at 37 °C under toluene in 0.1 mL of 50 mM sodium acetate buffer, pH 6.0, containing 0.1 mg of endo-β-galactosidase from *Escherichia freundii* (Nakagawa et al., 1980), which was kindly provided by Dr. Y.-T. Li (Tulane University School of Medicine). The glycopeptides were then diluted to 1.5 mL with 0.5 M NaCl and applied to a column of Sephadex G-50 (0.9 × 65 cm), which was eluted with 0.1 M NaCl. Fractions of 1.2 mL were collected, and radioactivity was monitored by liquid scintillation counting.

Specific cell-surface labeling of glycoproteins containing poly(*N*-acetyllactosaminyl) oligosaccharides was performed by using endo-β-galactosidase and galactosyltransferase according to the general procedure described by Viitala and Finne (1984). Briefly, 25 μL of packed cells was incubated with occasional stirring in an Eppendorf tube for 1 h at 37 °C with 130 μL of 10 mM phosphate-buffered saline, pH 7.4 (PBS), and 20 μL of 50 mM sodium acetate, pH 5.8, containing 2 milliunits of endo-β-galactosidase from *E. freundii* (obtained from Seikagaku Kogyo, Tokyo, through Miles Laboratories, Elkhart, IN). After being washed twice by centrifugation in 0.15 M NaCl and once with 20 mM tris(hydroxymethyl)aminomethane (Tris) buffered saline, pH 7.5, containing 10 mM $MnCl_2$ (TBS), the washed cells were suspended in 40 μL of this buffer containing 0.5 μCi of UDP-[^{14}C]galactose (New England Nuclear, 337 mCi/mmol). The reaction was started by adding 10 μL of TBS containing 17 milliunits of galactosyltransferase (Sigma, from bovine milk) and continued for 1 h at 37 °C with occasional stirring. (To block potential preexisting nonreducing terminal *N*-acetylglucosamine residues that might be present before endo-β-galactosidase treatment and act as acceptors for galactosyltransferase, experiments were also carried out in which cells were preincubated with 1 mM unlabeled UDP-galactose under the conditions described above). After being washed several times in PBS, the cells were suspended in sodium dodecyl sulfate (SDS) containing sample buffer, heated for 5 min at 100 °C, and electrophoresed on 7.5–15% SDS–polyacrylamide slab gels using the discontinuous buffer system of Laemmli (1970), followed by staining with Coomassie Blue and fluorography.

Results

Glycopeptides Derived from PC12 Cells and Sympathetic Neurons. Glycoprotein oligosaccharides in NGF-treated or untreated PC12 pheochromocytoma cells were labeled in their hexosamine and sialic acid residues by growth for 3 days in the presence of [^{3}H]glucosamine and [^{14}C]glucosamine, respectively. Glycopeptides were prepared by Pronase treatment of the lipid-free protein residue obtained from the culture medium and cell-soluble, trypsinate, and membrane fractions and, after removal of glycosaminoglycans, were fractionated by sequential lectin–agarose affinity chromatography. Gel filtration on Sephadex G-50 before and after endo-β-galactosidase treatment of the resulting glycopeptide fractions demonstrated that poly(*N*-acetyllactosaminyl) oligosaccharides are present exclusively in the trypsin-releasable and membrane glycoproteins but are apparently not shed into the culture medium in appreciable amounts over a period of 3 days (in comparison with the other labeled glycoproteins found there; Margolis et al., 1983a; Richter-Landsberg et al., 1984). While only tri- and tetraantennary complex oligosaccharides (not bound by concanavalin A–agarose) were partially degraded by endo-β-galactosidase, these included oligosaccharides both with and without a 2,6-substituted α-linked mannose residue together with fucose linked to the proximal *N*-acetylglucosamine on the chitobiose unit, as determined by their ability to bind to lentil lectin–agarose (Figures 1–3). Endo-β-galactosidase degraded a somewhat larger proportion of the oligosaccharides in nonneuronally differentiated PC12 cells that had not been treated with nerve growth factor as compared to NGF-treated cells (Figure 3). Although this dif-

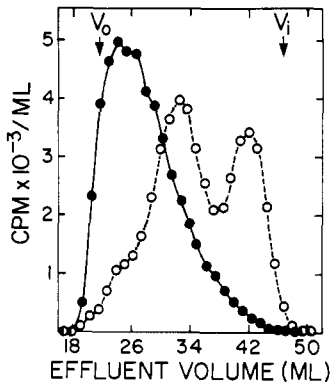

FIGURE 1: Glycopeptides prepared from the membranes of PC12 cells cultured in the presence of [^{3}H]glucosamine. The fractions that were not bound by either concanavalin A–agarose or lentil lectin–agarose were eluted from Sephadex G-50 (0.9 × 65 cm) with 0.1 M NaCl before (●) and after (○) treatment with endo-β-galactosidase. In endo-β-galactosidase-treated glycopeptides, approximately 33% more radioactivity eluted after 30 mL.

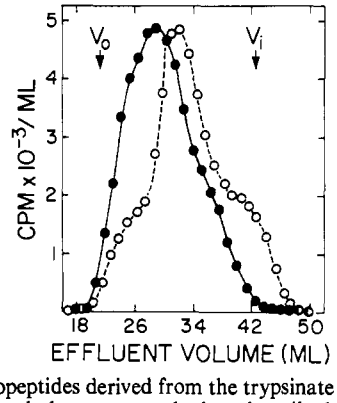

FIGURE 2: Glycopeptides derived from the trypsinate of labeled PC12 cells, prepared and chromatographed as described in the legend to Figure 1. Treatment with endo-β-galactosidase (○) resulted in a 14% increase in retarded radioactivity.

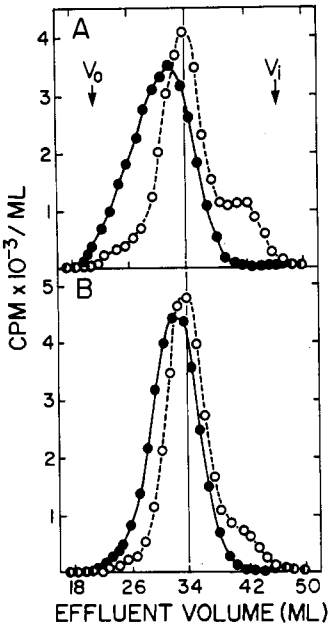

FIGURE 3: Gel filtration on Sephadex G-50 of glycopeptides derived from the trypsinate of untreated PC12 cells (panel A) and cells treated for 2 weeks with nerve growth factor (panel B), before (●) and after (○) incubation with endo-β-galactosidase. Tri- and tetraantennary complex oligosaccharides (not bound by concanavalin A–agarose) containing both a 2,6-substituted α-linked mannose residue together wtih fucose linked to the proximal *N*-acetylglucosamine of the chitobiose unit (bound by lentil lectin–agarose) were obtained by affinity chromatography and used for digestion with endo-β-galactosidase, which produced a 7% increase in retarded radioactivity in NGF-treated cells, as compared to 13% in cells not treated with NGF.

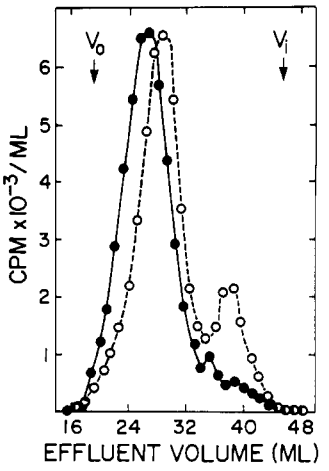

FIGURE 4: Glycopeptides containing tri- and tetraantennary complex oligosaccharides isolated by affinity chromatography (unbound by both concanavalin A– and lentil lectin–agarose) from the membranes of sympathetic ganglia cultured in the presence of [^{3}H]glucosamine. The figure shows their elution from Sephadex G-50 before (●) and after (○) treatment with endo-β-galactosidase, which produced a 12% increase in retarded radioactivity.

ference is probably due to a decreased proportion of poly(*N*-acetyllactosaminyl) oligosaccharides in the NGF-treated cells, it is also possible that NGF treatment results in structural alterations (e.g., sulfation or increased branching) that render these oligosaccharides partially resistant to degradation by endo-β-galactosidase. However, in previous studies we have not detected any major structural changes in PC12 cell glycoprotein oligosaccharides as a result of NGF treatment (Margolis et al., 1983a). It should also be noted that the use of glycopeptides labeled with [^{3}H]- or [^{14}C]glucosamine (for NGF-treated or untreated PC12 cells, respectively) permitted a more reliable comparison of the effects of NGF on the proportion of poly(*N*-acetyllactosaminyl) oligosaccharides. Insofar as endo-β-galactosidase-treated glycopeptides from both types of cells were eluted together from the gel filtration column, even relatively small differences in the resulting elution profiles can be considered to be significant. The presence of poly(*N*-acetyllactosaminyl) oligosaccharides in PC12 pheochromocytoma cells is apparently not merely a reflection of their tumor origin, since they were also found in cultured sympathetic neurons (Figure 4), which can be considered as a close nontumor counterpart to NGF-treated PC12 cells.

NILE Glycoprotein. One of the major components of PC12 cell membranes is a 230 000-Da cell-surface glycoprotein whose concentration increases approximately 3–5-fold in the presence of NGF (McGuire et al., 1978; Salton et al., 1983a). This surface glycoprotein has been named the NGF-inducible large external, or NILE, glycoprotein, and by use of indirect immunofluorescence it was found that polyclonal antisera against NILE glycoprotein purified from PC12 cells stained the PC12 cell surface in a uniformly distributed granular pattern (Salton et al., 1983a). Immunofluorescent staining of primary cell cultures and tissue whole mounts demonstrated that immunochemically cross-reactive NILE glycoprotein is expressed on the cell surfaces (somas and neurites) of all peripheral and central neurons examined but was not found on a wide variety of other cell types (Salton et al., 1983a; Stallcup et al., 1985). Recent findings indicate that the NILE glycoprotein is recognized by antisera against the L1 antigen (Bock et al., 1985; Sajovic et al., 1986) and that it may be homologous to or identical with a neuronal–glial cell adhesion molecule (Friedlander et al., 1986).

It has previously been reported that the greater molecular size of the PC12 cell NILE glycoprotein as compared to the immunochemically cross-reactive species present in postnatal brain (205 000 Da) is due to the greater size of the PC12 cell tri- and tetraantennary complex oligosaccharides (Margolis et al., 1983b). We now find that the largest of these oligosaccharides contain poly(*N*-acetyllactosaminyl) units and that, after endo-β-galactosidase treatment, most of the undegraded oligosaccharides that remain (Figure 5A, peak 2) are of the same size as the single population of tri- and tetraantennary oligosaccharides present in the NILE glycoprotein of brain (Figure 5B). (It should be noted in this connection that endo-β-galactosidase treatment had no effect on any of the glycopeptide fractions isolated from brain).

Labeling of PC12 Cell-Surface Poly(N-acetyllactosaminyl) Oligosaccharides. To obtain information concerning the number of PC12 cell glycoproteins containing poly(*N*-acetyllactosaminyl) oligosaccharides and their apparent molecular sizes, cells were incubated with endo-β-galactosidase to selectively expose *N*-acetylglucosamine residues in these glycoproteins, which were then labeled by incubation with galactosyltransferase and UDP-[^{14}C]galactose. After SDS–polyacrylamide gel electrophoresis and fluorography, 15–20 distinct PC12 cell glycoproteins were identified by this technique, with apparent molecular weights in the range of 25 000–250 000 (Figure 6). These glycoprotein bands were resolved considerably more distinctly than the total population of PC12 cell glycoprotein labeled biosynthetically with [^{3}H]glucosamine (or [^{3}H]fucose). Overall, the electrophoretic pattern of glycoproteins containing poly(*N*-acetyllactosaminyl) oligosaccharides appeared quite different from that of the total cell glycoproteins. The enzyme incubation procedure had no effect on the general glycoprotein pattern, as demonstrated by identical fluorographs of [^{3}H]glucosamine-labeled PC12 cell glycoproteins before and after incubation of the cells with

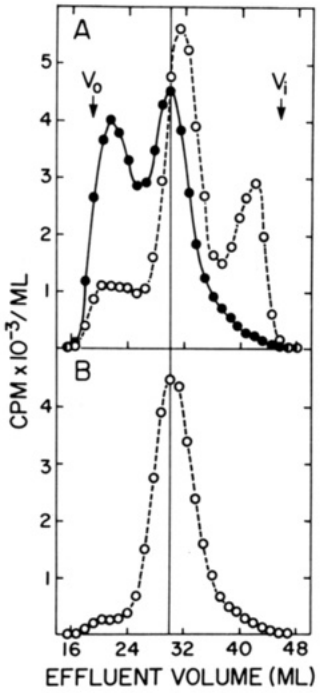

FIGURE 5: NILE glycoprotein biosynthetically labeled with [^{3}H]-glucosamine and immunoprecipitated from NGF-untreated PC12 cells (panel A) and rat brain (panel B). Glycopeptides containing tri- and tetraantennary complex oligosaccharides were isolated from each NILE glycoprotein species (i.e., the glycopeptides that did not bind to concanavalin A–, lentil lectin–, or leukoagglutinating phytohemagglutin–agarose) and eluted from Sephadex G-50 before (●) and after (○) treatment with endo-β-galactosidase. The enzyme digestion resulted in a 23% increase of PC12 cell NILE glycopeptide radioactivity eluting after 30 mL (panel A) but had no effect on the NILE glycopeptides from brain (panel B), which showed identical elution patterns both before and after treatment with endo-β-galactosidase.

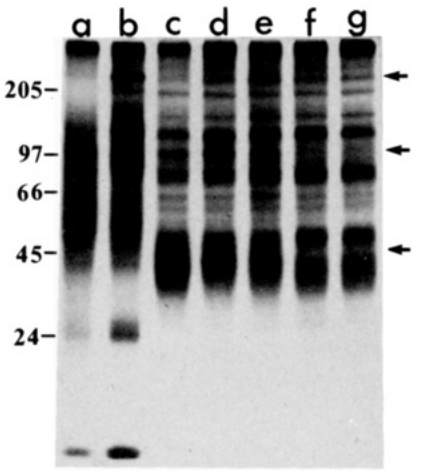

FIGURE 6: Effect of various times of exposure to NGF on the pattern of PC12 cell glycoproteins containing poly(*N*-acetyllactosaminyl) oligosaccharides. Cells were exposed to NGF for various times, and either they were metabolically labeled with [^{3}H]glucosamine (lanes a and b) or their poly(*N*-acetyllactosaminyl) oligosaccharides were specifically labeled as described in the text (lanes c–g). Total cell proteins were then dissolved in SDS-containing sample buffer and resolved by SDS–polyacrylamide gel electrophoresis as described under Experimental Procedures. Labeled glycoproteins were visualized by fluorography. Times of treatment with NGF were as follows: 0 (lane a), 17 (lane b), 0 (lane c), 1 (lane d), 3 (lane e), 8 (lane f), or 14 (lane g) days. Numbers at the left indicate the positions of molecular weight standards (given as $M_r \times 10^{-3}$). Arrows at the right indicate the positions of NGF-responsive species (in lanes c–f), and the top arrow also marks the position of the NILE glycoprotein.

endo-β-galactosidase and galactosyltransferase. In agreement with the results of endo-β-galactosidase treatment of purified glycopeptides described above, a band corresponding to the NILE glycoprotein ws among those apparent following the labeling of poly(*N*-acetyllactosaminyl) oligosaccharides of intact PC12 cells.

That only glycoproteins containing poly(*N*-acetyllactosaminyl) oligosaccharides were labeled by this procedure was confirmed by the absence of quantitatively significant labeling if either of the enzymes was omitted or if the galactosyltransferase treatment was carried out in the presence of 10 mM *N*-acetylglucosamine. The small amount of radioactivity that was incorporated under these conditions showed an electrophoretic pattern quite unlike that seen in the specifically labeled cells. Moreover, no radioactivity was incorporated when the usual enzyme incubation procedure was carried out by using collagen scraped from a culture dish without cells.

To test whether nonreducing terminal *N*-acetylglucosamine residues are present as potential galactose acceptors in PC12 cell glycoproteins before treatment with endo-β-galactosidase, such acceptors were blocked by pretreatment with galactosyltransferase in the presence of unlabeled UDP-galactose before incubation with endo-β-galactosidase and the subsequent labeling step. It would appear that relatively few such residues exist, insofar as there was a noticeable decrease in the labeling of only one band at 85–100 kDa. However, preincubation did, unexpectedly, result in the labeling of a few glycoproteins that were otherwise seen only faintly (i.e., the NILE glycoprotein at 230 kDa and bands at 140–160 and 75–85 kDa). This effect appears to be due to the increased "metabolic" uptake and incorporation of [^{14}C]galactose, released from UDP-[^{14}C]galactose during the final 1-h incubation period. Although 1 h of preincubation increases the subsequent metabolic incorporation (measured by incubation with UDP-[^{14}C]galactose in the absence of both endo-β-galactosidase and galactosyltransferase), such incorporation of [^{14}C]galactose was generally found to represent less than 10% of the specific labeling seen in the presence of the two enzymes.

PC12 cells do not appear to contain a significant proportion of poly(*N*-acetyllactosaminyl) oligosaccharides as components of glycolipids, most of which have recently been identified as globosides (Yu et al., 1985). This was evident from the absence of labeling in the region of the tracking dye in fluorographs of SDS–polyacrylamide gels (especially in cells not treated with NGF; Figure 6) and by the finding that less than 5% of the incorporated [^{14}C]galactose was extractable with chloroform–methanol in either NGF-treated or untreated PC12 cells.

Similar studies were performed on PC12 cells that had been pretreated with nerve growth factor for various time periods. Within several days of NGF exposure, PC12 cells extend neurites and acquire a sympathetic neuronlike phenotype (Greene & Tischler, 1976, 1982). As shown in Figure 6, labeling of the 230 000-Da (NILE) glycoprotein increased beginning at 1–3 days following treatment with NGF, and this increase is probably proportional to the known effects of NGF on the cellular concentration of the NILE glycoprotein (McGuire et al., 1978; Salton et al., 1983a). The labeling of a band at about 100 kDa decreased somewhat following 3 days of NGF treatment and had almost disappeared by 1 week. This does not clearly correspond to any known NGF-regulated protein. NGF treatment also led to a simplification of the 30–50-kDa region of the fluorograph with enhancement of a band at about 50 kDa. Two experiments showed even greater changes, involving a considerable sharpening of the glyco-

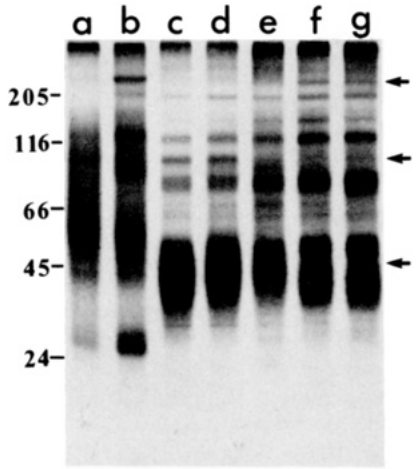

FIGURE 7: Effects of forskolin treatment and of exposure to NGF in suspension on the pattern of PC12 cell glycoproteins containing poly(*N*-acetyllactosaminyl) oligosaccharides. In lanes a and b, cells were metabolically labeled with [^{3}H]glucosamine and cultured without (lane a) or for 16 days with (lane b) NGF. Material in lanes c–g is from cells in which poly(*N*-acetyllactosaminyl) oligosaccharides were specifically labeled as described in the text: lane c, no exposure to additives; lanes d and e, exposure to 10 μM forskolin for 1 and 14 days, respectively; lanes f and g, exposure to NGF for 2 weeks on a substrate or in suspension, respectively. Other details were as in the legend to Figure 6.

protein pattern of cells treated for 2 weeks with NGF.

Cells in suspension treated for 2 weeks with NGF showed the same labeling pattern as seen in PC12 cells treated with NGF on a substrate (Figure 7), indicating that these changes do not require attachment or neurite outgrowth. To test the specificity of the NGF-induced changes, cells were exposed to epidermal growth factor (EGF) for 2 weeks. EGF elicits a variety of responses in PC12 cells (Huff et al., 1981) but does not cause neuronal differentiation. Treatment with epidermal growth factor (5 ng/mL) had no effect on the pattern of glycoproteins containing poly(*N*-acetyllactosaminyl) oligosaccharides nor on PC12 cell morphology or glycoprotein labeling with [^{3}H]glucosamine.

PC12 cells also show certain responses (but not neuronal differentiation) to agents that mimic or increase intracellular cyclic AMP [for a review, see Greene and Tischler (1982)]. After treatment for 2 weeks (but not after 2 or 19 h) with 10 μM forskolin, which results in an elevation in cellular levels of cyclic AMP in PC12 cells (Rabe et al., 1982), labeling of the 100-kDa band, which was also affected by NGF, is greatly diminished (Figure 7). However, in contrast to NGF, no effects were observed on the NILE glycoprotein. Effects of long-term forskolin treatment on the 30–50-kDa region were somewhat variable, ranging from no effect to a slight effect (Figure 7).

Discussion

These studies have revealed that PC12 cell membranes contain 15–20 glycoproteins whose tri- and tetraantennary complex oligosaccharides have one or more branches with a poly(*N*-acetyllactosaminyl) structure. We previously reported that the greater size of the 230-kDa NILE glycoprotein of PC12 cells, as compared to the immunochemically cross-reactive 205-kDa species present in brain, is due to the greater size of the PC12 cell tri- and tetraantennary oligosaccharides (Margolis et al., 1983b). Our present finding of poly(*N*-acetyllactosaminyl) oligosaccharides in the NILE glycoprotein of PC12 cells, but not that of postnatal brain (see below), is probably in itself sufficient to provide a structural basis for the difference in size of these two related glycoproteins.

In preliminary studies of 1- and 30-day-postnatal rat brain we have not been able to detect the presence of poly(*N*-acetyllactosaminyl) oligosaccharides, although such oligosaccharides were found in cultured neonatal sympathetic neurons, which can be considered to be a close nontumor counterpart of NGF-treated PC12 cells. These results indicate that the occurrence of this oligosaccharide structure reflects the common embryonic origin of pheochromocytoma cells and normal sympathetic neurons and that it is not necessarily related to the tumor origin of PC12 cells. Studies now in progress with attempt to determine whether poly(*N*-acetyllactosaminyl) oligosaccharides are characteristic only of sympathetic or peripheral nervous tissue or whether they might also be present in less differentiated embryonic central nervous tissue.

The relatively large number of PC12 cell membrane glycoproteins that our surface-labeling studies revealed to contain poly(*N*-acetyllactosaminyl) oligosaccharides was somewhat unexpected, but these presumably still represent only a very small proportion of the total number of PC12 cell glycoproteins, many of which are resolved only poorly or not at all in SDS–polyacrylamide gels of glucosamine- or fucose-labeled cells.

It was previously reported that the membranes of chromaffin granules isolated from bovine adrenal medulla contain a population of unusually large tri- and tetraantennary oligosaccharides with molecular sizes up to approximately 7000 Da, and it was suggested that the large size of these oligosaccharides might be due to the presence of poly(*N*-acetyllactosaminyl) chains (Margolis et al., 1984). Reexamination of the larger glycopeptides obtained by fractionation on Sephadex G-50 demonstrated that a significant proportion of this material was susceptible to treatment with endo-β-galactosidase (unpublished results). Since chromaffin granules are also present in PC12 cells (Tischler & Greene, 1978; Roda et al., 1980), chromaffin granule glycoproteins might account for some of the poly(*N*-acetyllactosaminyl) oligosaccharides detected in PC12 cell membranes.

The general neurobiological role of poly(*N*-acetyllactosaminyl) oligosaccharides remains to be determined, although in other systems their presence and fine structure frequently appear to be related to specific stages of cell and tissue differentiation (Fukuda, 1985; Feizi, 1985). Our preliminary finding that they do not occur in postnatal rat brain indicates that these oligosaccharides may be restricted to very early developmental periods or to only certain types of cells, such as neurons of sympathetic origin but not central nervous tissue. It is of interest in this regard that the "extension enzyme", β-galactoside:β1–3 *N*-acetylglucosaminyl transferase, which adds *N*-acetylglucosamine to C-3 of a galactose residue in Galβ1–4GlcNAc acceptors, is evidently present in brain, since a small proportion of keratan sulfate chains (having the same repeating unit with sulfate at C-6 of the *N*-acetylglucosamine) occur in the chondroitin sulfate proteoglycan of brain (Krusius et al., 1986). The apparent lack of poly(*N*-acetyllactosaminyl) oligosaccharides in other brain glycoproteins could therefore be due to the lack of acceptor proteins, rather than the lack of the necessary glycosyltransferases. The presence or absence of these oligosaccharides may also reflect the molecular organization of particular membrane glycoproteins (Fukuda, 1985), since in human erythrocytes glycophorin A, which traverses the lipid bilayer only once (Tomita et al., 1978), does not contain poly(*N*-acetyllactosaminyl) oligosaccharides, whereas they are present on the band 3

glycoprotein which is believed to cross the lipid bilayer several times (Jenkins et al., 1975; Steck et al., 1978).

It should also be noted that, in our studies, poly(*N*-acetyllactosaminyl) oligosaccharides were identified on the basis of their susceptibility to endo-β-galactosidase. Since certain highly branched or sulfated poly(lactosaminoglycans) (Fukuda & Matsumura, 1976; Scudder et al., 1984), as well as those that are close to the core portion of an oligosaccharide or contain only a limited number of poly(*N*-acetyllactosaminyl) units (Fukuda et al., 1984a,b), are relatively resistant to endo-β-galactosidase digestion, it is possible that our inability to detect appreciable amounts of these oligosaccharides in postnatal brain is due to the presence of such structural features in brain glycoproteins but not in those of PC12 cells or sympathetic neurons.

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