Distribution and Metabolism of Mucopolysaccharides and Glycoproteins in Neuronal Perikarya, Astrocytes, and Oligodendroglia†

Richard U. Margolis* and Renee K. Margolis

ABSTRACT: Neuronal perikarya, astrocytes, and oligodendroglia were isolated in bulk from calf brain and from 20- to 30-day-old rat brain. In bovine brain the highest concentration of mucopolysaccharides was found in the neurons (0.83 μmol of hexosamine/100 mg of lipid-free dry weight), while the mucopolysaccharide content of astrocytes and oligodendroglia was 68 and 17%, respectively, of that present in neurons. The percentage of the total mucopolysaccharide represented by chondroitin sulfate decreased from 64% in neurons to 24% in oligodendroglia, while the proportion of hyaluronic acid was greatest in the oligodendroglia (48%) and lowest in neurons (19%). Astrocytes had percentages of hyaluronic acid and chondroitin sulfate almost exactly intermediate between neurons and oligodendroglia. Heparan sulfate comprised 17% of the total mucopolysaccharide in both neurons and astrocytes, and 28% in oligodendroglia. The concentration of mucopolysaccharides was identical (0.64 μmol/100 mg of lipid-free dry weight) in both neurons and astrocytes from young rat brain, and the mucopolysaccharide composition in both cell types was very similar to that found in neuronal perikarya from calf brain. In contrast to their low content of mucopolysaccharides, oligodendroglia had the highest concentration of glycoprotein carbohydrate (2.4% of the lipid-free dry weight), followed by astrocytes (1.9%) and neuronal perikarya (1.1%). The sugar composition was generally similar in all three cell types, although the oligodendroglia had a lower molar ratio (to glucosamine) of fucose, mannose, and galactosamine, and a slightly higher amount of sialic acid. After a short period of labeling *in vivo* with glucosamine or sulfate, the specific activity of hyaluronic acid in rat neurons was greater than twice that of astrocytes, while the specific activity of hexosamine and sulfate in heparan sulfate was only 70–80% as high in neurons as in astrocytes. There was no significant difference in the specific activity of chondroitin sulfate. The labeling of glycoprotein hexosamine and sialic acid was 30% greater in neurons as compared to astrocytes.

There has recently been a greatly increased interest in the possible functional roles of mucopolysaccharides and glycoproteins in nervous tissue. As a result of our studies in this area, we have found that brain contains at least two major pools of mucopolysaccharides and glycoproteins, having rates of metabolism differing by 6- to 20-fold, and which exhibit different susceptibilities to extraction by various agents (Margolis and Margolis, 1972a,b; Margolis and Gomez, 1973). However, in order to understand the functional significance of these complex carbohydrates in brain, it is essential to know whether they are localized in specific cells or subcellular fractions. The development of methods for the bulk isolation of neuronal perikarya, astrocytes and oligodendroglia in good yields and having a high degree of purity (Norton and Poduslo, 1970; Poduslo and Norton, 1972a,b) has enabled us to investigate the distribution and metabolism of mucopolysaccharides and glycoproteins in neurons and in glial cells.

Experimental Section

Neuronal perikarya and astrocytes were isolated from the brains of 20- to 30-day-old rats (trimmed of cerebella) or from calf cerebrum, and oligodendroglia were isolated from calf brain white matter, as described by Norton and Poduslo (1970) and Poduslo and Norton (1972a,b). In these procedures the tissue is minced and treated with trypsin. Tissue suspensions are then prepared by sieving, and cells are isolated from the suspension by sucrose density gradient centrifugation. The yield of cells is approximately 15–20% for neurons, 7% for astrocytes, and 12% for oligodendroglia. The yield of oligodendroglia was increased from that obtained using the method as originally described (Poduslo and Norton, 1972a,b) by substituting 1.2 and 1.8 M sucrose for the 1.4 and 1.55 M sucrose used in the density gradient (T. Abe and W. T. Norton, personal communication). Although this modification also increased the contamination with capillaries, these could be completely removed with no detectable loss of oligodendroglia by filtering the final cell suspension through a short column of loosely packed glass wool. All cell preparations were finally concentrated from hexose phosphate medium without albumin to remove extraneous protein. Using 250-ml bottles in the Sorvall HS-4 rotor it was possible to process 24 g of cerebrum or 80 g of white matter per day.

Groups of 21–23 rats (25-days old) were also injected intraperitoneally with [1-^{3}H]glucosamine (3.7 μCi/g body weight) or [^{35}S]Na_2SO_4 (8 μCi/g body weight) and sacrificed after 16 hr. The yield of tritium- and of sulfate-labeled cells was noted, and they were then combined and mixed with unlabeled carrier neurons or astrocytes to provide 300 mg (lyophilized dry weight) of each cell fraction. Specific activities of sulfate and tritium in the isolated mucopolysaccharides and glycopeptides were corrected for dilution to calculate their specific activities in the labeled cells.

The identity and purity of the cell fractions were checked by phase-contrast microscopy and by examination of fixed and stained cells. These were prepared by streaking a cell suspension on a microscope slide coated with pigskin gelatin, fixing in formaldehyde vapor, and staining with Methylene Blue.

† From the Department of Pharmacology, New York University School of Medicine, New York, New York 10016, and the Department of Pharmacology, State University of New York, Downstate Medical Center, Brooklyn, New York 11203. *Received February 22, 1974.* This work was supported by research grants from the U. S. Public Health Service (MH-17018 and NS-09348).

TABLE I: Yields of Cells and of Lipid-Free Dry Tissue.

	Yield of Lyophilized Cells/100 g Wet Wt (mg)	% Lipid-Free Dry Wt
Neuronal perikarya	181[a]	64
Astrocytes	178[a]	41
Oligodendroglia	28[b]	60

[a] From rat cerebrum. [b] From calf brain white matter.

Cell preparations were pooled, dialyzed against deionized water, and lyophilized. They were then suspended in water (1 ml/250 mg of lyophilized dry weight) and extracted with chloroform–methanol (2:1, v/v) followed by chloroform–methanol in the reverse ratio of 1:2. The lipid-free protein residue was dried *in vacuo*, suspended at a concentration of 2% in boric acid–borax buffer (pH 7.8), and digested with Pronase for a total of 48 hr at 55° as described previously (Margolis and Margolis, 1970). A small amount of undigested material was removed by centrifugation and the Pronase digest was desalted by gel filtration on Sephadex G-15. The solution was then concentrated to a volume of 7 ml/100 mg of original lipid-free dry weight, and made 0.04 M in NaCl. Mucopolysaccharides were precipitated with cetylpyridinium chloride (Margolis and Margolis, 1972b), and excess cetylpyridinium chloride was removed from the supernatant solution, containing the glycopeptides derived from the glycoproteins, by extraction with *n*-amyl alcohol.

The sulfated mucopolysaccharides were separated from hyaluronic acid by differential precipitation with cetylpyridinium chloride from 0.3 M NaCl, and excess cetylpyridinium chloride was removed from the hyaluronic acid by precipitation with KSCN followed by dialysis. The concentration of hyaluronic acid in the different cell fractions was determined from the glucosamine content of the supernatant obtained after precipitating the sulfated mucopolysaccharides from 0.3 M NaCl, and heparan sulfate and chondroitin sulfate concentrations were based on the glucosamine and galactosamine content of the sulfated mucopolysaccharide fraction. Mucopolysaccharides were hydrolyzed for 3 hr at 100° in 6 N HCl, and glucosamine and galactosamine were determined using the amino acid analyzer.

Sialic acid in the glycopeptides was determined by the periodate–resorcinol method of Jourdian *et al.* (1971), glucosamine and galactosamine were quantitated using the amino acid analyzer after hydrolysis of the glycopeptides for 8 hr at 100° in 4 N HCl, and fucose was determined by the method of Gibbons (1955). Galactose and mannose were determined enzymatically as described previously (Margolis *et al.*, 1972).

To determine the specific activity of heparan sulfate and chondroitin sulfate, the sulfated mucopolysaccharide fraction was digested with chondroitinase ABC, and the resulting chondroitin sulfate disaccharides were separated from the undegraded heparan sulfate by gel filtration on Sephadex G-25 (Margolis and Margolis, 1972a, 1973a). Similarly, to determine the specific activity of hexosamine and sialic acid in the glycoproteins, glycopeptides were desialylated by mild acid hydrolysis (0.1 N H_2SO_4, 1 hr, 80°) and free sialic acid was separated from the desialylated glycopeptides by gel filtration on Sephadex G-15. This procedure yields radiochemically pure fractions of hexosamine-labeled glycopeptides and sialic acid (Margolis and Margolis, 1973a).

TABLE II: Distribution of Mucopolysaccharides in Neurons and Glia of Bovine Brain.

	Neuronal Perikarya		Astrocytes		Oligodendroglia	
Hyaluronic acid	0.159[a]	19.2%[b]	0.194	34.3%	0.069	47.9%
Heparan sulfate	0.137	16.5%	0.098	17.4%	0.040	27.8%
Chondroitin sulfate	0.534	64.3%	0.273	48.3%	0.035	24.3%
Total	0.830		0.565		0.144	

[a] Concentration in cells, expressed as μmol of constituent hexosamine per 100 mg of lipid-free dry weight. [b] Percentage of total mucopolysaccharide.

TABLE III: Distribution of Mucopolysaccharides in Neurons and Glia of Rat Brain.

	Neurons		Astrocytes	
Hyaluronic acid	0.108[a]	16.8%[b]	0.102	16.1%
Heparan sulfate	0.122	18.9%	0.087	13.8%
Chondroitin sulfate	0.414	63.4%	0.443	70.1%
Total	0.644		0.632	

[a] Concentration in cells, expressed as μmoles of constituent hexosamine per 100 mg of lipid-free dry weight. [b] Percentage of total mucopolysaccharide.

Results and Discussion

The yield and purity of cells obtained by us were comparable to that reported by Norton and Poduslo (1970, 1971) and Poduslo and Norton (1972a,b), although our dry weights are somewhat lower insofar as the cells were dialyzed before lyophilization, and the per cent lipid-free dry weight is correspondingly higher. These figures are presented in Table I. The yield of neurons and astrocytes from calf brain was approximately one-half of that obtained from young (20- to 30-day-old) rat cerebrum.

Mucopolysaccharides. The concentration and composition of the mucopolysaccharides in neurons and glia of bovine brain are summarized in Table II. Neuronal perikarya had more than five times the concentration of mucopolysaccharides found in oligodendroglia. Chondroitin sulfate decreased from 64% of the total mucopolysaccharide in neurons to 24% in oligodendroglia, while the reverse situation obtained for hyaluronic acid (19% in neurons and 48% in oligodendroglia). The relative percentage of heparan sulfate was identical in neurons and astrocytes (17%) but considerably greater (28%) in oligodendroglia.

There was very little difference in the mucopolysaccharide content and composition of neurons and astrocytes isolated from young rat brain, although the neurons had slightly more heparan sulfate and less chondroitin sulfate than the astrocytes (Table III). It is possible that at 20–30 days of age the biochemical differentiation, with respect to mucopolysaccharides, of neurons and astrocytes in rat brain has not progressed to the same extent as in the older bovine brains, thus accounting for the different results obtained in these two species.

Glycoproteins. Although oligodendroglia had a low concen-

TABLE IV: Glycoprotein Carbohydrate Composition of Neurons and Glia from Bovine Brain.

	Neuronal Perikarya		Astrocytes		Oligodendroglia	
	μmol/100 mg of LFDW[a]	Molar Ratio	μmol/100 mg of LFDW	Molar Ratio	μmol/100 mg of LFDW	Molar Ratio
N-Acetylglucosamine	1.373	1.00	2.623	1.00	3.396	1.00
N-Acetylgalactosamine	0.097	0.07	0.122	0.05	0.109	0.03
N-Acetylneuraminic acid	0.608	0.44	1.019	0.39	1.687	0.50
Fucose	0.552	0.40	1.177	0.45	0.573	0.17
Galactose	0.704	0.51	1.234	0.47	1.734	0.51
Mannose	2.184	1.59	3.284	1.25	3.636	1.07
Per cent carbohydrate[b]	1.1		1.9		2.4	

[a] LFDW = lipid-free dry weight. [b] % by weight of lipid-free dry weight.

tration of mucopolysaccharides they had the highest amount of glycoprotein carbohydrate (2.4% of the lipid-free dry weight), while neurons had less than half of that amount (1.1%) and astrocytes were again intermediate in concentration (Table IV). It can also be seen from Table IV that the average sugar composition of the glycoproteins was generally similar in all three cell types, although the oligodendroglia had a lower molar ratio (to glucosamine) of fucose, mannose, and galactosamine, and a slightly higher amount of sialic acid.

The amounts of the various glycoprotein sugars in neurons and astrocytes of rat brain (Table V) were very similar to those found in bovine brain.

The trypsin used in the initial cell preparation might be expected to remove most or all of the cell surface glycoproteins. However, the glycoprotein carbohydrate in the isolated neurons and glia from rat brain (5.2 and 8.2 μmol per 100 mg of lipid-free dry weight, respectively; Table V) is very close to that found in the lipid-free protein residue from whole rat brain (7.4 μmol/100 mg). Moreover, the concentration of mucopolysaccharides in neurons and astrocytes prepared by trypsin treatment (Table III) is 40% greater than that present in 25-day-old rat cerebrum (0.455 μmol of hexosamine/100 mg of lipid-free dry weight, comprising 24% hyaluronic acid, 13% heparan sulfate, and 63% chondroitin sulfate). These data indicate that most of the mucopolysaccharides and glycoprotein carbohydrate in brain is internal or otherwise inaccessible when minced brain is treated with trypsin according to the procedure of Norton and Poduslo (1970), although they do not exclude the possibility that trypsinization of isolated cells might have much greater effects on their content of these complex carbohydrates. The high concentration of lipids in brain may also have some influence on the effects of trypsinization.

Metabolism. To determine whether differences exist in the metabolic activity of mucopolysaccharides and glycoproteins in neurons and glial cells, these complex carbohydrates were labeled *in vivo* for 16 hr in their hexosamine, sulfate, and sialic acid residues, and neurons and astrocytes were then isolated from rat cerebra in the usual manner. The specific activities of the mucopolysaccharides and glycoproteins are summarized in Table VI. The only major difference between the two cell types concerns hyaluronic acid, whose specific activity in neurons is greater than twice that in astrocytes. The specific activities of sulfate and hexosamine in heparan sulfate are 20–30% less in neurons than in astrocytes, while the specific activities of hexosamine and sialic acid in the glycoproteins are 30% greater in neurons.

We have previously demonstrated that two major metabolic pools of hyaluronic acid, heparan sulfate, and glycoproteins are present in brain (Margolis and Margolis, 1973a; Margolis and Gomez, 1973). One of these has a rapid turnover rate, with a half-time of 1–6 days, while the half-time for the slow compo-

TABLE V: Glycoprotein Carbohydrate Composition of Neurons and Glia from Rat Brain.

	Neuronal Perikarya		Astrocytes	
	μmol/100 mg of LFDW[a]	Molar Ratio	μmol/100 mg of LFDW	Molar Ratio
N-Acetylglucosamine	1.482	1.00	2.475	1.00
N-Acetylgalactosamine	0.089	0.06	0.130	0.05
N-Acetylneuraminic acid	0.657	0.44	1.038	0.42
Fucose	0.471	0.32	0.845	0.34
Galactose	0.664	0.45	1.183	0.48
Mannose	1.855	1.25	2.573	1.04
Per cent carbohydrate[b]	1.1		1.7	

[a] LFDW = lipid-free dry weight. [b] % by weight of lipid-free dry weight.

TABLE VI: Specific Activities of Mucopolysaccharides and Glycoproteins in Neuronal Perikarya and Astrocytes Isolated from Rat Brain 16 hr after the Administration of Labeled Precursors.[a]

	dpm/μmol		Neurons: Astrocytes
	Neurons	Astrocytes	
Hyaluronic acid	195,300	87,200	2.2
Chondroitin sulfate			
Hexosamine	76,100	81,100	0.9
Sulfate	136,500	121,900	1.1
Heparan sulfate			
Hexosamine	252,000	366,900	0.7
Sulfate	355,700	452,400	0.8
Glycoproteins			
Hexosamine	280,400	215,400	1.3
Sialic acid	100,200	80,100	1.3

[a] Specific activities are corrected for dilution with carrier but not for radioactivity decay (2 months).

nent ranges from 2 weeks in the glycoproteins to 6 weeks for hyaluronic acid. Based on these half-times, the specific activities of the mucopolysaccharides and glycoproteins in neurons and astrocytes after a relatively short period (16 hr) of labeling should be roughly proportional to their turnover rates.

From our data we can conclude that the turnover of hyaluronic acid is considerably more rapid in neurons than in astrocytes. More than one-third of the hyaluronic acid in brain is resistant to extraction with either water or Triton X-100 (Margolis and Margolis, 1973b), and attains only 44% of the specific activity of the more soluble pool after 24-hr labeling with glucosamine (Margolis and Margolis, 1973a). From our present results demonstrating that the hyaluronic acid in astrocytes also has only 45% of the specific activity of that in neurons after 16-hr labeling it would appear that the pool of hyaluronic acid with a slow turnover, and present in a structurally stable location in the cell, may be specifically localized in astrocytes. (It was not possible to study the composition and metabolism of oligodendroglia from rat brain, since large amounts of white matter are required for their isolation in a sufficient yield and degree of purity.)

We have also previously reported that in contrast to the other mucopolysaccharides and the glycoproteins, there was no evidence from turnover or differential extraction studies for the existence of more than a single metabolic pool of chondroitin sulfate in brain (Margolis and Margolis, 1972a, 1973a). It is therefore of interest that chondroitin sulfate is the only one of the four products studied which exhibited no significant difference in specific activity between neurons and glia. This finding also indicates that the pools of precursor hexosamine and sulfate are of approximately equal size and specific activity in the two types of cells.

General Discussion. Dorfman and Ho (1970) have reported the synthesis of hyaluronic acid, chondroitin 4-sulfate, and heparan sulfate by a clonal strain of glial cells from a rat glial tumor induced by injection of *N*-nitrosomethylurea, and Stoolmiller (1972; Stoolmiller *et al.*, 1973) later reported the isolation of small amounts of hyaluronic acid, chondroitin sulfate, and heparan sulfate from a clonal cell strain (NB41A) of mouse neuroblastoma C1300 grown in tissue culture. On the basis of the relatively low rates of incorporation of labeled acetate and sulfate into the mucopolysaccharides of neuroblastoma as compared to the previous findings with rat glial tumor cells, Stoolmiller and coworkers (1972, 1973) suggested that the mucopolysaccharides in brain are synthesized almost entirely by glial elements.

Although histochemical (Young and Abood, 1960; Castejón, 1970a,b) and autoradiographic (Hirosawa and Young, 1971) studies have indicated that mucopolysaccharides are present in neurons as well as in glial cells, no data were available on the mucopolysaccharides and glycoproteins of these cells in normal brain. Our results reported here demonstrate that the mucopolysaccharide content of neuronal perikarya from young rat brain is equal to that of astrocytes, while in bovine brain the mucopolysaccharide concentration in neurons is 50% greater than in astrocytes and almost six times that of oligodendroglia. The metabolism of chondroitin sulfate in rat brain was generally similar in both neurons and astrocytes, but it would appear that the turnover of hyaluronic acid is more than twice as rapid in neurons as in astrocytes. Our data suggest that a metabolically active pool of hyaluronic acid may have a specific functional association with neurons, while chondroitin sulfate appears to be present in a single metabolic pool throughout the brain, possibly in the cytoplasm or in the extracellular space. A portion of the mucopolysaccharides found in the isolated cells may also represent an intracellular biosynthetic (or degradation) pool of compounds destined for an extracellular fate. Glycoproteins are mainly membrane constituents of both cell types, but are present in higher concentration in glial cells. Their carbohydrate composition in oligodendroglia differs somewhat from that of neurons and astrocytes, and the turnover of glycoprotein hexosamine and sialic acid is more rapid in neurons.

Our analyses of neurons refer only to the cell body since in the isolation procedure most of the processes are lost. Although the axons, synaptic membrane, synaptic vesicles, and synaptic junctional complexes may constitute a relatively small part of the neuronal mass, they are of great functional importance, and we are therefore currently extending our studies of mucopolysaccharides and glycoproteins in nervous tissue to include these subcellular fractions.

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