Journal of Neurochemistry, 1969, Vol. 16, pp. 1529 to 1532. Pergamon Press. Printed in Northern Ireland

ISOLATION AND CHARACTERIZATION OF GLYCOSAMINOGLYCANS IN PERIPHERAL NERVE AND SPINAL CORD OF MONKEY

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(*Received* 28 *May* 1969. *Accepted* 4 *June* 1969)

Abstract—The isolation of uronic acid-containing glycosaminoglycans from peripheral nerve and spinal cord of monkey was done by combining the cetyl pyridinium procedure and DEAE-Sephadex column chromatography. The constituent analyses of the isolated GAG-fractions indicated that hyaluronic acid, chondroitin-4-sulphate, chondroitin-6-sulphate, heparan sulphate and a testicular hyaluronidase-resistant galactosamine-containing GAG were present in both tissues. Hyaluronic acid was the predominant GAG (63 per cent) in both tissues and its level was much higher than in brain. Chondroitin-4-sulphate constituted 16 per cent in both tissues. The levels of heparan sulphate and hyaluronidase-resistant galactosamine-containing GAG in these tissues were much lower than in brain. The results indicate that the patterns of GAGs in peripheral nerve and spinal cord of monkey are similar but differ from that of brain.

SEVERAL laboratories have reported the identification of glycosaminoglycans (GAGs) in different nervous tissues. ABOOD and ABUL-HAJ (1956) showed histochemically the presence of hyaluronic acid in peripheral nerve of bullfrogs. The work of SZABO and ROBOZ-EINSTEIN (1962) indicated the occurrence of sulphated and non-sulphated GAG in bovine spinal cord and brain. Further they noticed that the concentration of sulphated GAG in spinal cord was much less than in brain. This finding prompted us to extend our investigation on the nature of GAG in brain to spinal cord as well as to peripheral nerve. The present paper reports on the isolation and characterization of uronic acid-containing GAGs in spinal cord and peripheral nerve of monkey.

MATERIALS AND METHODS

Cetyl pyridinium bromide was obtained from Mann Research Laboratories, U.S.A. and chondroitin-6-sulphate from Miles Research Laboratories. Glucosamine-HCl, galactosamine-HCl, hyaluronic acid and testicular hyaluronidase type I were supplied by Sigma Chemicals, U.S.A. Sephadex G-75 and DEAE-Sephadex A-25 were obtained from Pharmacia Uppsala, Sweden. Papain (crude) and pronase B were received respectively from Central Food Technological Research Institute, Mysore, and California Biochemical Corporation, U.S.A. Other reagents used were of analytical grade.

The processing of the tissues for the isolation of GAGs was carried out as described earlier (SINGH and BACHHAWAT, 1968), and 12·2 g of lipid free dry tissue were obtained from 77·3 g spinal cord and 11·1 g from 52·7 g peripheral nerves (median and lateral popliteal).

Analyses. The modification of the carbazole reaction (DISCHE, 1947) by BITTER and MUIR (1962) was used to estimate uronic acid. Dermatan sulphate was identified by making use of its very low colour yield in carbazole reaction without borate. The total hexosamine, galactosamine, sulphate, non-acetylated hexosamine (N-sulphate), chondroitin-6-sulphate and hyaluronidase resistant GAG were estimated as described earlier (CHANDRASEKARAN and BACHHAWAT, 1969).

RESULTS

Table 1 presents the concentration of the three GAG fractions isolated from the tissues by the cetyl pyridinium procedure. Peripheral nerve contains twice the amount of GAG present in spinal cord. In both tissues the ratios of fractions I to II are

Abbreviation used: GAG, glycosaminoglycans.

TABLE 1.—CONCENTRATION OF GAG-FRACTIONS IN NERVOUS TISSUES

Tissue	μg uronic acid/g dry defatted tissue				Ratio I/II
	Fraction I	Fraction II	Fraction III	Total	
Spinal cord	486	209	14	709	2·33
Peripheral nerve	1010	415	31	1456	2·44
Brain*	1550	1320	2	2872	1·18

* For comparison, the values taken from an earlier paper (SINGH *et al.*, 1969).

roughly the same and fraction III is very low. Further resolution of fractions I and II by DEAE-Sephadex chromatography (Table 2) shows the fraction I-SB is very low in peripheral nerve and fraction II-SB constitutes 28–33 per cent of fraction II-SC in both tissues. The results of the analyses of fractions are given in Table 3.

Fraction I-SA. In both tissues this fraction consists of glucosamine only and very little non-acetylated amino sugar. It is completely digestible by hyaluronidase indicating the glycosaminoglycan in this fraction is hyaluronic acid.

Fraction I-SB. Amino sugar analyses indicate that this fraction in both tissues consists of 70 per cent glucosamine and 30 per cent galactosamine. The molar ratio of non-acetylated amino sugar to uronic acid is 0·36 in this fraction from both tissues showing that all the glucosamine-containing GAG can be accounted for as heparan sulphate. The presence of 0·59 molar sulphate with respect to uronic acid in this fraction from peripheral nerve indicates that the galactosamine-GAG is

TABLE 2.—THE LEVELS OF DEAE-SEPHADEX FRACTIONS IN NERVOUS TISSUES

Tissue	μg uronic acid/g dry defatted tissue				I-SA / I-SB	II-SB / II-SC
	I-SA	I-SB	II-SB	II-SC		
Spinal cord	447	39	52	157	11·5	0·33
Peripheral nerve	970	40	91	324	24·3	0·28
Brain*	1368	182	528	792	7·5	0·66

* The values reported from an earlier paper for comparison (SINGH *et al.*, 1969).

TABLE 3.—ANALYTICAL DATA OF THE DEAE-SEPHADEX FRACTIONS ISOLATED FROM SPINAL CORD AND PERIPHERAL NERVE

Tissue and fraction	Expressed as molar ratio of uronic acid				As percentage of total GAG in the fraction	
	Hexosamine	Galactosamine / Glucosamine	Non-acetylated hexosamine	Sulphate	Chondroitin-6-sulphate	Hyaluronidase resistant
Spinal cord:						
I-SA	0·75	Glucosamine	0·04	0·12	—	Nil
I-SB	0·75	0·43	0·36	N.D.	31*	N.D.
II-SB	0·70	1·00	0·27	0·49	25*	N.D.
II-SC	0·90	Galactosamine	0·02	0·93	20	8
Peripheral nerve:						
I-SA	0·80	Glucosamine	0·04	0·10	—	Nil
I-SB	0·70	0·40	0·36	0·59	30*	N.D.
II-SB	0·80	0·80	0·28	0·51	14*	N.D.
II-SC	0·80	Galactosamine	0·03	0·90	12	23

* Would include low-sulphated chondroitin-4-sulphate (SINGH and BACHHAWAT, 1968).
N.D.—Not done because of insufficient material.

low-sulphated. The chondroitin-6-sulphate value obtained from hyaluronidase-digestion method would include low-sulphated chondroitin-4-sulphate also (SINGH and BACHHAWAT, 1968). It is 30 per cent of the fraction in both tissues.

Fraction II-SB. In spinal cord this fraction consists of 50 per cent each of glucosamine and galactosamine and in peripheral nerve glucosamine is 56 per cent of the total hexosamine and the rest is galactosamine. The molar ratio of non-acetylated hexosamine to uronic acid is 0·27 in spinal cord and 0·28 in peripheral nerve, indicating that the glucosamine-GAG is heparan sulphate. The molar ratio of sulphate to uronic acid is 0·49 to 0·51 in this fraction from both tissues indicating that the galactosamine-GAG is low-sulphated. Chondroitin-6-sulphate as estimated by hyaluronidase-digestion method was found to be 25 per cent of the fraction in spinal cord and 14 per cent in peripheral nerve. These values would include low-sulphated chondroitin-4-sulphate that may be present in this fraction.

Fraction II-SC. The presence of galactosamine only as the amino sugar and very little non-acetylated amino sugar indicates the absence of heparan sulphate in this fraction. Sulphate is present in approximately equimolar ratio to uronic acid. The hyaluronidase-resistant GAG as estimated by Sephadex G-75 filtration of the hyaluronidase-digest comprises 8 per cent of the fraction in spinal cord and 23 per cent in peripheral nerve. The resistant GAG from peripheral nerve gave 41 per cent colour only in the carbazole reaction without borate indicating its nature to be dermatan sulphate. Chondroitin-6-sulphate as estimated by hyaluronidase-digestion was found to be 25 per cent of this fraction in spinal cord and 12 per cent in peripheral nerve.

The quantities of the identified GAGs in the various fractions are presented in Table 4. The major GAG is hyaluronic acid (63 per cent of the total GAG) and chondroitin-4-sulphate is 16 per cent in both tissues. In spinal cord, heparan sulphate is 5·5 per cent and chondroitin-6-sulphate is 7·9 per cent; in peripheral nerve they are 7·5 per cent and 5·2 per cent respectively. The chondroitin-6-sulphate value includes

TABLE 4.—THE NATURE AND QUANTITY OF THE GLYCOSAMINOGLYCANS IN THE PERIPHERAL NERVE, SPINAL CORD AND BRAIN OF MONKEY

GAG-Fractions	μg uronic acid/g lipid free dry tissue		
	Peripheral nerve	Spinal cord	Brain*
I-SA:			
Hyaluronic acid	970	447	1176
I-SB:			
Heparan sulphate	28	27	73
Chondroitin-6-sulphate including low-sulphated chondroitin-4-sulphate	12	12	42
II-SB:			
Heparan sulphate	52	26	253
Chondroitin-6-sulphate including low-sulphated chondroitin-4-sulphate	13	13	95
Chondroitin-4-sulphate	26	13	180
II-SC:			
Hyaluronidase-resistant, Galactosamine-GAG	75	13	292
Chondroitin-6-sulphate	39	31	136
Chondroitin-4-sulphate	210	113	364

* Values given from an earlier paper (SINGH *et al.*, 1969).

low-sulphated chondroitin-4-sulphate. The hyaluronidase resistant galactosamine-GAG is 3 per cent in spinal cord and 8 per cent in peripheral nerve.

DISCUSSION

The present study and our earlier investigations (SINGH and BACHHAWAT, 1968; SINGH, CHANDRASEKARAN, CHERIAN and BACHHAWAT, 1969) on the nature of GAG in brain indicate that all types of GAG are present in central and peripheral nervous systems. The present finding that the concentrations of total GAG in spinal cord and peripheral nerve are 25 per cent and 50 per cent respectively of the concentration of GAG in brain indicates the variation in the concentration of GAG in different parts of the central nervous system (brain and spinal cord) as well as in the two nervous systems (central and peripheral). This is further supported by the finding of SINGH (1967) that the concentration of GAG in human spinal cord is much less than in frontal grey and frontal white matter of human brain.

Our work on the nature of GAG in brain of different species (SINGH *et al.*, 1969) showed that the patterns of GAGs in various brains are the same except in chicken brain. In the present study, it is noticed that the levels of various GAGs in spinal cord and peripheral nerve differ from that in brain. Hyaluronic acid comprises 63 per cent of the total GAG in spinal cord and peripheral nerve whereas it is 41 per cent only in brain. Though hyaluronic acid is the major GAG and chondroitin-4-sulphate is the next prominent GAG in all nervous tissues, the levels of heparan sulphate and chondroitin-6-sulphate are lower in spinal cord and peripheral nerve, when compared to brain. Further, the hyaluronidase-resistant galactosamine-GAG is less in peripheral nerve and is in trace amount in spinal cord. The hyaluronidase-resistant GAG in peripheral nerve was found to be dermatan sulphate.

SZABO and ROBOZ-EINSTEIN (1962) reported the ratio of hyaluronic acid to chondroitin sulphate as 3 in bovine spinal cord and 0·8 in bovine brain. Comparable with these values, we find the ratio of hyaluronic acid to sulphated galactosamine-GAG in spinal cord and peripheral nerve as 2·5 whereas the ratio is 1·1 in monkey brain.

ABOOD and ABUL-HAJ (1956) have identified hyaluronic acid histochemically in a comparatively high concentration in the axoplasm and between the myelin and neurilemma sheath of the peripheral nerves from bullfrogs. In myelin sheath of rat peripheral nerve, WOLMAN (1957) found a weakly acidic polysaccharide. Consistent with these findings, the present study indicates that hyaluronic acid is the most predominant GAG in peripheral nerve.

Acknowledgements—The work was supported by Grant No. NIH-IND 6X4324 from the National Institute of Health, U.S. Public Health Service. Dr. G. M. TAORI's help in obtaining the tissues is gratefully acknowledged.

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