slices showed absolutely no spontaneous electrical activity in field potentials. It has been shown that the paroxysmal electrical activity can be produced in hippocampal slices in vitro by increasing the potassium concentration in the medium[3,8-10]. Figure, A illustrates the paroxysmal discharge observed in high potassium medium. The rate of occurrence of the paroxysmal discharge was usually less than 0.5/sec, and the interval between 2 consecutive paroxysmal discharges was relatively regular. When chloride was removed from the high potassium medium, both amplitude and total duration of the paroxysmal discharge were explosively augmented, and, in addition, clonic relapsing discharges, each of which had relatively short duration, appeared subsequently to the original discharge (figure, B). The number of the clonic relapsing discharges ranged from 3 to 10 arbitrarily in an unpredictable manner during the observation from the same preparation. The same phenomenon as the above observations which were seen in spontaneous electrical activities could also be observed in case of the evoked response by mossy fibre stimulation (figures, C and D). In this case, the evoked response in high potassium and normal chloride medium never developed into the clonic relapsing discharges, even by an extremely strengthened stimulation. Both spontaneous and evoked clonic relapsing discharges disappeared reversibly when the concentration of chloride in the medium was restored to normal. Further development of the discharges into full-brown seizure was not observed in both spontaneous and evoked activities. This might be partly ascribed to the metabolic limitations, such as accumulation of CO_2 and depletion of O_2 or high-energy phosphate intermediates, because metabolic capacity of the sliced preparation from the mammalian brain is assumed to be poor compared with that of the intact brain. It might also be plausible that a neural circuit involved in the sliced preparation is incomplete for the generation of full-brown seizure. The possibility that the clonic relapsing discharges were due to deterioration or injury of cells might be excluded, because discharges in these cases showed completely irregular patterns and occurred irreversibly.

In a series of previous reports[3,8-10], the following findings have been confirmed by the intracellular recordings from pyramidal cells in vitro. a) IPSP is retained in high potassium medium in spite of the emergence of the paroxysmal depolarization shift. b) On the other hand, in chloride-deficient medium with normal potassium concentration, IPSP is replaced by a small depolarizing potential accompanied by spike generation, and the paroxysmal depolarization shift was not observed. Therefore, it was indicated that, although chloride depletion causes the change in excitability by facilitating the synaptic transmission, it is not essential to the initiation of the focal paroxysm. Furthermore, changes in electrical activity of the paroxysmal discharge after the removal of chloride from the medium (figure) seem to be essentially identical to that observed at the transitional stage from interictal spikes to seizure in hippocampus in vivo (see figure 13 of Dichter and Spencer[5]). Thus, the present experiments support the notion that the reduction of inhibitory processes in hippocampus may play a causative factor in the evolution of the focal paroxysm into an ictal event, whereas it does not appear to be indispensable for the generation of the focal paroxysm.

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Proteoglycans in ovine brain

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Summary. Sulphated proteoglycans were isolated using a dissociative method of extraction. Cerebrum accounted for the major portion of proteochondroitin sulphate in brain, whereas the brain stem fraction contained over half the proteoheparan sulphate. Chemical characterization studies demonstrated that heparan sulphate from cerebellum contain more N-sulphate glucosamine residues.

The existence of chondroitin and heparan sulphate in nervous tissue has been firmly established. Owing to problems in isolation little is known of the molecular organization of these compounds. However, transferases associated with the addition of neutral sugars to a protein acceptor at the potentially reducing ends of glycosaminoglycans have been demonstrated[3]. We have recently reported that chondroitin sulphate and heparan sulphate exist as proteoglycans in brain tissue using a nondisruptive method of isolation[4,5]. In this present work the distribution of proteoglycans within specific locations have been studied.

Materials and methods. Fresh adult sheep brains were freed from adipose tissue, blood vessels and other adhering tissue. Brain tissue was carefully dissected into cerebrum, cerebellum and brain stem and each fraction was defatted with chloroform-methanol (3:1, v/v). Lipid-free batches (about 10 g) were suspended in 0.1 M citrate, pH 3.1, and further extracted with 4 M guanidinium chloride[4]. On removal of guanidinium ions by dialysis against water an insoluble residue formed was collected by centrifugation and dried over acetone. This residue was then extracted with 0.4 M citrate, pH 5.0, for 12 h at room temperature and the suspension clarified by centrifugation at 35,000 × g. The supernatant (fraction B) was then chromatographed on DEAE-cellulose (figure).

Free glycosaminoglycan chains were released by papain

Distribution and chemical analysis of proteoglycans in ovine brain

Fraction	Protein*	Galactose/ xylose (molar ratio)	Hexuronic acid**	Sulphate**	N-substituted sulphate (%)	Yield (% dry lipid-free)
Cerebrum Bm	20.8	1.8	0.9	1.0	–	0.38
Cerebrum Bn	12.1	1.7	0.85	1.75	65	0.20
Cerebellum Bm	21.6	2.0	1.0	1.1	–	0.21
Cerebellum Bn	11.8	2.1	1.1	1.8	72	0.17
Brain stem Bm	20.5	1.9	1.0	1.0	–	0.15
Brain stem Bn	12.0	1.8	0.9	1.7	70	1.7

* Percent dry weight ash-free sample. ** Expressed as molar ratio hexosamine.

hydrolysis in 0.1 M acetate, pH 5.5, containing 2 mg/ml cysteine for 24 h at 55 °C using 5 mg of enzyme per g of insoluble material. After dialysis, samples were separated on a DEAE-cellulose column (100 mm × 26 mm; Whatman DE-52) using a linear gradient (0–2 M NaCl) in 0.1 M citrate, pH 5.0, at a flow rate of 30 ml/h. Hexuronic acid-containing fractions were precipitated by the addition of 4 vol. ethanol, 1 vol. being saturated with potassium acetate.

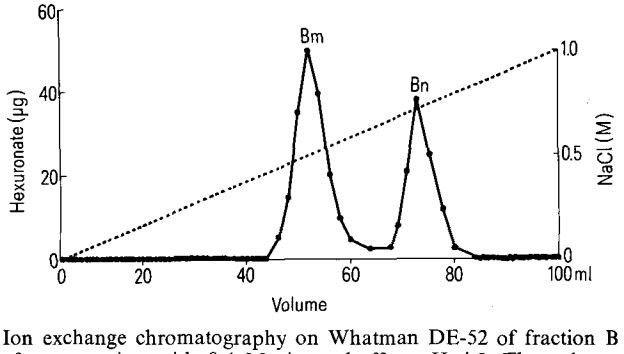
Amino acid and hexosamine analyses were carried out on an amino autoanalyzer. N-sulphated hexosamine residues were determined by the nitrous acid procedure[6] and hexuronic acid by the carbazole reaction[7]. All other analytical procedures have been described elsewhere[4].

Analytical gel chromatography was carried out on a column (800 mm × 16 mm) of Sepharose 4B (Pharmacia) which had been equilibrated in 0.5 M sodium acetate, pH 7.0. Samples (2 mg/ml) were run at a flow rate of 6 ml/h. Alkali-induced β-elimination incubations were done at 25 °C for 24 h and the mixture desalted on a sephadex G-10 column. Products eluted in the void volume were chromatographed on a DEAE-cellulose using a salt elution gradient as described above. Cellulose acetate electrophoresis was performed at 6 V/cm for 2 h using 0.1 M barium acetate, pH 7.2, following staining with 0.6% toluidine blue in 1% acetic acid. Digestion of samples with chondroitinase ABC (Sigma) was carried out in 0.1 M sodium acetate, pH 7.0. After incubation at 37 °C for 8 h the unsaturated oligosaccharides released were measured spectrophotometrically at 232 nm.

Results and discussion. Delipidation with chloroform/methanol accounted for about 10% wet wt of each fraction. Analysis of brain fractions for hexuronic acid-containing material released after papain hydrolysis showed whole brain to contain 0.2% cerebrum 0.16%, cerebellum 0.18% and brain stem 0.27% lipid-free dried material. Suspension of defatted material in guanidinium chloride solubilized 80% of the glycosaminoglycans present in the various fractions studied. However, recent studies in my laboratory have shown that only 70% of glycosaminoglycans in a rat brain preparation were solubilized under similar dissociative conditions. Ion exchange chromatography of extracts yielded 2 discrete fractions Bm and Bn (figure). Furthermore both fractions were eluted as monodispersed peaks when chromatographed on 4% agarose. Distribution coefficients (Kav) of 0.32 for Bm and 0.67 for Bn were estimated for all brain fractions. All Bm-fractions contained galactosamine and when incubated with chrondroitinase resulted in the degradation of glycosaminoglycan chains which were detected after gel filtration on Sephadex G-25. Protein fractions were eluted in the void volume with unsaturated oligosaccharide fractions being retained by the column. Only glucosamine was detected in Bn-fractions which were also resistant to chondroitinase activity. Electrophoresis showed that both Bm- and Bn-fractions migrated as single species with mobility rates similar to chondroitin 6-sulphate (Sigma) and heparan sulphate from bovine lung respectively.

Yields and chemical composition of the isolated fractions are shown (table). The protein moieties of Bm- and Bn-fractions did vary in amino acid composition with differences in serine, glycine, aspartate and glutamate. Moreover antisera against proteoglycans from bovine nasal septum cross-reacted with only Bm. Treatment of brain proteoglycans with alkali resulted in partial reductions of serine (Bm, 50% decrease; Bn, 19% decrease) with no loss of threonine. Thus it seems that all Bm- and Bn-fractions exist as multichained complexes in which glycosaminoglycans chains are covalently linked through a neutral sugar moiety to specific serine residues in the protein core. Distribution studies showed that the cerebrum contained 86% of the total proteochondroitin sulphate in brain whereas 60% of the proteoheparan sulphate was concentrated in brain stem.

Little is known of the biological role of these macromolecules in nervous tissue. However, they have been implicated with extracellular cation interactions[8] and also with the storage of catecholamines[9]. The distribution of proteoglycans may therefore influence both inter- and intracellular events within specific regions in the brain.



Ion exchange chromatography on Whatman DE-52 of fraction B after extraction with 0.4 M citrate buffer, pH 4.5. The column (300 mm × 26 mm) was eluted at 20 ml/h with 0.1 M citrate, pH 5.0, containing increasing concentrations of NaCl.

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