

ENCEPHALITOGENIC BASIC PROTEIN FROM SCIATIC NERVE

Beverley BENCINA and P.R.CARNEGIE

*Russell Grimwade School of Biochemistry, University of Melbourne,
Parkville, Victoria 3052, Australia*

and

T.A.McPHERSON* and G.ROBSON

*Clinical Research Unit of the Walter and Eliza Hall, Institute of Medical Research,
University of Melbourne, Parkville, Victoria 3052, Australia*

Received 5 May 1969

Experimental allergic encephalomyelitis (EAE) can be produced by injecting animals with either a homogenate of whole central nervous tissue or basic protein extracted from central nervous tissue myelin together with Freund's complete adjuvant (FCA) [1,2]. Experimental allergic neuritis (EAN) is induced by injecting homogenate of whole sciatic nerve with FCA [3-6]. Because EAE and EAN have many similarities we thought that the antigen responsible for EAN might be a basic protein from peripheral nerves.

Bovine sciatic nerve (30 g) was frozen in liquid nitrogen and ground to a powder. The powder was defatted with cold chloroform: methanol (2:1, v/v), dried and extracted with 450 ml HCl (0.05 M) containing NaCl (0.2 M). The acid extract (pH 2.5-3) was adjusted to pH 5 and dialysed against distilled water at 5°C overnight. The extract was concentrated and mixed with CM-Sephadex (C-25, 2 g). The non-adsorbed material (SN1) contained proteins of high molecular weight. The adsorbed fraction (SN2) was eluted with buffer (0.25 M Na₂HPO₄, 1 M NaCl, pH 8.0) and contained basic proteins of low molecular weight. Fraction SN2 was chromatographed on Sephadex G-50 with 1M acetic acid as solvent and yielded three fractions (fig. 1). All fractions were freeze-dried and from the dry-weights it was estimated that 100 mg fresh sciatic nerve contained 31 µg of

fraction SN2C. All fractions were examined by polyacrylamide gel electrophoresis at pH 2.5 [7] (fig. 2). It can be seen that fraction SN2A, the high molecular weight fraction from chromatography on Sephadex G 50 (fig. 1) contains slow moving proteins. Fraction SN2B contains the major basic protein of fraction SN2, while fraction SN2C, the low molecular weight fraction from Sephadex chromatography contains a fast moving component contaminated with some of the major component.

Using gel filtration with reference proteins (fig. 1), fraction SN2C was found to have a molecular weight of approximately 10,000, which is slightly less than

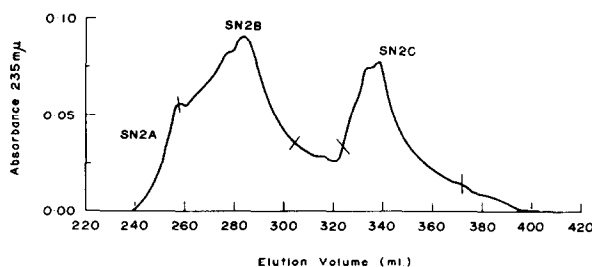


Fig. 1. Elution profile of fraction SN2 on Sephadex G-50 column (2.5 cm X 138.5 cm). Elution was carried out at room temperature with 1M acetic acid. The peak of blue dextran appeared at 228 ml and that of cytochrome c at 257 ml under these conditions.

* Present address: Department of Medicine, University of Alberta, Edmonton 61, Alberta, Canada.

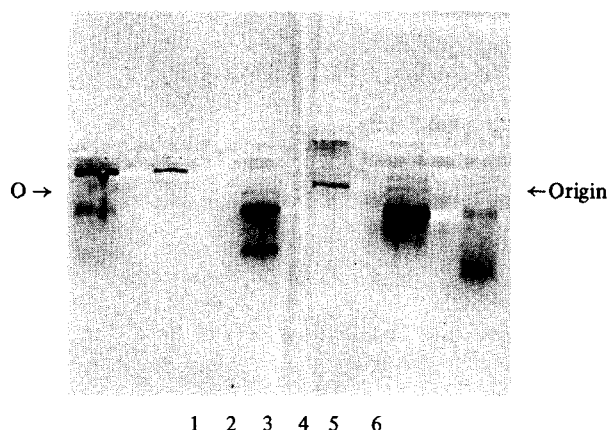


Fig. 2. Polyacrylamide gel electrophoresis of sciatic nerve fractions. Electrophoresis was carried out in 15% polyacrylamide gel in formic acid:acetic acid:water (1:4:45, by vol.), 4M urea, pH 2.5 for 2 hr at 900 V (1) Sciatic nerve acid extract, (2) SN1, (3) SN2, (4) SN2A, (5) SN2B, (6) SN2C.

that of the encephalitogenic basic protein from bovine spinal cord [2]. Amino acid analysis (table 1) showed marked differences between the compositions of the basic proteins of sciatic nerve and spinal cord. Recently peripheral and central myelin have been shown to contain basic proteins of markedly different amino acid composition [8,9]. It is probable that fraction SN2C is derived from peripheral myelin. Electrophoresis of central and peripheral nervous tissue basic protein in polyacrylamide gel was carried out at three different pH values. Mobilities relative to cytochrome *c* (M_c values) [7] are given in table 1.

Five groups of adult guinea pigs were used to test sciatic nerve fractions for ability to produce disease (table 2). Samples were injected into both hind foot pads. Each guinea pig received 0.1 ml of a mixture of 0.05 ml of an aqueous solution of a sciatic nerve fraction (containing protein equivalent to 100 mg fresh sciatic nerve) and 0.05 ml FCA.

The animals were observed daily and killed when disease was apparent or at 30 days. Brain, spinal cord and sciatic nerve were immediately fixed in formalin; at least six sections from each tissue were stained with haematoxylin and eosin and examined. All six guinea pigs injected with the whole homogenate of sciatic

Table 1
Amino acid composition and electrophoretic behaviour of basic proteins from bovine sciatic nerve (SN2C) and bovine spinal cord [2].

| | SN2B | SN2C | Spinal cord Basic protein |
|--|------|---------|------------------------------|
| Amino acid composition (moles/100 moles) | | | |
| Asp | 8.4 | 9.9 | 6.9 |
| Thr | 7.9 | 9.0 | 4.4 |
| Ser | 7.3 | 7.6 | 9.8 |
| Glu | 11.5 | 10.2 | 6.8 |
| Pro | 0.7 | 1.5 | 7.4 |
| Gly | 10.5 | 10.2 | 14.5 |
| Ala | 7.5 | 5.9 | 8.4 |
| Val | 6.8 | 6.7 | 2.2 |
| ¹ Cys | 1.3 | 0.3 | 0.0 |
| Met | 1.7 | 1.6 | 1.1 |
| Ile | 4.0 | 4.3 | 1.8 |
| Leu | 7.8 | 7.8 | 6.2 |
| Tyr | 1.5 | 1.5 | 2.4 |
| Phe | 3.8 | 4.0 | 4.6 |
| Lys | 14.0 | 13.1 | 7.9 |
| His | 1.0 | 0.9 | 5.7 |
| Arg | 5.9 | 5.5 | 9.5 |
| Trp | None | Present | 0.6 |

| Electrophoretic behaviour | | | |
|---------------------------|---------------|------|------|
| pH | M_c values* | | |
| 2.5 | 0.81 | 1.32 | 0.75 |
| 4.6 | 0.70 | 0.71 | 0.45 |
| 9.0 | 0.85 | 0.85 | 0.56 |

* Mobility relative to cytochrome *c* [5].

nerve (SN) developed signs of neurological disease. Five had histological peripheral nerve lesions consisting of myelin degeneration and axis cylinder fragmentation; and of these, two with marked degeneration had associated infiltration of lymphocytes and polymorphonuclear leucocytes (polymorphs). One animal had minimal central nervous system lesions consisting of margination of vessels by polymorphs.

Animals injected with the mixture of basic protein (SN2) or the low molecular weight fraction (SN2C) developed incontinence and paralysis of the hind limbs, and on histological examination were found to have vascular lesions in the brain and spinal cord but no peripheral nerve lesions. The vascular lesions seen,

Table 2
Results of injection of sciatic nerve fractions into guinea pigs.

| Antigen | No.* | Clinical | Neurological Disease** | | | |
|---------|------|----------|------------------------|---|-----|---|
| | | | Histological | | | |
| | | | CNS | | PNS | |
| | | | P | M | P | M |
| SN | 7 | 6 | 1 | 0 | 5 | 2 |
| SN2 | 7 | 6 | 6 | 6 | 0 | 0 |
| SN2A | 5 | 0 | 1 | 0 | 0 | 0 |
| SN2B | 5 | 0 | 0 | 0 | 0 | 0 |
| SN2C | 5 | 5 | 3 | 5 | 0 | 0 |

* Number of animals injected.

** Number of animals with symptoms.

CNS = Brain and spinal cord; PNS = Sciatic nerve; P = Margination of vessels with polymorphonuclear leukocytes and/or infiltration of tissue with polymorphonuclear leukocytes; M = Perivascular or diffuse infiltration of tissue with mononuclear leukocytes; SN = Whole sciatic nerve; SN2 = Mixed basic proteins; SN2A, SN2B and SN2C = fractions from gel filtration of SN2, see fig. 1.

consisted of migration of polymorphs through vessels into the perivascular brain substance, vascular endothelial proliferation and mononuclear cell infiltration into the vessel walls and perivascular brain substance, these being lesions usually seen in EAE. Animals injected with fractions SN2A and SN2B showed no disease, although one animal injected with SN2A showed mild histological lesions in the central nervous system. It appears that the protein responsible for the induction of the disease is the fast moving component of fraction SN2C.

The surprising finding that basic protein from peripheral nerve could induce a disease in the central nervous system rather than in the peripheral system led us to examine the sera from the five experimental groups for antibody to encephalitogenic basic protein. The technique of gel filtration radio-immunoassay with iodinated (^{125}I) encephalitogenic protein from central nervous tissue (^{125}I -EP) as antigen was used [10]. The sera taken at death from five guinea pigs injected with fraction SN2 and one injected with fraction SN2C had antibodies which reacted with ^{125}I -EP, whilst serum from animals in the other groups did not. The antigen binding capacities, expressed as μg ^{125}I -EP bound/ml undiluted serum, for

the reactive sera were 0.2, 0.5, 2, 2.8 and 8 $\mu\text{g}/\text{ml}$ for animal injected with SN2 and 0.3 for SN2C.

Antigenic cross-reactivity between the encephalitogenic basic proteins of central nervous tissue and sciatic nerve was studied by competitive inhibition using radio-immunoassay [10]. Samples were prepared containing a standard amount of anti-EP rabbit serum and ^{125}I -EP (0.1 μg) diluted to 1 ml with a mixture of normal rabbit serum (1 part) and Tris-acetate buffer (0.2 M, pH 7.3, 9 parts). Various amounts of unlabelled EP were added to these samples, the mixtures let stand for 12 hr at 4°C and tested by radio-immunoassay. Similar series of tests were carried out using SN2C and lysozyme (a protein unrelated to EP but of similar charge and molecular size) instead of EP. The degree of inhibition of the binding between ^{125}I -EP and anti-EP serum was determined by plotting the amount of ^{125}I -EP bound against the weight of added EP, SN2C and lysozyme. It was found that 0.6 μg EP and 6 μg SN2C caused 50% inhibition of the binding between ^{125}I -EP and anti-EP serum, whilst no inhibition was caused by the addition of up to 600 μg lysozyme. Thus, antigenic cross-reactivity was shown to exist between EP and SN2C.

We have shown that the injection of a whole homogenate of sciatic nerve produced histological lesions in the sciatic nerves with only minimal changes in the spinal cord in one animal. Hall [6] found histological lesions confined to the peripheral nervous tissue when he injected guinea pigs with homogenised sciatic nerve with FCA. However, Waksman [11] reported histological changes in both the central and peripheral nervous tissue in about 50% of guinea pigs injected with sciatic nerve homogenates with FCA.

Unlike the sciatic nerve homogenate, the basic protein isolated from sciatic nerve produced lesions only in central nervous tissue. It seems that during the fractionation the capacity to induce EAN was lost. This unexpected and unusual result was supported by our finding that basic protein from sciatic nerve shared antigenic determinants with a known encephalitogenic basic protein from central nervous tissue (EP) as shown by its capacity to induce antibody reactive with ^{125}I -EP and to inhibit the binding between ^{125}I -EP and anti-EP serum when tested by radio-immunoassay. It is now known that intact EP is not required for the induction of EAE. Polypeptides from this protein are highly encephalitogenic [12-14].

It is possible that the protein from sciatic nerve shares a common encephalitogenic determinant with EP

Acknowledgments

We thank Dr. R.M.Butterfield, University of Queensland for supplying the bovine sciatic nerves. This work was supported by grant No. 421 from the National Multiple Sclerosis Society, New York and by grants from the National Health and Medical Research Council, Australia. This is publication No. 1299 from the Walter and Eliza Hall Institute.

References

- [1] E.C.Alvord, in: The Central Nervous System, eds. O.T. Baily and D.E.Smith (Williams and Co., Baltimore, 1968) p. 52.
- [2] P.R.Carnegie, B.Bencina and G.Lamoureux, *Biochem. J.* 105 (1967) 559.
- [3] S.Levine and E.J.Wenk, *Proc. Soc. Exp. Biol. Med.* 113 (1963) 898.
- [4] O.Palacios and E.Pette, *Z. Imm. Allerg.* 126 (1964) 122.
- [5] B.H.Waksman and R.D.Adams, *J. Exp. Med.* 102 (1955) 213.
- [6] J.I.Hall, *Brain* 90 (1967) 297.
- [7] P.R.Carnegie, G.Lamoureux and B.Bencina, *Natura (London)* 214 (1967) 407.
- [8] L.F.Eng, F.C.Chao, B.Gerstl, D.Pratt and M.G.Tavaststjerna, *Biochemistry* 7 (1968) 4455.
- [9] F.Wolfgram and K.Kotorii, *J. Neurochem.* 15 (1968) 1281 and 1291.
- [10] T.A.McPherson and P.R.Carnegie, *J. Lab. Clin. Med.* 72 (1968) 824.
- [11] B.H.Waksman and R.D.Adams, *J. Neuropath. Exp. Neurol.* 15 (1956) 293.
- [12] P.R.Carnegie, T.A.McPherson and G.Robson, in preparation.
- [13] E.H.Eylar and G.A.Hashim, *Proc. Natl. Acad. Sci. U.S.* 61 (1968) 644.
- [14] E.R.Einstein and L.P.Chao, *J. Biol. Chem.* 243 (1968) 6050.