IDENTIFICATION AND DIFFERENTIAL DISTRIBUTION OF COLLAGEN TYPES IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEMS

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1. Introduction

In peripheral nerve the connective tissue component is composed of the epineurium which surrounds and invests the large nerve trunk, the perineurium enclosing separate bundles or fascicles of nerve fibres and the endoneurium which surrounds the individual nerve fibres. In the central nervous system there is not the same intimate degree of contact between nerve fibres and connective tissue as is found in peripheral nerve. This may be because the axial skeleton (vertebral column and cranium) provides the necessary physical support to this part of the nervous system. The connective tissue in the central nervous system takes the form of three meningeal membranes – the highly vascular dura mater and the arachnoid mater which line the vertebral canal, and the pia mater which is closely applied to the exterior face of the brain and spinal cord.

The demonstration of genetically different collagen types has led to investigations into their occurrence in neural connective tissue. The fibrous collagens, types I and III have been identified in human peripheral nerve [1]. However, this report made no attempt to determine the distribution of these collagen types in nerve although other studies have shown that the endoneurium is composed of a fibrous element [2] as well as an amorphous basement membrane [3]. In this paper we have used immunofluorescence microscopy to investigate the distribution of types I and III collagen in bovine central and peripheral nervous systems. We have also identified and described the distribution of the more recently characterised basement membrane associated collagens, types IV and V, in these tissues.

2. Materials and methods

Both the sciatic nerve and spinal cord were removed within 1 h of slaughter from an 18 month old steer. Adherent fatty tissue was dissected from the sciatic nerve and the dura and arachnoid matres were removed from the spinal cord.

Separation of the different collagen types was performed by fractional precipitation of the pepsin digest of defatted (2:1 chloroform:methanol for 5 days) nerve tissue [4,5]. The collagen types were characterised by their mobilities on SDS–polyacrylamide gel electrophoresis [4].

Rabbit antibodies to bovine types III, IV and V collagens and goat antibodies to bovine type I collagen were prepared as in [6]. Nerve tissue was processed and wax-embedded [7]. Serial sections were stained with the type-specific anti-collagen antibodies as in [6]. Non-immune serum was used as control.

3. Results

3.1. *Biochemical analysis*

3.1.1. Sciatic nerve

SDS–polyacrylamide gel electrophoresis demonstrated types I and III collagen in the 0.7 M NaCl precipitate while types IV and V were found in the

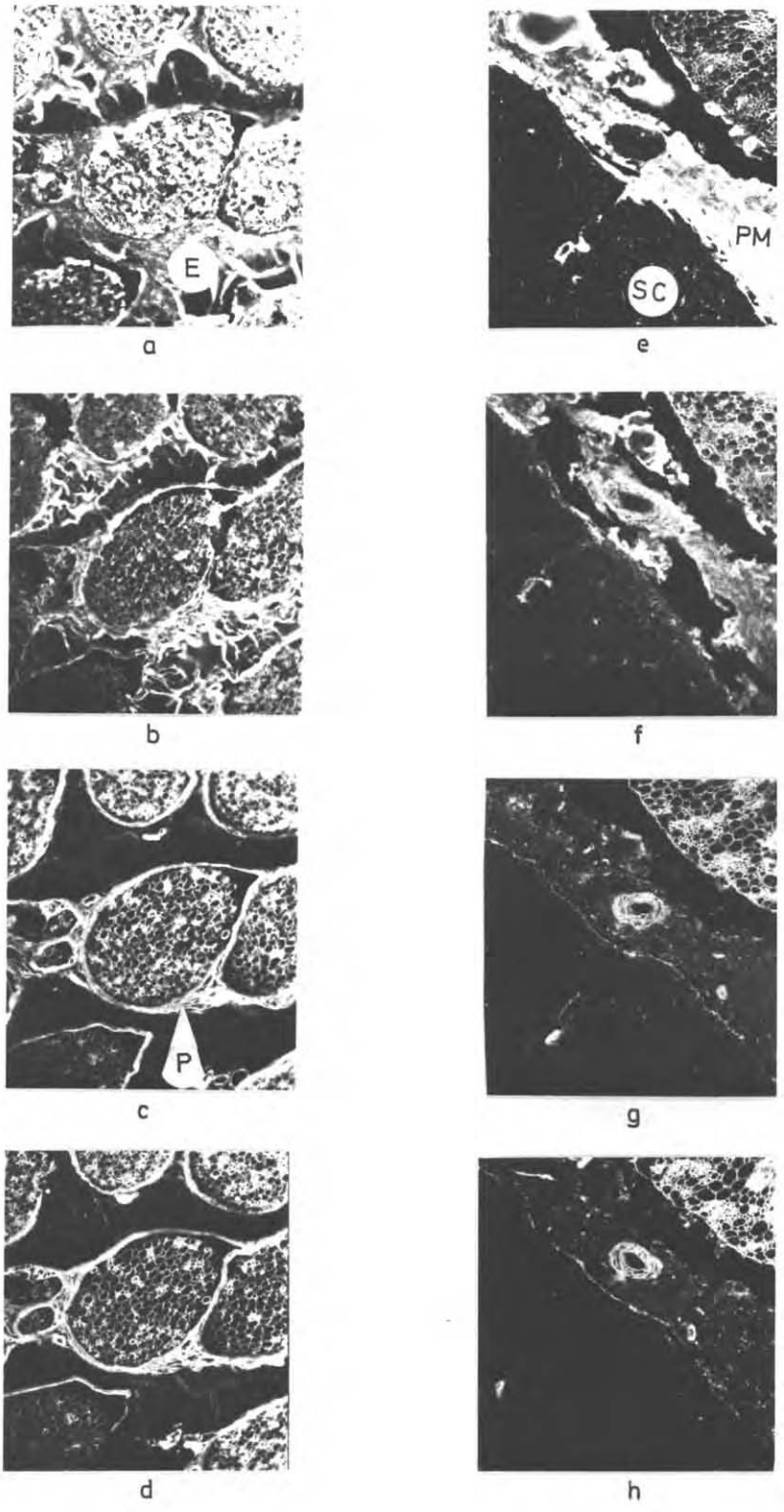

Fig.1

supernatant. The type IV bands were identified by comparison with the mobilities of the 3 bands obtained from lens capsule and placenta [8,9].

3.1.2. Spinal cord

Analysis of spinal cord by similar techniques revealed types I and III collagen only. The small amounts of types IV and V present in the fine blood vessels and apparent with immunofluorescence (see below) were not detected biochemically.

3.2. *Immunofluorescence staining*

3.2.1. Sciatic nerve

There was a clear localisation of the collagen types within the different components of the neural connective tissue. Type I collagen was generally distributed in the epi-, peri- and endoneurium while type III collagen was more apparent in the peri- and endoneurium than in the epineurium (fig.1a,b). Both the basement membrane-associated collagens, types IV and V were found in the endoneurium as sharply delineated rings around the single nerve fibres and as distinct laminae in the perineurium (fig.1c,d).

3.2.2. Spinal cord

In sharp contrast to the sciatic nerve there was very little staining of the neural compartment itself (fig.1e–h). Close examination revealed that the areas of fluorescence (present with all anti-collagen types) corresponded to small blood vessels. Some very small structures were only stained by anti type IV and V collagen, and these represent the finer capillaries where the proportion of fibrous collagen is very much reduced.

The pia mater showed strong fluorescence with anti-types I and III collagen and only weak staining with anti-types IV and V (fig.1e–h). There was an especially clear delineation of both types IV and V at the pia mater/spinal cord junction (fig.1g,h), an area of specialised cell contacts and basement membrane which has been termed the 'pia intima' to distinguish it from the rest of the pia mater (the 'epi-pia').

Figure 1e–h also includes part of a ventral nerve root which demonstrates the same distribution of collagen types as in the sciatic nerve. However, the perineurium is much reduced in the spinal nerve to a thin membrane particularly well defined with anti-types IV and V collagen.

4. Discussion

Our results provide information on the structure and distribution of connective tissue in both central and peripheral nerve.

With respect to the spinal cord, we have demonstrated a total absence of collagen in the neural tissue itself. Immunofluorescent staining was restricted to the blood vessels within the cord and to the meninges. Our observation that small capillaries stained with anti-type IV and V collagen (basement membrane-associated) but not with anti-types I and III collagen confirms earlier findings that fibrous elements of the pia mater accompany the blood vessels invading the cord only as far as their capillary networks, and that after this level there is only a basement membrane separating the vascular endothelium from the astrocyte end-feet of the nervous tissue.

In contrast to the nervous tissue itself, the pia mater is heavily stained with anti-types I and III collagen, in agreement with earlier reports on its fibrous nature. It is interesting in view of the structural continuity between the pia mater and the connective tissue of the peripheral nerve that types I and III collagens are also present as the major extracellular component of the endo- and perineurium.

Electron microscope investigations into the structure of the peripheral nerve connective tissue have revealed that the endoneurium has two compartments, a basement membrane on the external face of the Schwann cell sheath and an extracellular matrix largely composed of collagen which fills the spaces between the nerve fibres, reviewed in [3]. On the evidence presented here we can state that both the fibrous types I and III collagen are present in the latter and that both

Fig.1. Near-consecutive sections of bovine sciatic nerve (a–d) and spinal cord (e–h) stained with antibodies against (in order, top to bottom) types I, III, IV and V collagen. E, epineurium; P, perineurium; PM, pia mater; SC, spinal cord. Magnification 90 ×.

the basement membrane associated types IV and V are restricted to the former. A similar distribution of fibrous and basement membrane associated collagen types is present in the perineurium.

The relative contribution of types IV and V collagen to any particular basement membrane may vary considerably in different tissues. We have shown that both types are present in the Schwann and perineurial cell basement membranes. However, type V is barely detectable by immunofluorescence in lens capsule and kidney glomeruli, but is readily demonstrated both immunologically and biochemically in human placenta, lung, muscle and skin [6,8–11]. It may well be that there is a family of basement membrane collagen types and that their proportions in any particular basement membrane depends on its function.

It has long been held that the development of neural connections depends on some system of pathway guidance, e.g., [12]. One possibility is that some activity of connective tissue may provide the necessary positional cues, as has been proposed for certain aspects of muscle pattern formation [13–15]. With regard to the Schwann cell basement membrane it has been suggested that the reformation of accurate connections by peripheral nerves depends upon the integrity of this structure during regeneration. In vitro studies [16] have shown that ensheathment and myelination of nerve fibres occurs much more readily if Schwann cells have contact with an extracellular collagenous matrix. We feel that the characterisation and localisation of the various collagenous components of neural connective tissue is a necessary first step in finding out which cells are involved in their production. This information should clarify the mechanisms by which nerves form, maintain and regain their normal connections.

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References

[1] Seyer, J. M., Kang, A. H. and Whitaker, J. N. (1977) Biochim. Biophys. Acta 492, 415–425.
[2] Friede, R. L. and Bischausen, R. (1978) J. Neurol. Sci 38, 83–88.
[3] Peters, A., Palay, S. L. and Webster, H. de F. (1976) The Fine Structure of the Nervous System, W. B. Saunders, Philadelphia.
[4] Bailey, A. J. and Sims, T. J. (1976) Biochem. J. 153, 211–215.
[5] Rhodes, P. K. and Miller, E. J. (1978) Biochem. 17, 3442–3448.
[6] Duance, V. C., Restall, D. J., Beard, H., Bourne, F. J. and Bailey, A. J. (1977) FEBS Lett. 79, 248–252.
[7] Sainte-Marie, G. (1962) J. Histochem. Cytochem. 10, 250–256.
[8] Bailey, A. J., Duance, V. C., Sims, T. J. and Beard, H. (1979) in: Frontiers of Matrix Biology (Robert, A. M. et al. eds) vol. 7, pp. 49–59, Karger, Basel.
[9] Bailey, A. J., Sims, T. J., Duance, V. C. and Light, N. D. (1979) FEBS Lett. 99, 361–366.
[10] Burgeson, R. E., El Adli, F. A., Kaitila, L. L. and Hollister, D. W. (1976) Proc. Natl. Acad. Sci. USA 73, 2579–2583.
[11] Bentz, H., Bachinger, H. P., Glanville, R. and Kuhn, K. (1978) Eur. J. Biochem. 92, 563–567.
[12] Ghysen, A. (1978) Nature 274, 869–872.
[13] Shellswell, G. B. (1977) PhD Thesis, University of London.
[14] Shellswell, G. B. (1977) J. Embryol. Exp. Morphol. 41, 269–277.
[15] Wolpert, L. (1978) Sci. Am. 239, 124–137.
[16] Bunge, R. P. and Bunge, M. B. (1978) J. Cell Biol. 78, 943–950.