IMMUNOCYTOCHEMICAL LOCALIZATION STUDIES OF MYELIN BASIC PROTEIN

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ABSTRACT

The location of myelin encephalitogenic or basic protein (BP) in peripheral nervous system (PNS) and central nervous system (CNS) was investigated by immunofluorescence and horseradish peroxidase (HRP) immunocytochemistry. BP or cross-reacting material could be clearly localized to myelin by immunofluorescence and light microscope HRP immunocytochemistry. Fine structural studies proved to be much more difficult, especially in the CNS, due to problems in tissue fixation and penetration of reagents. Sequential fixation in aldehyde followed by ethanol or methanol provided the best conditions for ultrastructural indirect immunocytochemical studies. In PNS tissue, anti-BP was localized exclusively to the intraperiod line of myelin. Because of limitations in technique, the localization of BP in CNS myelin could not be unequivocally determined. In both PNS and CNS tissue, no anti-BP binding to nonmyelin cellular or membranous elements was detected.

KEY WORDS immunocytochemical · horseradish peroxidase · myelin · basic protein · peripheral nervous system · central nervous system

The chemical composition of central nervous system (CNS) and peripheral nervous system (PNS) myelin has been extensively investigated, but the topographical arrangement of proteins and the stabilizing forces in the ordered structure of myelin lamellae are unknown. Myelin differs substantially from other membranes in its high lipid-to-protein ratio (28, 42, 18). The major proteins of CNS myelin are proteolipid, encephalitogenic or basic protein (BP), and Wolfgram protein which comprise approx. 50%, 30%, and 20%, respectively, of myelin proteins (24). Much lower concentrations of other proteins, such as the DM-20 (3) or intermediate band protein (32, 12) and a glycoprotein (46), may also be present in CNS myelin.

The most completely characterized of the CNS myelin proteins is BP which induces experimental allergic encephalomyelitis (28, 35, 21). In most mammals BP has a monomer mol wt of 18,500 and contains 169 amino acids. The rat, mouse, and related species have in addition a second smaller BP with a molecular weight of 14,300 (38). The amino acid sequence (19) has been established for BP from human (13) and bovine (26) brain and for the small BP of the rat (23). The protein has been postulated to be of major importance in myelin structure because its grouping of apolar and charged amino acids would provide for both hydrophobic and hydrophilic interaction (27). BP was initially isolated from the CNS, but more recent studies have demon-

strated that there is a PNS myelin protein, designated P1, very similar to, if not identical with, BP (31, 9, 49). The amount of P1 in PNS myelin varies considerably among species (28, 31), attaining a maximum of approx. 50%, in the guinea pig, of the concentration of BP in CNS myelin. The two other major proteins of PNS myelin are a glycoprotein (25, 54), Po (31, 10), of 30,000 daltons and a smaller protein, P2, with a mol wt of 12,000 (11, 10, 36). BP and its PNS counterpart are restricted to neural tissue and appear to be the only major myelin protein common to both CNS and PNS. The present study was undertaken to localize this protein(s) in the PNS and CNS.

MATERIALS AND METHODS

Preparation of Basic Proteins

Monkey and guinea pig brains were purchased (Pel-Freeze Bio-Animals, Inc., Rogers, Ark.). Bovine brain was obtained within 2 h of death from a local slaughterhouse. BP from guinea pig, monkey, and cow was isolated according to previously described methods (21, 51, 15). Briefly, this consisted of defatting the brain specimens in chloroform-methanol (2/1) followed by treatment with acetone, washing with H_2O and extraction in dilute HCl at pH 3. The acid extracts were chromatographed on carboxymethylcellulose (CM-C) (Whatman CM 52, H. Reeve Angel Co., Inc., Clifton, N.J.) in 0.05 M glycine-NaOH buffer, pH 10.5, containing 6 M urea. BP, which binds to CM-C under these conditions, was eluted with a linear gradient of 0–0.2 M NaCl in the same buffer containing 2 M urea. The last fraction, termed component 1 (20), of BP eluting from the column was desalted on Sephadex G-25 coarse (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N.J.) in 0.5% acetic acid, lyophilized, and used for immunochemical tests. Purity of the BP preparations was monitored by disk electrophoresis in 5% acrylamide gels at pH 2.5 in the presence of 8 M urea (21). Component 1 of guinea pig and bovine BP were used without further purification. The monkey BP preparation was filtered through a 1.5 × 90-cm column of Sephadex G-100 (Pharmacia) equilibrated with 0.01 N HCl to remove materials, presumably BP breakdown products, which had a greater cathodal migration in the acid urea gels. Bovine BP used for preparation of the immunoadsorbent (see below) was obtained by combining fractions 1–4 (20) eluted from the CM-C.

Antiserum to Basic Protein

Rabbit (R47) antiserum to monkey BP was prepared by immunizing with BP complexed to deoxyribonucleic acid (49). This antiserum has been previously characterized as to specificity, tissue reactivity, and antigenic regions of bovine BP recognized (49, 51). The extent of cross-reaction of R47 (anti-monkey BP) with guinea pig and bovine BP was assessed by the quantitative microcomplement fixation test (QMCT) (49, 48).

In order to demonstrate specificity of reactions by selectively removing anti-BP, a BP-immunoadsorbent was prepared. 1,500 mg of AH Sepharose 4B (Pharmacia) were washed with 300 ml of 0.5 M NaCl and 100 ml of distilled H_2O and then suspended in 10 ml of distilled H_2O. 100 mg of bovine BP, fractions 1–4, were added and the pH was adjusted to 4.5 with 0.1 N HCl. With the pH maintained at 4.5, 5 ml of distilled H_2O containing 60 mg of 1-ethyl-3 (3-dimethyl-aminopropyl)-carbodiimide HCl (Sigma Chemical Co., St. Louis, Mo.) were added. This mixture was gently agitated at 25°C for 18 h, washed successively with 200 ml of distilled H_2O, 100 ml of 0.5% acetic acid, and 200 ml of borate-saline buffer (41), and stored in the same buffer.

The bovine BP-AH-Sepharose was placed in a 1.5 × 5-cm column at 25°C and washed thoroughly with the borate-saline buffer. 2 ml of R47 serum were passed through the column, and the effluent was monitored at 280 nm. The initial peak of antiserum which had been absorbed with BP was concentrated by vacuum dialysis to the starting volume.

Horseradish Peroxidase Conjugates

Goat antiserum to rabbit IgG, its selective purification on a column of rabbit IgG attached to Sepharose 2B (Pharmacia), formation of Fab, and the conjugation through glutaraldehyde of the Fab to horseradish peroxidase (HRP) (type VI, Sigma) by the one-step method of Avrameas (5), were performed as previously described (49, 41). Some of the studies were also conducted with Fab conjugated to HRP by the two-step method of Avrameas and Ternyck (6, 50). The immunochemical reactants which have goat anti-rabbit IgG activity and are linked to HRP will be referred to as HRP-Fab.

Immunofluorescence

Human stellate ganglion and femoral nerve were obtained postmortem and quick-frozen in 2-methylbutane cooled in liquid N_2. 8-μm cryostat sections were cut, air-dried, and fixed in 95% ethanol or 100% methanol for 5 min at 25°C. The sections were washed in 0.01 M phosphate buffer, pH 7.2, containing 0.15 M NaCl and processed by an indirect immunofluorescence technique (52). R47 anti-BP was used in the first reaction and goat anti-rabbit IgG (49) which had been conjugated with fluorescein (41, 14) was the second antibody. Sections were viewed with epi-illumination.

Immunoperoxidase Studies

All the indirect immunoperoxidase studies for localization of antibody to BP were performed with normal adult guinea pigs weighing 400–800 g.

Light Microscope Studies

Fresh frozen and fixed frozen material were studied. Guinea pigs were anesthetized with sodium pentobarbital (38 mg/kg), and portions of the sciatic nerve, spinal cord, and basal ganglia were removed and frozen in 2-methyl-butane cooled in liquid N_2. Fresh frozen material was used in an effort to arrange fixation for optimal immunocytochemical reactions. Cryostat sections, 10 μm, were placed on glass slides and fixed for 3 min in one of the following fixatives: 95% ethanol, 100% methanol; 0.1 M phosphate-buffered formalin (5% and 10%), glutaraldehyde (1% and 2.5%) and paraformaldehyde (1% and 4%); Bouin's fixative (8), formol-calcium solution (8), Clarke's solution (8), Carnoy's solution (8), 6% formalin-ethanol, 8% formalin-ethanol-glacial acetic acid, 2.5% glutaraldehyde-ethanol, 2.5% glutaraldehyde-methanol, 2.5% glutaraldehyde-ethanol-glacial acetic acid; and 1% glutaraldehyde-4% formalin-ethanol-glacial acetic acid.

Fixed frozen material was obtained from anesthetized animals perfused through the heart with 0.1 M phosphate buffer, pH 7.2, containing 10% formalin, 2.5% glutaraldehyde, or 4% paraformaldehyde. Portions of the sciatic nerve, brachial plexus, spinal cord, and basal ganglia were frozen in 2-methylbutane cooled in liquid N_2. Cryostat sections, 10 μm, were placed on glass slides. These fixed frozen sections were refixed in 95% ethanol for 3 min.

Both the fresh frozen and fixed frozen tissues were then processed at 25°C as follows: the sections were washed in 0.01 M phosphate buffer, pH 7.2, in 0.15 M NaCl (PBS) for 10 min. They were then incubated for 1 h in a 1:25–1:50 dilution of rabbit anti-BP; washed for 15 min in PBS, and incubated for 1 h in a 1:75–1:100 dilution of HRP-Fab. After another 15-min wash in PBS, the sections were reacted for 30 min at 4°C in a solution containing 5 mg 3-3'-diaminobenzidine (DAB) (Sigma) in 10 ml of Tris-HCl buffer, pH 7.4, with 0.1 ml of freshly prepared 1% H_2O_2 (41, 30). The sections were washed in PBS for 15 min, postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 5 min, dehydrated in graded ethanol solutions and xylene and mounted in Permount (41).

Electron Microscope Studies

Guinea pigs were anesthetized and perfused through the heart with 0.1 M phosphate buffer, pH 7.4, containing 10% formalin or 2.5% glutaraldehyde. Portions of the sciatic nerve, spinal cord, and basal ganglia were removed and sectioned at 50 μm on a Smith-Farqhuar tissue sectioner. The sections were then refixed in 95% ethanol for 10 min and washed in PBS for 10 min. These sections were incubated in a 1:25–1:50 dilution of rabbit anti-BP for 8 h at 25°C and washed for 18 h at 25°C in PBS. This was followed by an 8-h incubation in a 1:75–1:100 dilution of HRP-Fab and a subsequent overnight wash in PBS. The sections were refixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 for 30 min, washed in PBS for 10 min, and reacted with a solution of DAB as described above. After postfixation in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4, for 1 h, the sections were dehydrated in graded ethanols and propylene oxide and embedded in Spurr low-viscosity embedding media (Polysciences, Inc., Warrington, Pa.). Thin sections were viewed with and without counterstaining with uranyl acetate and lead citrate (47) at 50 and 75 kV in a Hitachi Hu-11B electron microscope.

RESULTS

Immunochemical Characterization of Anti-BP

Since guinea pig neural tissue was selected for the immunoperoxidase studies, it was necessary to show that the rabbit (R47) anti-BP cross-reacted well with guinea pig BP. Proof of cross-reactivity seemed particularly necessary when the reaction with guinea pig tissue was limited or absent, a problem ultimately related to difficulties in fixation (*vide infra*). Cross-reactivity with bovine BP was assessed because the immunoadsorbent was prepared with bovine BP.

As demonstrated by the QMCT at dilutions of 1:300 or less, R47 cross-reacted essentially equally well with monkey, guinea pig, and bovine BP (Fig. 1). On further dilution, this cross-reactivity was less, especially with bovine BP (49);

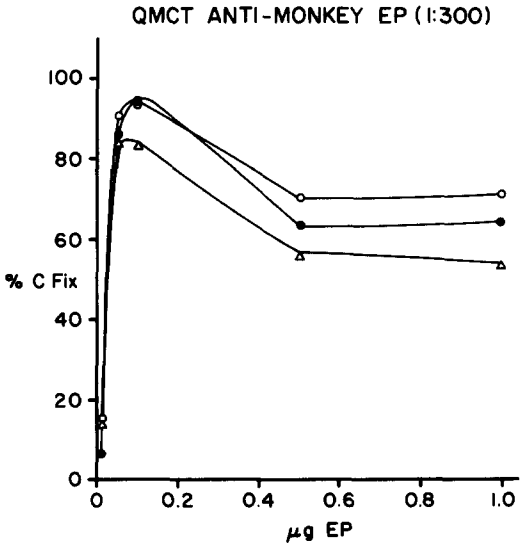

FIGURE 1 Quantitative complement fixation (C Fix) between anti-BP, diluted 1:300, and monkey (●), guinea pig (○), and bovine (△) EP.

however, for the immunocytochemical studies in which much lower dilutions were used, the cross-reaction of anti-monkey BP with guinea pig BP should have been virtually complete.

Immunocytochemical Tissue Studies

PERIPHERAL NERVOUS SYSTEM: In order to define a method of fixation which would be applicable for preservation of tissue detail for ultrastructural examination, different methods of fixation were tried on fresh frozen PNS tissue. The results of these various methods of fixation in relationship to tissue binding site of antibodies to BP are shown in Table I. Fixation of tissue in 95% ethanol, absolute methanol, or Clarke's and Carnoy's solutions demonstrated the reaction of anti-BP with myelin. In the sciatic nerve and brachial plexus, the staining reaction was confined to the myelin sheath without evidence of staining of Schwann cell cytoplasm or nuclei (Figs. 2 and 3). The endoneurial, perineurial or epineurial connective tissue, or blood vessel walls showed no localization of anti-BP. Immunofluorescence studies of human femoral nerve (Fig. 4) and stellate ganglion showed similar reactions restricted to the myelin sheath. The thick myelin sheaths of femoral nerve and the myelin sheaths of varying thickness in the stellate ganglion were sites of antibody reaction. No reaction with the unmyelinated fibers or Schwann cells in the stellate ganglion was detected.

TABLE I

Effects of Fixatives on BP Localization

Fixative	BP localized to myelin sheath
Ethanol (95%)	+
Methanol (100%)	+
Clarke's solution (ethanol-acetic acid)	+
Carnoy's solution (ethanol-acetic acid-chloroform)	+
Formalin (10%)	0
Paraformaldehyde (1% and 4%)	0
Formol-calcium	0
Bouin's fixative (picric acid, formalin, acetic acid)	0
Glutaraldehyde (1% and 2.5%)	0
Formalin (6%)-ethanol	+
Formalin (8%)-ethanol-acetic acid	+
Glutaraldehyde (2.5%)-ethanol or methanol	+
Glutaraldehyde (2.5%)-ethanol-acetic acid	+
Glutaraldehyde (1%)-formalin (1%)-ethanol-acetic acid	+

By immunoperoxidase technique, fresh frozen PNS tissue fixed in other solutions including formalin, paraformaldehyde, glutaraldehyde, or Bouin's fixative either failed to react with anti-BP or led to a variable staining of many morphologic structures. This included sporadic staining of axis cylinders and interstitial tissue elements by both preimmune and postimmunization serum from R47.

Since alcohol appeared to preserve the localization of the BP in fresh frozen tissue, studies were undertaken to examine the effects of combining an aldehyde fixative in varying concentrations with ethanol, methanol, or ethanol-acetic acid. By use of these mixtures, specific myelin sheath staining with anti-BP could still be preserved (Table I). However, none of these mixtures could be successfully used as perfusates for retention of fine structural detail.

Fixative studies were then conducted on fresh frozen sections sequentially exposed to an aldehyde followed by an alcohol. In sequentially fixed sections, anti-BP reacted with the myelin sheath but not with other neural tissue elements. This procedure for sequential fixation provided the best morphological preservation for light microscope studies and, subsequently, for ultrastructural investigations. In this procedure the PNS tissue was removed after cardiac perfusion with 10% formalin, 2.5% glutaraldehyde, or 4% paraformaldehyde. The tissue was frozen, and the cryostat sections were refixed in 95% ethanol. Specific localization of antibodies to BP was preserved as illustrated in Fig. 5.

For ultrastructural studies of PNS, the sciatic nerve was removed from animals perfused with 2.5% glutaraldehyde or 10% formalin. 50-μm sections were immediately immersed in 95% ethanol and processed as described above. After either method of fixation, the compact regions of the myelin sheath were well preserved. However, the transitional area of continuity between these regions of compact myelin and the inner and outer mesaxons could not be clearly demonstrated because of tissue disruption. In control sections reacted with preimmune serum, anti-BP absorbed with BP, or DAB alone, no HRP-Fab deposits were found. In control sections (Fig. 6), major dense and intraperiod lines were distinct and the double membrane structure of the intraperiod line was evident. The periodicity of the major

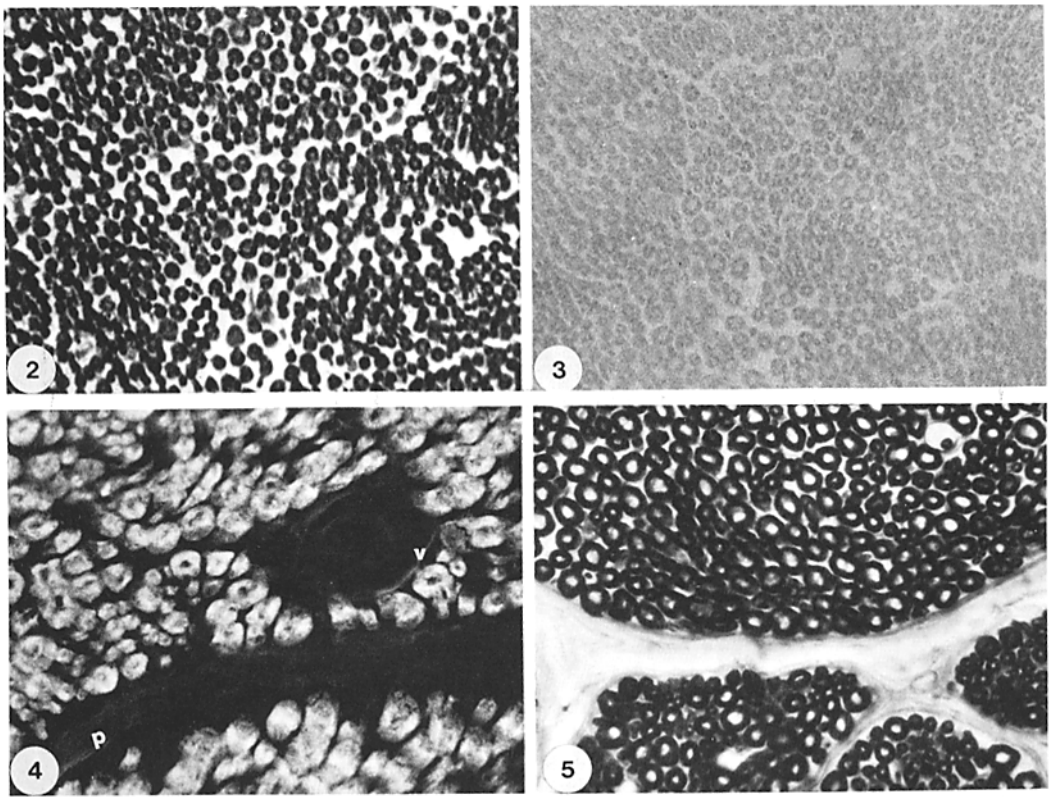

FIGURE 2 Fresh frozen cryostat section of guinea pig sciatic nerve fixed in 95% ethanol and processed by HRP immunocytochemical technique after anti-BP. Only myelin sheaths show staining with HRP. × 200.

FIGURE 3 Control section of sciatic nerve; processing identical to that for Fig. 2 but reacted with R47 preimmune serum. The myelin sheaths are unstained. × 200.

FIGURE 4 Fresh frozen cryostat section of human femoral nerve fixed in absolute methanol. Indirect immunofluorescence with anti-BP shows binding restricted to myelin sheaths. Blood vessel (v) wall and perineurium (p) show some autofluorescent areas. × 360.

FIGURE 5 Cryostat section of sciatic nerve from guinea pig perfused with 10% formalin, sequentially fixed in 95% ethanol and processed by HRP immunocytochemical technique with anti-BP. The myelin sheaths are better preserved than in Fig. 2. HRP deposits are restricted to myelin sheaths. × 300.

dense lines was 13.5–14.5 nm, and the centers of the major dense and intraperiod lines were separated by 6.5–7 nm. BP was localized in the intraperiod line (Figs. 7–10) of PNS myelin from animals perfused with glutaraldehyde or formalin. Fine structure details were generally better preserved in glutaraldehyde-fixed tissue (Fig. 7). The reaction product appeared to be deposited on both laminae of the intraperiod line with less deposition in the area between laminae. In formalin-fixed material (Figs. 8–10), more lamellae were sites of reaction for anti-BP. In Fig. 8, the HRP-Fab deposits appear on alternate lamellae of the myelin sheath. This interpretation is substantiated in Fig. 9, not counterstained with uranyl acetate or lead citrate, showing anti-BP localization confined to only one of the myelin lamellae with a periodicity of 13.5–14.5 nm. This was determined to be the intraperiod line by careful comparison with controls reacted with preimmune serum (Fig. 6) where no reaction product was found in the intraperiod line. Further evidence that the intraperiod line was the site of anti-BP localization was ascertained from the areas of the

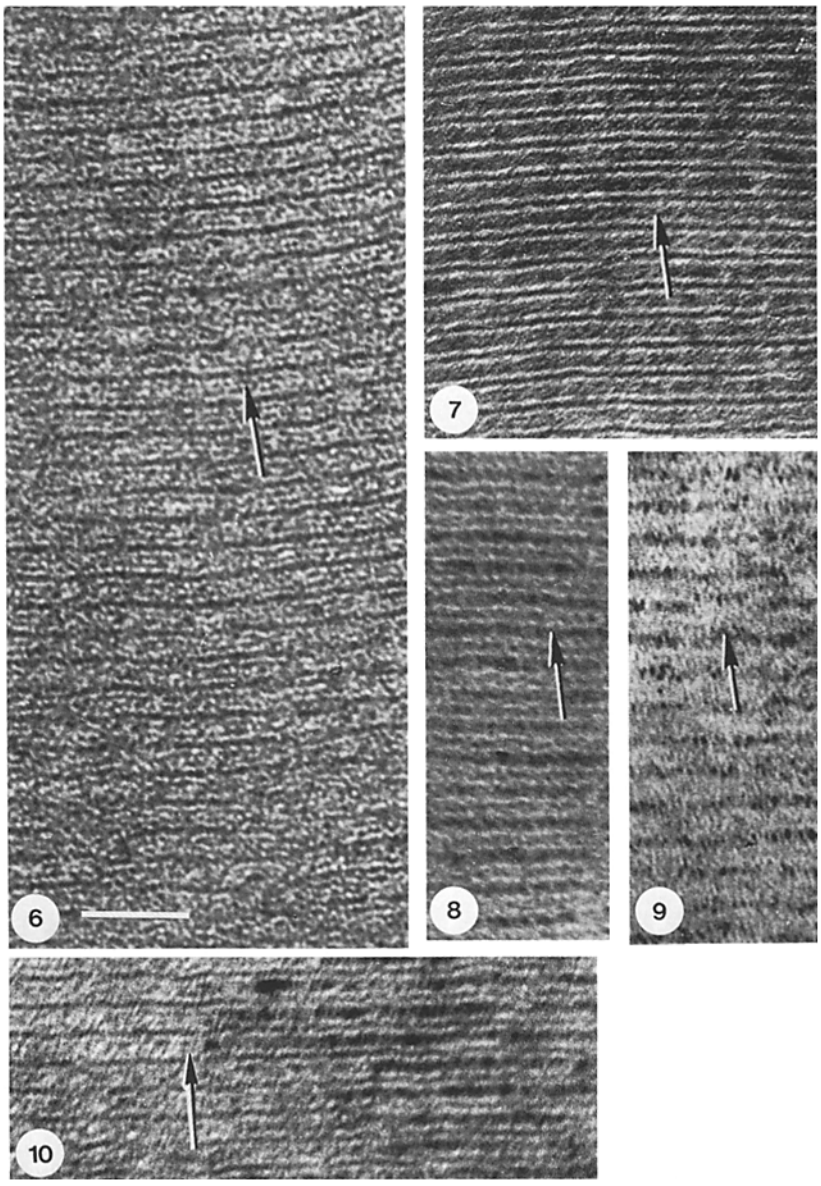

FIGURE 6 Electron micrograph of sciatic nerve myelin perfused with 10% formalin, sequentially fixed in 95% ethanol and processed by HRP immunocytochemical technique with R47 preimmune serum. Intraperiod line shows double membrane structure (arrow). Stained with uranyl acetate and lead citrate. Bar, 0.05 μm. × 285,000.

FIGURE 7 Sciatic nerve myelin perfused with 2.5% glutaraldehyde, sequentially fixed in 95% ethanol, exposed to anti-BP and processed by HRP immunocytochemical technique. HRP deposits are seen in the intraperiod line (arrow). Stained with uranyl acetate and lead citrate. × 285,000.

FIGURE 8 Processing of myelin sheath identical to that in Fig. 7 except that initial perfusate was 10% formalin. HRP deposits are seen in the intraperiod line (arrow). Stained with uranyl acetate and lead citrate. × 285,000.

FIGURE 9 Myelin sheath processed as in Fig. 8 without counterstaining with uranyl acetate and lead citrate. HRP deposits are seen in the intraperiod line (arrow). × 285,000.

FIGURE 10 Processing of myelin identical to that in Fig. 8. Area of myelin sheath where HRP deposition is contiguous with unpenetrated region. HRP deposits can be seen in alternate lamellae of the myelin sheath at the right. Where the deposition terminates, the intraperiod line can be clearly identified (arrow). × 285,000.

myelin sheath where HRP deposition was contiguous with unpenetrated regions. In these sites, HRP deposition terminated to expose the intraperiod line (Fig. 10).

Central Nervous System

Once having established a suitable methodology for light and electron microscope examination of PNS tissue, similar studies were attempted on CNS tissue. In order to have comparable conditions for studies of PNS and CNS and since more extensive areas of the PNS were stained when formalin was the initial fixative, 10% phosphate-buffered formalin was exclusively used for CNS studies. As with PNS tissue, the formalin-perfused brain was removed and 10-μm (light microscope) cryostat sections or 50-μm (electron microscope) "tissue-chopped" sections were refixed in 95% ethanol. At the light microscope level, localization of antibodies to BP was confined to the myelin sheaths as illustrated in Fig. 11 which shows HRP staining in the bundles of myelinated nerve fibers coursing through the caudate nucleus and corpus callosum. White matter of cerebellum, brainstem, and spinal cord also showed reaction with anti-BP. As previously noted (49), no staining was seen in the cytoplasm or nuclei of CNS cells (neurons, oligodendroglia, or astrocytes). Control sections incubated with R47 preimmune serum (Fig. 12) or R47 absorbed with BP revealed no reaction with myelin.

In ultrastructural studies of CNS, it proved to be much more difficult to obtain penetration of reagents for an immunocytochemical reaction. Despite the use of HRP-Fab conjugates to reduce the size of the labeled second antibody, acceptable penetration and reactivity for unequivocal identification of the localization of BP in the myelin sheath was not possible.

DISCUSSION

This investigation has demonstrated that BP or an immunologically related protein, presumably P1 (31, 10), could be detected only in the intraperiod line of the compact region of PNS myelin. Numerous technical problems, especially concerning fixation, were encountered. An aldehyde fixative and exposure to ethanol or methanol were required for satisfactory morphology, penetration of reagents, and preservation of tissue antigens for reaction with antiserum. The effect of alcohol on the reaction remains to be defined but could be related to the extraction of certain myelin lipids that mask BP or to rearrangements within the myelin membrane to make antigen-binding sites more accessible (2, 16). Alterations in myelin composition, especially lipids, effected by aldehyde and alcohol may have influenced our results, but this treatment was essential for immunocytochemical reactions.

In this immunocytochemical system, no reaction was detected to indicate the presence of BP in the

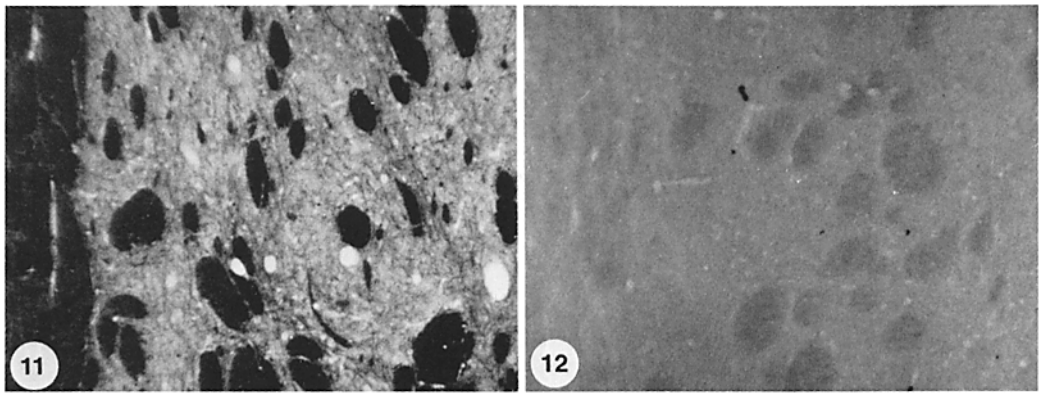

FIGURE 11 Cryostat section from guinea pig brain perfused with 10% formalin, sequentially fixed in 95% ethanol, reacted with anti-BP and processed by HRP immunocytochemical technique. Large bundles of myelinated nerve fibers stained with HRP can be seen coursing through the caudate nucleus with corpus callosum at extreme left. × 125.

FIGURE 12 Control section of caudate nucleus processed as in Fig. 11 except for reaction with R47 preimmune serum. Bundles of myelinated nerve fibers can be faintly seen. Small black dots represent peroxidase staining of some remaining erythrocytes. × 125.

oligodendroglia or P1 in the Schwann cell. Small quantities could have gone undetected or been altered by fixation so that they were no longer demonstrable. Glutaraldehyde alters the immunoreactivity of BP in solution (50), but its effect on BP in the myelin sheath may not be so extensive (53). By immunofluorescence and HRP immunocytochemistry conducted under a variety of conditions, reaction with cellular elements in CNS or PNS tissue was never observed. These findings suggest that the cells of origin of CNS and PNS myelin do not contain BP or P1 in the same state in which it occurs in the myelin sheaths. Previous studies on isolated oligodendroglia have indicated that BP is not found in calf oligodendroglial plasma membrane (43). It has been reported that BP is present in preparations of human oligodendroglia (34), but the possible attachment of BP to human oligodendroglia during the purification process (40) limits the interpretation of such findings. Antibodies raised against oligodendroglia do not react with CNS myelin (45) or BP (1). The remarkable chemical differences between plasma and myelin membranes indicate that these structures are very different. Our findings in the PNS would be consistent with the speculation that formation of the CNS myelin sheath involves a precursor phase (17, 39, 4) in which BP exists in a form (in the cell of origin) that cannot react with anti-BP. Unfortunately, fixation of tissue and reliability of penetration by reagents were not suitable to identify with certainty the transition point between negatively staining Schwann cell and the positively staining compact PNS myelin.

Despite our relative success with PNS myelin, the ultrastructural localization of BP in the CNS was not successfully delineated. The immunocytochemical reaction in CNS was very limited and usually seen best only with tissue disruption. Others (33) have made similar observations. Cells in the CNS, whether disrupted or not, showed no reaction. An accurate immunocytochemical localization of BP in CNS must await technical improvements which we have not been able to achieve.

Localization of BP or P1 to the intraperiod line of PNS myelin differs from some of the results of investigations of the distribution of BP in CNS myelin. Two of these studies (33, 37) involved an immunocytochemical approach; impaired penetration of reagents was a major problem in both. Although BP was localized in paraformaldehyde-fixed CNS myelin to the major dense line, staining occurred only when myelin was disrupted. The fixative used or the specificities of the anti-BP could have restricted the detection of BP. Reaction product was also present on the oligodendroglial plasma membrane and on postsynaptic densities in sections treated with both immune and normal rabbit serum.

Studies of myelin structure after partial autolysis (22) or treatment with enzymes (55, 7) have not localized BP with certainty and are in disagreement over the position of BP in the CNS myelin lamellar array. Surface labeling of intact CNS myelin membrane by nonpermeant probes failed to label BP well but did label other myelin proteins (44, 29, 56). Negative results by these techniques are difficult to interpret, but such findings would be expected in a myelin membrane model in which BP is not openly exposed on the external surface but located in the major dense line. Alternatively, the lack of BP labeling by nonpenetrating probes, rather than indicating BP localization in the major dense line, could be the result of lack of suitable exposure in the membrane of amino acid residues, e.g., tyrosine, histidine or lysine, of BP for attachment of radiolabel, or the masking of BP by associated myelin lipid. Our observation that treatment with alcohol was requred for satisfactory reaction might also suggest that an alcohol-soluble material shielded BP. Whether or not there are dissimilarities in the topography of similar proteins such as BP or P1 in the CNS or PNS myelin cannot yet be resolved.

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