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Nerve injury stimulates the secretion of apolipoprotein E by nonneuronal cells

(37-kDa protein/glia/nerve regeneration)

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ABSTRACT **Nerve trauma initiates significant changes in the composition of proteins secreted by nonneuronal cells. The most prominent of these proteins is a 37-kDa protein, whose expression correlates with the time course of nerve development, degeneration, and regeneration. We now report that the 37-kDa protein is apolipoprotein E (apoE). We produced a specific antiserum against the 37-kDa protein isolated from previously crushed nerves. This antiserum recognizes a 36-kDa protein in rat serum that we have purified and identified as apoE. The anti-37-kDa antiserum also recognizes apoE on electrophoretic transfer blots of authentic samples of high and very low density lipoproteins. The nerve 37-kDa protein comigrates with apoE by two-dimensional electrophoresis, shares a similar amino acid composition, and reacts with an antiserum against authentic apoE. The purified apoE specifically blocks the immunoprecipitation of [^{35}S]methionine-labeled 37-kDa protein synthesized by nonneuronal cells. Thus, on the basis of its molecular mass, isoelectric point, amino acid composition, and immunological properties, we conclude that the 37-kDa protein is apoE. We also used light microscopic immunohistochemistry to localize apoE following nerve injury. In rats with optic nerve lesions, the 37-kDa antiserum bound specifically to the degenerating optic tracts and to the retinorecipient layers of the lateral geniculate nucleus and the superior colliculus. We propose that apoE is synthesized by phagocytic cells in response to nerve injury for the purpose of mobilizing lipids produced as a consequence of axon degeneration.**

Nonneuronal cells in the mammalian nervous system are estimated to outnumber neurons by 10:1 and may account for nearly half of the volume of the brain (1). Although the functions of some nonneuronal cells [which include astrocytes, oligodendrocytes, and microglia in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS)] are beginning to be understood, thus far little is known at the molecular level of the roles such cells play in the development and in the normal functioning of the nervous system or in response to injury. Recently, it has been shown that nonneuronal cells in the PNS and CNS release soluble proteins into their microenvironment during development (2–4) and following nerve injury (3–5). The fact that the synthesis and secretion of some of these proteins is significantly enhanced during nerve regeneration in the PNS leads naturally to the hypothesis that they may play an important role in determining the ability of an axon to regenerate following injury. The poor regenerative response of most CNS neurons in higher vertebrates might be related to the failure of CNS nonneuronal cells to secrete the requisite proteins necessary for regeneration or the failure to secrete them in sufficient amounts. These considerations serve to emphasize the importance of identifying and characterizing the proteins released by nonneuronal cells in the PNS and CNS and of elucidating how the synthesis and secretion of these proteins are regulated normally and following nerve injury.

The most prominent soluble extracellular protein released by nonneuronal cells following nerve injury is an acidic 37-kDa protein. Two weeks following injury to the sciatic nerve in adult rats, the 37-kDa protein undergoes a 250- to 350-fold increase in synthesis and constitutes 2–5% of the total extracellular protein synthesized by nonneuronal cells remaining in the distal nerve stump (5). A presumably identical protein, having the same molecular mass and pI, is synthesized by CNS nonneuronal cells following injury of the adult rat optic nerve (3, 5, 6). The synthesis and release of a 37-kDa protein is also developmentally regulated in neonatal rat optic and sciatic nerves (2–4).

We have pursued the characterization of the 37-kDa protein as a necessary initial step toward elucidating its cellular role in development and in response to injury. In the present study, we present evidence that the 37-kDa protein released by nonneuronal cells following injury is physically and immunologically identical to the plasma protein apolipoprotein E (apoE). Furthermore, using light microscopic immunohistochemistry, we have shown that apoE is localized specifically in association with degenerating fiber tracts following nerve injury in the adult rat CNS. We propose that apoE is involved in the regulation of the lipid environment of the nervous system and that following injury, this regulation is governed by similar, but not identical, mechanisms in the PNS and CNS.

Preliminary reports of some of this work have been published (2, 7–9).

MATERIALS AND METHODS

Surgery. Adult Sprague–Dawley rats were anesthetized with ketamine hydrochloride (250–500 mg/kg, i.p.). In nerve crush experiments, optic nerves were crushed intraorbitally and sciatic nerves were crushed at the midthigh level. Animals were sacrificed by cervical dislocation while they were under halothane anesthesia 10 days–3 weeks following surgery.

Electrophoresis. Proteins were precipitated by the addition of an equal volume of 10% trichloroacetic acid. The proteins were centrifuged at 10,000 × *g* for 10 min and the pellets were washed twice with diethyl ether. Pellets of proteins for two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) were solubilized and run by the O'Farrell (10)

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Abbreviations: apoE, apolipoprotein E; CNS, central nervous system; PNS, peripheral nervous system; 1D-PAGE, one-dimensional polyacrylamide gel electrophoresis; 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; HDLs, high density lipoproteins; VLDLs, very low density lipoproteins; IEF, isoelectric focusing.

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procedure as modified by Skene and Shooter (5), except that the heating step was omitted from the sample preparation, the isoelectric focusing (IEF) gels contained 3% pH 4–6 and 3% pH 3.5–10 Ampholines (LKB), and the "transfer buffer" consisted of 2% $NaDodSO_4$, 100 mM Tris·HCl (pH 8.0), 2 mM dithiothreitol, 20% (vol/vol) glycerol, and a trace of bromophenol blue. IEF pH gradients were determined from triplicate blank tube gels.

One-dimensional polyacrylamide gel electrophoresis (1D-PAGE) in 10% acrylamide/0.3% bisacrylamide gels was performed as described by Laemmli (11), except that 50 mM dithiothreitol replaced 2-mercaptoethanol in the sample buffer. Molecular mass standards used were bovine serum albumin (68 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), and soybean trypsin inhibitor (20 kDa).

Purification of the 37-kDa Protein and Production of Antiserum. Two to 3 weeks following sciatic nerve crush, the portion of the nerve segment (20 nerves) distal to the crush was excised, cut into 2- to 4-mm segments, and washed twice for 15 min in Dulbecco's phosphate-buffered saline at 37°C in order to collect a soluble extracellular protein fraction. Washes were pooled, prepared, and run on 2D-PAGE as described above. One milligram of protein was loaded onto a 11.5 cm × 4 mm IEF tube gel. After focusing, the tube gel was soaked in transfer buffer for 40 min before second-dimension electrophoresis. A rabbit antiserum was prepared against the nerve 37-kDa protein by using a procedure similar to that described by Boulard and Lecroisey (12). Briefly, the preparative gels were stained with Coomassie blue and the stained spot corresponding to the 37-kDa protein was excised. The 37-kDa protein, in acrylamide, isolated from five gels (≈100 μg of protein), was homogenized in 0.5 ml of Dulbecco's phosphate-buffered saline/0.5 ml of incomplete Freund's adjuvant and injected equally into two New Zealand White rabbits at multiple subcutaneous sites on days 0, 14, 21, and 28. The animals were bled for the 37-kDa antibody 10 days after the final injection.

Electrophoretic Transfer Blot Analysis. Transfer of proteins to nitrocellulose (Schleicher & Schuell, BA83, 0.2 μm) was performed as described by Burnette (13). Electrophoretic transfer blots were processed as suggested by Bio-Rad (reference manual for Bio-Rad Immuno-Blot assay kit) and stained by the indirect avidin/biotin/peroxidase procedure (Vector Laboratories, Burlingame, CA). Rat lipoproteins were isolated by sequential ultracentrifugation (14) of fresh rat serum adjusted with solid KBr at densities $\rho < 1.006$ (130,000 × g, 18 hr) for very low density lipoproteins (VLDLs) and from $1.063 < \rho < 1.215$ (160,000 × g, 48 hr) for high density lipoproteins (HDLs). The lipoproteins were washed by recentrifugation. A rabbit antiserum against preparative gel-purified apoE from rat VLDLs was obtained from Henry Wilcox (Department of Pharmacology, University of Tennessee, Memphis).

Purification of the Serum 36-kDa Protein. A 36-kDa protein in rat serum with a similar pI to the nerve 37-kDa protein was identified by 2D-PAGE and purified. To purify the 36-kDa protein from rat serum, ammonium sulfate fractionations were performed by making the diluted (1:1) rat serum 20%, 30%, and 40% (weight/starting volume) in ammonium sulfate. The 40% ammonium sulfate fraction was dissolved in distilled water and dialyzed overnight against 50 mM sodium phosphate (pH 7.0) at 4°C. The dialysate was applied to a DEAE (Whatman DE-52)-cellulose column (8.0 × 2.5 cm) that was equilibrated with the 50 mM sodium phosphate buffer. Unbound proteins were washed from the column with the 50 mM sodium phosphate buffer and the column was eluted with a 250-ml linear gradient of 0–250 mM sodium chloride in 50 mM sodium phosphate buffer at pH 7.0. Fractions containing the immunoreactive 36-kDa protein were identified by electrophoretic transfer blots, pooled, and further purified by preparative 1D-PAGE.

Amino Acid Analysis. Samples of the nerve 37-kDa protein and the serum 36-kDa protein were isolated from preparative 2D and 1D gels, respectively, by electroelution into dialysis membranes in buffer containing 20 mM Tris base, 150 mM glycine, and 0.1% $NaDodSO_4$ at 7 V/cm for 12 hr. The eluted proteins were prepared for amino acid analysis by exhaustive dialysis against 0.005% $NaDodSO_4$ followed by lyophilization (15). The samples were subjected to pre-column derivitization according to the method of Heinrikson and Meredith (16) and 1–2 μg of total protein was analyzed by using a Waters Associates Pico-Tag amino acid analysis system.

Immunoprecipitations. Adult rat optic and sciatic nerves were excised, washed briefly in Dulbecco's phosphate-buffered saline, and labeled in methionine-free Dulbecco's modified Eagle's medium supplemented with 250 μCi (1 Ci = 37 GBq) of [^{35}S]methionine per ml (200 μl of medium per nerve) at 37°C in a humidified atmosphere of 5% CO_2 in air for 3 hr. Labeled nerves were homogenized in 1% $NaDodSO_4$/1 mM *o*-phenanthroline/4 mM phenylmethylsulfonyl fluoride and heated to 100°C for 4 min. The immunoprecipitations were then performed exactly as described by Anderson and Blobel (17) with 20 μl of anti-37-kDa serum per nerve. Slab gels for fluorography were processed according to Jen and Thatch (18).

Immunohistochemical Localization of the 37-kDa Protein. Brains from rats that had received a unilateral intraorbital optic nerve crush 12 days earlier were removed and frozen in OCT compound. Frozen sections (10 μm) were fixed in 2:1 chloroform/methanol and treated with 1:100 hydrogen peroxide/methanol to block endogenous peroxidase activity. The sections were rehydrated and postfixed with 1% paraformaldehyde in phosphate-buffered saline for 1 hr. The sections were then incubated with the anti-37-kDa serum (1:100 dilution) and were visualized by standard peroxidase-antiperoxidase methods (19) using 3,3'-diaminobenzidine as the chromagen.

RESULTS

Production and Characterization of an Antiserum to the Nerve 37-kDa Protein. A specific antibody was made in rabbit to the purified 37-kDa protein obtained from the distal segment of injured rat sciatic nerves. The specificity of the antibody is shown by a comparison of a Coomassie blue-stained 2D gel of extracellular proteins isolated from regenerating sciatic nerves (Fig. 1*A*) with an anti-37-kDa antibody-stained electrophoretic transfer blot of an identically prepared sample (Fig. 1*B*). No other proteins from the 2D gel reacted with the anti-37-kDa serum. Control blots incubated with nonimmune rabbit serum also showed no reaction. When the anti-37-kDa serum was preabsorbed with rat serum to block potential binding to nonspecific rat proteins, staining of the 37-kDa band was significantly reduced. This led us to investigate whether a protein with similar antigenic properties to the nerve 37-kDa protein was present in rat serum. By additional 2D electrophoretic transfer blot analysis, the 37-kDa antiserum was shown to recognize a similar 36-kDa protein in rat serum (Fig. 1*C*), which virtually comigrates with the nerve 37-kDa protein when a mixture of the serum 36-kDa protein and the nerve 37-kDa protein is resolved by 2D-PAGE (Fig. 1*D*).

Purification and Characterization of the Immunoreactive Serum 36-kDa Protein: Identification as apoE. The similarities in molecular mass, pI, and immunological properties suggested that the nerve 37-kDa and the serum 36-kDa proteins were functionally identical. To investigate this further, the amino acid composition was determined for purified 37-kDa nerve and purified 36-kDa serum proteins. As shown in Table

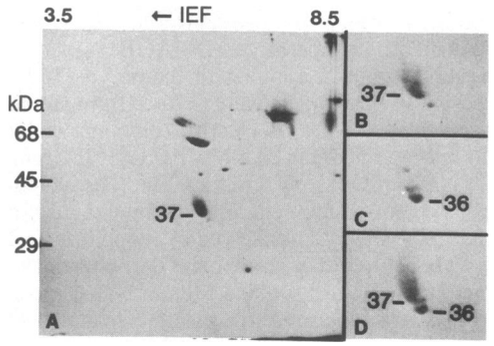

FIG. 1. Comparison of the nerve 37-kDa protein and the serum 36-kDa protein by 2D-PAGE. (*A*) Coomassie blue-stained 2D gel of extracellular proteins isolated from regenerating sciatic nerves 18 days "postcrush". (*B–D*) Vignettes of anti-37-kDa serum immunoperoxidase-stained electrophoretic transfer blots from 2D gels of extracellular proteins from regenerating sciatic nerves (*B*), rat serum (*C*), and a 1:1 mixture of rat serum and extracellular proteins from regenerating sciatic nerves (*D*). The numbers 36 and 37 refer to the apparent molecular masses (in kDa) of the only serum and nerve proteins detected by using the anti-37-kDa serum (1:750) in these 2D blots.

1, the amino acid composition of these two proteins is very similar overall and most closely resembles that of the arginine-rich plasma apolipoprotein apoE. To ascertain whether the serum 36-kDa protein and the nerve 37-kDa protein were immunologically related to apoE, we performed an electrophoretic transfer blot analysis of rat HDLs and VLDLs, the serum 36-kDa protein, and the soluble extracellular proteins from normal and injured sciatic nerves, using the anti-37-kDa serum and an authentic anti-apoE serum. Fig. 2 shows that the anti-37-kDa and the anti-apoE antibodies specifically recognize apoE in rat VLDLs and HDLs, the serum 36-kDa protein, and the 37-kDa protein. In control blots incubated with nonimmune serum as the primary antiserum, no staining was observed. It is apparent from Fig. 2 that the anti-37-kDa serum and the anti-apoE serum have identical specificities. Thus, we conclude, on the basis of their molecular masses [rat apoE molecular mass is 35 kDa (22)], pI values [rat apoE pI = 5.3–5.5 (23), serum 36-kDa protein apparent pI = 5.5, nerve 37-kDa protein apparent pI = 5.4], amino acid analyses, and immunological cross-reactivities that the serum 36-kDa protein and the nerve

Table 1. Amino acid composition (residues per molecule)

Amino acid	Nerve 37-kDa protein	Serum 36-kDa protein	apoE*
Glu	63.6	66.7	66
His	2.5	1.6	1
Arg	31.8	32.3	36
Thr	16.3	14.9	16
Ala	31.5	29.5	25
Pro	10.1	9.0	8
Asp†	13.7	20.2	21
Tyr	5.3	4.9	4
Val	20.1	18.1	18
Met	7.3	9.0	10
Ile	7.9	8.0	7
Leu	33.6	34.8	36
Phe	4.1	4.4	3
Lys	12.1	11.1	11

Amino acid composition was calculated based on a molecular mass estimate of 34 kDa for unglycosylated rat apoE (20).
*Calculated from the sequence of rat apoE mRNA (20).
†These values may be low because acidic residues are particularly sensitive to acid hydrolysis (21).

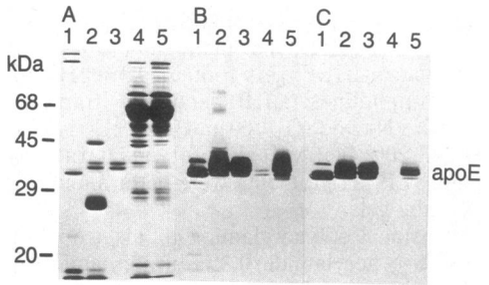

FIG. 2. Comparison of rat apoE from equivalent samples of VLDLs (lanes 1), HDLs (lanes 2), the purified 36-kDa protein (lanes 3), the soluble extracellular proteins (40 μg) from normal adult sciatic nerves (lanes 4), and the soluble extracellular proteins (40 μg) from injured (16 days postcrush) adult sciatic nerves (lanes 5) visualized by Coomassie blue staining (*A*) and electrophoretic transfer blot analysis utilizing an authentic antiserum to rat apoE (1:1500) (*B*) and the anti-37-kDa serum (1:500) (*C*) as probes.

37-kDa proteins are apoE. Unambiguous proof of the identity of the nerve 37-kDa protein and apoE will require the comparison of their entire primary amino acid sequences. It remains possible, therefore, that the 37-kDa protein is a member of a family of apoE-like proteins. All of our data are consistent, however, with the assignment of the 37-kDa protein as apoE.

The molecular mass heterogeneity of mature rat serum apoE, which is evident from Fig. 2, has been described (22), but the chemical basis for it is not understood. In view of our identification of the 37-kDa protein as apoE, the difference observed between the molecular masses and pI values of the serum 36-kDa protein (serum apoE) and the nerve 37-kDa protein seen in Fig. 1 most likely reflects the increased sialic acid content of newly synthesized and secreted apoE, which, in humans, is also more acidic and has a higher molecular mass than the mature serum form (24). In support of this interpretation, we have obtained evidence that part of the molecular mass heterogeneity of the nerve 37-kDa protein is sensitive to *Clostridium perfringens* neuraminidase treatment (unpublished observations).

Origin of Nerve apoE. Because apoE is found normally in plasma, the apoE identified in optic and sciatic nerves following injury could have arisen from extravasation subsequent to vascular injury rather than from the local synthesis and secretion by nonneuronal cells. To distinguish between these two possibilities, we radiolabeled proteins specifically synthesized by nonneuronal cells by incubating excised optic and sciatic nerves in [^{35}S]methionine. The [^{35}S]methionine-labeled nerves were homogenized and divided into two equal samples: one sample was immunoprecipitated with the anti-37-kDa antibody, whereas the other was precipitated with the anti-37-kDa antibody preblocked with purified serum apoE. The anti-37-kDa serum precipitated several species with molecular masses between 35 and 38 kDa, which were not precipitated with the apoE-blocked antiserum (Fig. 3). Thus, as apoE is an ≈35-kDa protein (22) that exhibits molecular mass heterogeneity on 1D-PAGE (Fig. 2; ref. 22), we con-

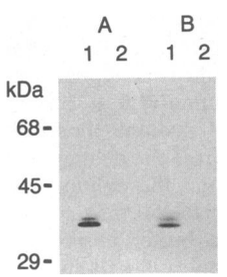

FIG. 3. Immunoprecipitation of apoE from [^{35}S]methionine-labeled whole nerve homogenates. apoE synthesized by the nonneuronal cells in optic (*A*) and sciatic (*B*) nerves was immunoprecipitated with the anti-37-kDa serum (lanes 1) but not with the anti-37-kDa serum preblocked with purified apoE (50 μg of apoE per 20 μl of antiserum) (lanes 2).

clude that the 37-kDa protein identified in normal and in injured nerves represents apoE synthesized by nonneuronal cells present in the nerves.

Immunohistochemical Localization of apoE after Nerve Injury. The specificity of the increased expression of apoE following nerve injury was examined by using immunohistochemistry. The anti-37-kDa serum was used to localize apoE in frozen sections of the brains of adult rats that had previously received a unilateral intraorbital optic nerve crush. The 37-kDa antiserum bound specifically to areas undergoing Wallerian degeneration. Immunoreactivity was seen in the optic tracts and in the retino-recipient zones of the lateral geniculate nuclei and the superior colliculus (Fig. 4 *a* and *c*). As shown in Fig. 4 *b* and *d*, control sections in which the anti-37-kDa serum had been preabsorbed with purified serum apoE showed no immunoreactivity. These results strongly suggest that apoE synthesis is stimulated by the presence of degenerating axons and that following injury, the increased expression of apoE synthesis and release is limited to areas undergoing degeneration.

DISCUSSION

In the present study, we have presented evidence that the previously described 37-kDa protein whose expression is associated with periods of nerve growth and degeneration is apoE. This assignment was arrived at in a stepwise fashion. First, we identified a 36-kDa protein in rat serum that is physically and immunologically related to the nerve 37-kDa protein. Next, we purified the serum 36-kDa protein and identified it as apoE by amino acid analysis and immunoreactivity with an authentic antiserum to rat apoE. Finally, we present direct evidence that the 37-kDa protein produced in response to nerve injury is also apoE. This identification is based on the similar molecular masses, pI values, and amino acid compositions of the nerve 37-kDa protein and serum apoE as well as on their immunological properties. We have also provided direct evidence that apoE is synthesized by nonneuronal cells of optic (CNS) and sciatic (PNS) nerves following injury and that apoE is seen in association with degenerating pathways in the CNS.

apoE is a major component of several classes of lipoproteins in the rat, including HDLs and VLDLs (25), and at least two specific cell surface receptors have been identified for apoE (26). Although most apoE is synthesized in the liver, the brain, adrenal, testis, and spleen are also major sites of apoE mRNA synthesis (27). There is a growing list of cell types that have been shown to synthesize apoE, including tissue macrophages (28, 29), smooth muscle cells (30), and ovarian granulosa cells (22). In the nervous system, Boyles *et al.* (31) have reported recently that apoE is found specifically within astrocytes in the adult CNS. Previously, Carey and Bunge (32) reported the release of a protein of an apparent molecular mass of 40 kDa, which may be apoE (5), from pure Schwann cell–neuron cultures. Thus, it appears likely that CNS astrocytes and PNS Schwann cells can synthesize and secrete apoE. We have also found that apoE can be localized to phagocytic cells that resemble macrophages in primary brain cell cultures from neonatal rat (unpublished observations).

Results from studies in neuronal and nonneuronal tissues suggest at least two possible roles for apoE during nervous system development, following injury, or in regeneration. First apoE may play a role in removing the by-products of degenerating axons from the brain. In nonneuronal tissues, it has been proposed that apoE helps to mobilize lipids and to mediate the transport of cholesterol from peripheral tissue to the central vascular pool (28, 29, 33). In the nervous system, cholesterol is a major by-product of the degeneration of myelinated axons (34). We hypothesize that following injury, phagocytosis of degenerating axons and myelin stimulates an increase in the synthesis and secretion of apoE by reactive

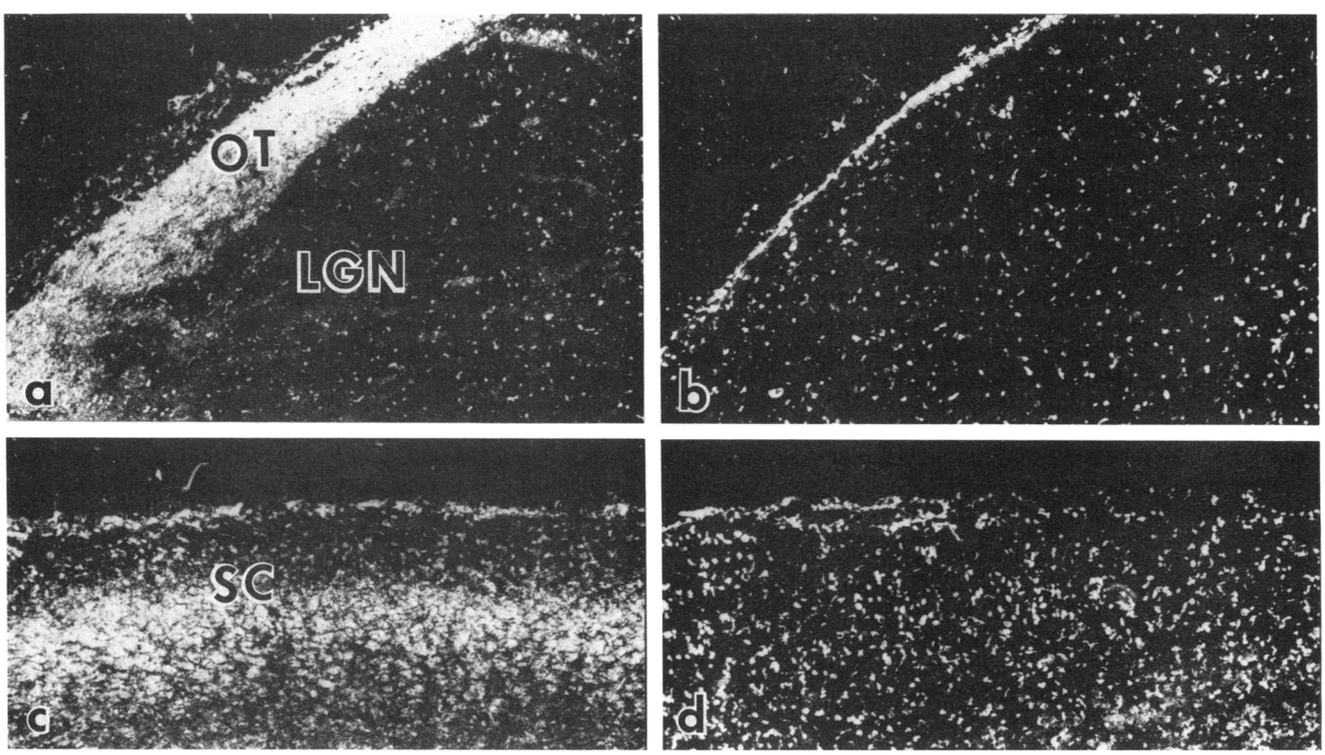

FIG. 4. Immunohistochemical localization of apoE. These are dark-field photomicrographs of anti-37-kDa immunoreactivity in the contralateral optic tract (OT) (*a*) as it enters the lateral geniculate nucleus (LGN) and the contralateral superior colliculus (SC) (*c*). Rats had received a unilateral optic nerve crush 12 days previously. Control sections (*b* and *d*) were allowed to react with the anti-37-kDa serum after preblocking with purified apoE. Peroxidase-antiperoxidase method. (×87.)

astrocytes [and possibly by other nonneuronal cells that are phagocytic (35)] in the CNS and Schwann cells in the PNS as they become laden with cholesterol and other ingested lipids. Indirect support for this hypothesis has been obtained from studies showing that macrophages, which also phagocytose cell debris, increase their secretion of apoE in response to cholesterol loading (28, 29). An analogous mechanism in the brain would explain why nonneuronal cells increase their synthesis and secretion of apoE in the distal, but not the proximal, nerve stump after nerve crush (5). Similarly, our immunohistochemical study of the localization of apoE following optic nerve lesions confirms that apoE appears to be associated only with that part of CNS pathways undergoing Wallerian degeneration. Likewise, in the PNS, the time course of expression of apoE can be correlated with the process of Wallerian degeneration (5, 36). The increased synthesis and secretion of apoE by nonneuronal cells that appears to be regulated developmentally (2–4) may be due to naturally occurring cell death or other regressive events that occur during the normal development of the nervous system (37).

apoE may also play a role in neuron growth by providing neurons, by way of a receptor-mediated endocytosis, with lipids necessary for axon growth. In this way, stable extracellular lipid complexes formed by apoE and cholesterol that were not removed from the brain could be reutilized by growing axons. Although apoE receptors have not been identified in brain, endocytosis of apoE lipoproteins has been demonstrated in nonneuronal systems. Furthermore, receptor-mediated endocytosis of apoE lipoproteins is believed to contribute to the availability of fatty acids and cholesterol, which are necessary to growing cells (38). Since neurons require cholesterol for the formation of new membrane during growth and for subsequent myelination of axons, a similar mechanism involving apoE may operate in nerves undergoing growth or regeneration. That apoE may be involved in axon growth is supported by a number of correlations: (*i*) apoE (37 kDa) synthesis and release is high during neonatal development in the CNS and PNS when axons are actively growing (2–4); (*ii*) an enhanced apoE synthesis and release occurs over the same time period as regeneration of the sciatic nerve in the PNS (5); (*iii*) the increased expression of apoE in the adult optic nerve (CNS) following injury is correlated with the time course of growth cone formation (3 days) and abortive sprouting (20 days) of mammalian optic axons (39); and (*iv*) apoE synthesis, which normally declines by 8 weeks following sciatic nerve crush as the nerve regenerates, remains elevated if the sciatic nerve is prevented from regenerating (4).

apoE has only recently been identified in brain (27, 31). The available data on the nerve 37-kDa protein and on apoE regulation in nonneuronal tissues, however, strongly suggest that apoE released during CNS and PNS development and following nerve injury primarily acts to mobilize degeneration by-products. apoE may also play a direct role in promoting axon growth by providing lipids that can be incorporated into the growing axonal membrane. It appears certain, however, that the regulation of apoE synthesis and secretion in the nervous system involves a functional interaction between axons and the nonneuronal cells that surround them.

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