THE EFFECT OF INJECTIONS OF
LYSOPHOSPHATIDYL CHOLINE INTO WHITE
MATTER OF THE ADULT MOUSE SPINAL
CORD

SUSAN M. HALL
Department of Anatomy, Guy's Hospital Medical School,
London, S.E. I, England

SUMMARY

The action of lysophosphatidyl choline, LPC, on myelinated fibres in the dorsal white matter of the spinal cord of the adult mouse has been studied electron microscopically, and compared with the recently described activity of LPC in the peripheral nerve fibre.

Control injections of sterile isotonic saline and injections of LPC both produced oedematous zones in the white matter; within these zones, many fibres exhibited the characteristic changes of Wallerian degeneration. After injection of LPC, however, an area of demyelination was observed, extending within and beyond the Wallerian degeneration. Ultrastructurally, demyelination involved progressive disruption of the previously-compact sheath, observed initially as a splitting of the intraperiod line within 30 min. Subsequent breakdown was indicated by the appearance of strands of 4-6 nm repeat lamellar material, itself further degraded through quintuple- and triple-layered lamellar units to disorganized membranous networks around undamaged axons.

The significance of the demyelinating activity of LPC is discussed in terms of its known action in in vitro systems of isolated central nervous tissue, and its action in vivo in the peripheral nervous system.

INTRODUCTION

The local action of some myelinolytic factor, or factors, possibly hydrolytic enzymes, or non-enzymic surface active agents, has been implicated in the pathogenesis of the 'demyelinating diseases' (Marburg, 1906; Thompson, 1961). In the latter category, the activity of the lysophosphatides, particularly lysophosphatidyl choline, LPC, has attracted some interest. It has been demonstrated that LPC can bring about demyelinating change in vitro in the central nervous system (Morrison & Zamecnik, 1950; Birkmayer & Neumayer, 1957); more recently, Périer (1965) obtained demyelination and subsequent remyelination in vitro with cultured rat cerebellum treated with LPC. Further, in vitro studies with brain homogenates in saline have demonstrated the rapid 'clearing' effect of LPC on such preparations (Webster, 1957); moreover, LPC will produce complete solubilization of brain myelin (Gent, Gregson, Gammack & Raper, 1964).

Recently, the demyelinating capacity of LPC has been demonstrated in the sciatic nerve of adult mice (Hall & Gregson, 1971). It was found that LPC produced a pure, demyelinating lesion in which the other non-myelin cellular components of the fibre
were apparently not involved. The demyelinated fibres became remyelinated within 14 days of microinjection of LPC. Wallerian degeneration was observed only as a consequence of axonal damage deliberately induced by multiple microinjections of LPC.

This study reports the effects of similar microinjections of LPC into the dorsal white matter of the adult mouse spinal cord, in an attempt to compare the reactivity of peripheral and central myelin to this substance.

**MATERIALS AND METHODS**

**Preparation of LPC**

LPC was prepared from egg lecithin following treatment with snake venom (Ankistrodon p. piscivorus) (W. L. G. Gent, N. A. Gregson & C. Lovelidge, in preparation). Solutions of LPC were made up with sterile, isotonic saline, to give a final LPC concentration of 10 mg/ml. Freshly prepared solutions of LPC were made up for each batch of injections.

**Injection of LPC**

Twenty-six adult mice were used in this experiment. The experimental animal was anaesthetized with Nembutal intraperitoneally, secured on a cork block, the skin of the back was shaved, and a mid-line incision made, exposing the thoracolumbar fascia and underlying musculature. Short, bilateral incisions were made in the fascia and in sacrospinalis, and the muscles were reflected and sutured back. Using a pair of fine scissors, the spinal processes of two adjacent lumbar vertebrae were cleaned of adherent muscle and then gently partially removed, exposing a small area of the dorsal surface of the spinal cord.

The animal was then positioned on the stage of a Meopta binocular dissecting microscope (final magnification x 22.4). Using a micromanipulator, the 1-μm tip of a freshly pulled hard-glass micropipette was introduced through the dura mater into the right dorsal column of white matter, and the volume of fluid contained within the tip (about 0.0002 ml) injected, by applying slight positive pressure to the syringe plunger. The micropipette was then withdrawn, the wound closed, and the animal allowed to recover for periods ranging from 30 min to 5 days. Three animals were examined at each of the following times; 30 min, 2 h, 24 h, 48 h, 72 h and 5 days, and 2 animals were examined after 1 h. Six animals were used as controls. In 3 animals the operative procedure was performed as described above, and the wound was immediately closed without injecting LPC into the cord; one animal was examined at 24 h, 48 h and 72 h. In a further 3 animals, sterile isotonic saline was substituted for the 10 mg/ml solution of LPC, and 1 animal was examined at each of the times stated for the uninjected controls.

**Preparation for electron microscopy**

Under Nembutal anaesthesia, animals were perfused through the left ventricle with Karnovsky's formaldehyde-glutaraldehyde solution, pH 7.3 (Karnovsky, 1965). Perfusion was continued for 15 min, after which the previous operative site was reopened, and complete transverse segments of the cord were removed and placed in fresh fixative for a further 75 min, at 4 °C. At the end of this time, the specimens were cut into 1–2 mm thick blocks and washed overnight in 0.2 M cacodylate buffer, pH 7.3. Specimens were postfixed in 1 % OsO₄ in Millonig's phosphate buffer, pH 7.3, for 1 h, dehydrated in an ascending series of ethanol, carried through 2 15-min changes of propylene oxide, and infiltrated and embedded in TAAB resin, polymerized at 60 °C for 48 h. The tissue was sectioned on an LKB ultramicrotome, sections were stained with uranyl acetate and lead citrate and examined in an RCA EMU 4 electron microscope.
RESULTS

Operated, uninjected controls

In the dorsal columns of operated, uninjected mice, the myelinated fibres were packed closely together (Fig. 1), so that at points of close contact adjacent myelin lamellae apparently fused, producing additional intraperiod lines. The triangular spaces separating neighbouring fibres were occupied by numerous slender glial processes, some, containing bundles of filaments, and, less frequently microtubules and glycogen granules, were identified as astroglial; others, from the continuity of their plasma membrane with the outer layer of the sheath, and their content of microtubules, were oligodendroglial. The myelin sheaths were composed of 10–11 nm repeat lamellar material of alternating major dense and intraperiod lines, in which the intraperiod line was always single (Fig. 8). Occasional irregular splits, limited to short stretches of a few lamellae, were observed in the sheaths, and were assumed to be preparative artifacts. The axoplasm contained numerous filaments, microtubules and occasional small mitochondria; smooth-walled vesicles and components of the endoplasmic reticulum were not observed.

Injected dorsal columns

The injection zone was oedematous in all specimens examined, irrespective of whether saline or LPC had been injected. This resulted from an enlargement of the extracellular space, accompanied by glial swelling, the oedema involving both the dorsal column and the posterior part of the ipsilateral lateral column. There was, however, a marked difference in the subsequent reaction of the myelinated fibres to the presence of the 2 substances.

In both cases, fibre damage consistent with previous descriptions of Wallerian change in the spinal cord (Lampert & Cressman, 1966; Lampert, 1967), was observed within 48 h of injection in the immediate injection zone. This damage was primarily reflected in the dramatic alteration in axonal morphology. Whereas in the uninjected controls the axoplasm had contained few organelles besides filaments and microtubules, the axons were now stuffed full of dense bodies, lamellar forms, degenerating mitochondria and granules of all sizes (Fig. 2). There was little initial change in the lamellar pattern of the myelin sheath. By 48 h, however, there was some splitting of the sheath, generally along the intraperiod lines, frequently accompanied by the collapse of loops and whorls of 10-nm repeat material into dilated periaxonal space.

Within 48 h, myelin debris was sometimes observed in vacuoles in perivascular macrophages. During the same period there was a rapid glial response, as indicated by the swollen tongues of oligodendrocyte cytoplasm associated with the outer aspect of the degenerating myelin sheath, and the swelling of astrocyte perivascular feet.

However, while saline injections resulted in only a limited zone of degeneration and oedema, consistent with traumatization of a small area of white matter, LPC produced a demyelinating lesion extending within and beyond the degeneration initiated by the mechanical disruption of its injection. The earliest response of the sheath, occurring within 30 min of injection of LPC, was a loss of the previously compact myelin
architecture, resulting from the splitting of the intraperiod lines (Figs. 3, 9, 10). Although the electron micrographs have been arranged sequentially, all stages of demyelination were observed in the LPC-treated white matter during the 24–72 h time interval, and it is therefore not possible to state the time taken for individual steps in the process.

Splitting of the intraperiod lines, within the range 25–55 nm, produced relatively orderly regions of quintuple-layered lamellar units, consisting of 2 outer hemi-intraperiod lines separated from a central major dense line by 2 pale interzones. While in some of the smaller fibres this change involved the total width of the fibre, in most fibres these regions were separated by and continuous with strands of 4–6 nm repeat lamellar material composed of alternating dark and light bands of equal width, in which it was not possible to distinguish between the original major dense or intraperiod line (Fig. 10). Subsequent progressive widening of the intraperiod line gap, the appearance of large, irregular electron-translucent spaces between the layers of the 4–6 nm repeat material and the breakdown of any remaining 10–11 nm repeat compact myelin, resulted in a swollen structure composed of quintuple- and triple-layered units (Fig. 4). Rounding-off of these strands of lamellar material, in a manner similar to that observed in the myelin sheaths of peripheral nerve fibres after injection of LPC (Hall & Gregson, 1971), produced a foam-like system around the apparently normal, if somewhat shrunk, axon (Fig. 5).

Over the 3-day period, much of this material disappeared from the extracellular space: the various lamellar forms of degenerating myelin and the membranous elements of disrupted glial cells were all seen within vacuoles in the cytoplasm of macrophages which were present in the area after 24 h. Further degradation of the components of the sheath was indicated by the large lipid droplets, some of which were membrane-bound, present in these macrophages by the third day (Fig. 7). Small whorls of lamellar debris, predominantly the 4–6 nm repeat material, were also observed infrequently in the cytoplasm of both astrocytes and oligodendrocytes, although there was never any indication of subsequent degradation, i.e. to lipid globules, in these cells.

The axons remained apparently undamaged throughout the period of demyelination, although in many cases, the increased density of axoplasmic organelles, and the irregular axonal outline were indicative of some degree of shrinkage. Most probably, this shrinkage reflects an osmotic response to the altered ionic environment of the axon, resulting from the loss of the sheath, and from the presence of various myelin-degradation products. Similar axonal changes in axons otherwise considered to be viable, have been described in experimental allergic neuritis (Allt, Evans & Evans, 1971), and in LPC-induced demyelination in peripheral nerve fibres (Hall & Gregson, 1971). In most instances in the present material, this shrinkage appeared to be a transient feature, which was less evident by 5 days (compare the relative axoplasmic densities in Figs. 8 and 9).

Within 5 days the original area of densely packed myelinated fibres had been replaced by an oedematous zone containing denuded axons (Fig. 6), still surrounded by the filamentous remnants of their original investing sheaths, swollen and dis-
Effects of lysophosphatidyl choline on white matter

ruptured glial processes, and the watery cytoplasm of the occasional 'reactive clear glial cell' (Gonatas, Zimmerman & Levine, 1962). The lesion was sharply delineated, and was bordered by normal myelinated fibres, morphologically indistinguishable from the uninjected controls. Unlike the LPC-treated peripheral nerve fibres, no evidence of remyelination was obtained in any of the specimens examined.

DISCUSSION

The demyelinating capacity of LPC, already described in CNS tissue in vitro (Morrison & Zamecnik, 1950; Birkmayer & Neumayer, 1957; Périer, 1965), and more recently in mature, myelinated peripheral nerve fibres in vivo (Hall & Gregson, 1971), has been demonstrated in dorsal white matter of the adult mouse spinal cord. The experimental results indicate both the overall morphological similarity between central and peripheral LPC-dependent demyelination, and also the rapidity - within 30 min - with which one form of experimentally induced central demyelination can occur. The latter finding is interesting in view of a recent report of the rapid demyelination produced within 2 days of the direct introduction of diphtheria toxin into the CNS (Harrison, McDonald, Ochoa & Sears, 1970).

As in peripheral nerve, the action of LPC would seem to be restricted primarily to the myelin sheath, with no damage, on morphological criteria, to the previously myelinated axons. It is more difficult to determine what action, if any, LPC has on the glial population. In the normal, uninjected control dorsal columns, there was little evidence of glial cytoplasm; it was almost entirely restricted to microtubule-bearing tongues of oligodendrocyte cytoplasm continuous with the outer layer of the myelin sheaths, and narrow cytoplasmic processes between adjacent myelinated fibres. In the oedematous zones produced by the mechanical disruption of injecting either saline or LPC, many of these processes appeared intact, albeit somewhat swollen. Again in the demyelinating lesion, many processes, particularly of astrocyte cytoplasm, although swollen, remained identifiable on the basis of their content of organelles. Others, however, were undoubtedly damaged, contributing to the membranous debris surrounding axons and contained within macrophages by the third day. Since the 1:1 relationship between the myelinated internode and its satellite cell found in the peripheral nervous system does not necessarily exist in central tissue (Peters, 1964), and further, since individual glial cell territories may involve the extension of long cytoplasmic processes for variable distances of up to 12 μm from the cell body (Bunge, 1968), it follows that glial cells already damaged in the zone of Wallerian degeneration would exhibit change in all their processes, even where these extended beyond this zone into the demyelinating lesion. It was considered that in the peripheral nerve fibres the apparent lack of interaction between LPC and the Schwann cell membranes was attributable to a rapid reacylation of any bound LPC by the Schwann cells (Hall & Gregson, 1971). Neural tissues have been shown to possess the ability to acylate LPC quite rapidly (Webster & Alpern, 1964). However, it is not known whether all components of such tissue possess this ability equally, or whether membranes already damaged, e.g. in Wallerian degeneration, retain this activity.
It seems reasonable to consider that the demyelination produced by LPC reflects a primary attack on the myelin sheath, rather than a secondary consequence of a non-viable oligodendrocyte. Even allowing for a higher binding of LPC to extraneous protein in the cord, the results have indicated that LPC acts as rapidly on central myelin in vivo as it does on peripheral myelin. One reason for this may be the relatively greater surface area of 'unprotected' sheath immediately available in white matter, since unlike the Schwann cell in the peripheral nerve fibre, oligodendroglial cytoplasm typically covers only a fraction of the myelin circumference.

The ultrastructural alterations in the sheath, splitting of the intraperiod lines and subsequent breakdown of quintuple- and triple-layered units to disorganized reticular systems around undamaged axons, are similar to those seen in peripheral fibres (Hall & Gregson, 1971). Disruption of the intraperiod line is a well documented feature of both peripheral and central myelin following various forms of experimental manipulation, and is generally interpreted as indicating an increase in the hydration of the sheath at the intraperiod line. It is likely, however, that this extreme separation of the intraperiod line reflects an increase in the hydrophilic nature of the molecular components of the sheath attributable to the addition of LPC (Gent, Gregson, Lovelidge & Winder, 1971).

The changes described may be correlated with those seen during the solubilization of central myelin in vitro: on addition of approximately half the total amount of LPC required, an expanded structure is produced and a component containing the principal part of the basic protein is lost (W. L. G. Gent, N. A. Gregson & C. Lovelidge, in preparation). Subsequently, this expanded structure collapses to give a 4-nm repeat structure in which the bands of electron-dense material are narrow, and of identical density.

It is interesting, in the context of the sequence of morphological changes described above, to consider Lampert's report of demyelination in experimental allergic encephalitis (Lampert, 1965), in which the myelin sheath was found to undergo a 'vesicular transformation and dissolution'... in the presence of mononuclear cells. Many of the changes in the sheath resemble those produced by LPC, the latter appearing in the absence of any gross cellular infiltration.

I wish to thank Dr N. A. Gregson of this Department for supplying the lysophosphatidyl choline.

REFERENCES


Effects of lysophosphatidyl choline on white matter


(Received 9 August 1971)
Fig. 1. Operated, uninjected dorsal column. At points of close contact additional intraperiod lines are observed. Note relative paucity of axoplasmic organelles. \( \times 23250 \).

Fig. 2. Dorsal column, white matter, 48 h after injection of sterile isotonic saline. Compare the axoplasm with that seen in Fig. 1. Numerous dense bodies, lamellar forms and degenerating mitochondria are present in the granular axoplasm. The myelin sheath, initially unchanged, has become disorganized, and exhibits large spaces, due to separation of the intraperiod lines. \( \times 23250 \).
Effects of lysophosphatidyl choline on white matter
Fig. 3. Dorsal white matter 30 min after injection of LPC. While axoplasm appears undamaged, the myelin sheath has already undergone extensive alteration, as a result of gross splitting of the intraperiod lines. × 19600.

Fig. 4. Dorsal white matter 48 h after injection of LPC. Progressive widening of the intraperiod line gap has occurred; however, the lamellae remain approximately concentrically arranged. × 22050.

Fig. 5. Dorsal white matter 48 h after injection of LPC. Axon is surrounded by a network of membranous profiles. × 21700.

Fig. 6. Dorsal white matter 72 h after injection of LPC. Axon is surrounded by membranous remnants of its myelin sheath, swollen glial processes and possible glial debris. × 31500.

Fig. 7. Dorsal white matter 48 h after injection of LPC. Macrophage (m) contains several large lipid globules. Axon appears undamaged, and is still partially surrounded by myelin debris. × 42900.
Fig. 8. Operated, uninjected dorsal column. The intraperiod line is single throughout the sheath. × 90,000.

Fig. 9. Dorsal column 30 min after injection of LPC. The intraperiod line is widely split, producing quintuple-layered lamellar units. Despite a grossly dilated periaxonal space, the axoplasm appears normal. × 120,000.

Fig. 10. Dorsal column 48 h after injection of LPC. Several stages of myelin breakdown are present in this sheath, from simple splitting of the intraperiod line (a), through quintuple-layered units (b), to strands of 4–6 nm repeat lamellar material (c). × 180,000.