Rapid accumulation of acetylcholine in nerve above a crush

An accumulation of axoplasmic material in crushed or cut axons has been attributed to interruption of a proximo-distal movement of material in the axons (Weiss & Hiscoe, 1948). The transmitter noradrenaline has been found to accumulate rapidly after ligation of nerves containing adrenergic fibres (Dahlström & Häggendal, 1966; Kappeller & Mayor, 1966). The noradrenaline is probably stored in amine granules that are transported distally in the axon. These neurons have been extensively studied with respect to this transport, which is of the "fast" type (several mm per hour, Dahlström & Häggendal, 1970). In cholinergic nerves, Sastry (1956) observed that acetylcholine increased above a nerve section. Further studies by Evans & Saunders (1967) revealed an increase in acetylcholine proximal to a crush made in nerves 3–25 days earlier. The present report describes some results from studies of acetylcholine accumulation in nerves up to 24 h after crushing, with an approach similar to that used by Dahlström & Häggendal (1966, 1970).

Sciatic nerves of rats (Sprague-Dawley, 200–230 g males) were used. Axons in both sciatic nerves were crushed by applying pressure on the nerve with a fine thread (diameter 0·1 mm) pulled against a metal rod for 5 s (Dahlström & Häggendal, 1966). One crush was placed at a level 1–2 mm below the *foramen infra-piriformis*. In some experiments a second crush was made at the same time 15 mm distal to the first. Control nerves were either uncrushed or crushed just before removal ("0 h crush").

The nerves were divided into 10 mm lengths above and below a single crush (Fig. 1) or into 5 mm lengths relative to double or single crushes as shown in Fig. 2. In the experiments of Fig. 1, 10 mm lengths were pooled from both sciatic nerves of 2 rats; in experiments of Fig. 2, 5 mm lengths from both sciatic nerves of 5 rats were pooled. Acetylcholine was extracted (MacIntosh & Perry, 1950), and the activity estimated on the guinea-pig ileum in the presence of antihistamine using a modification of the method of Blaber & Cuthbert (1961).

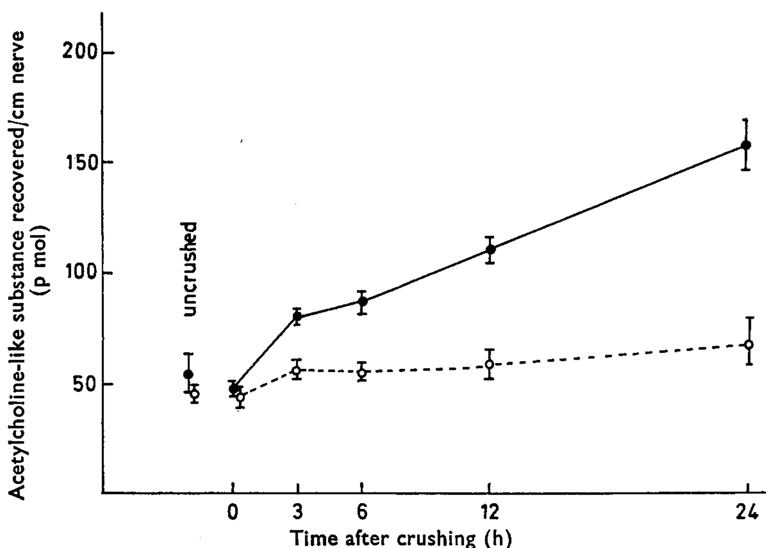

FIG. 1. The amount of acetylcholine (ACh)-like substance in the 1 cm of sciatic nerve of rat just above (●——●) and just below (○ - - - ○) a single crush. Means ± one s.e. are given, n = 4 in all cases. The ordinate indicates the amount of ACh-like substance in pmol per 1 cm of nerve (uncorrected for losses). The abscissa indicates the time interval between crushing and removal for extraction. "Uncrushed" indicates the values for the uncrushed nerve parts above (●) and below (○) the level of the crush.

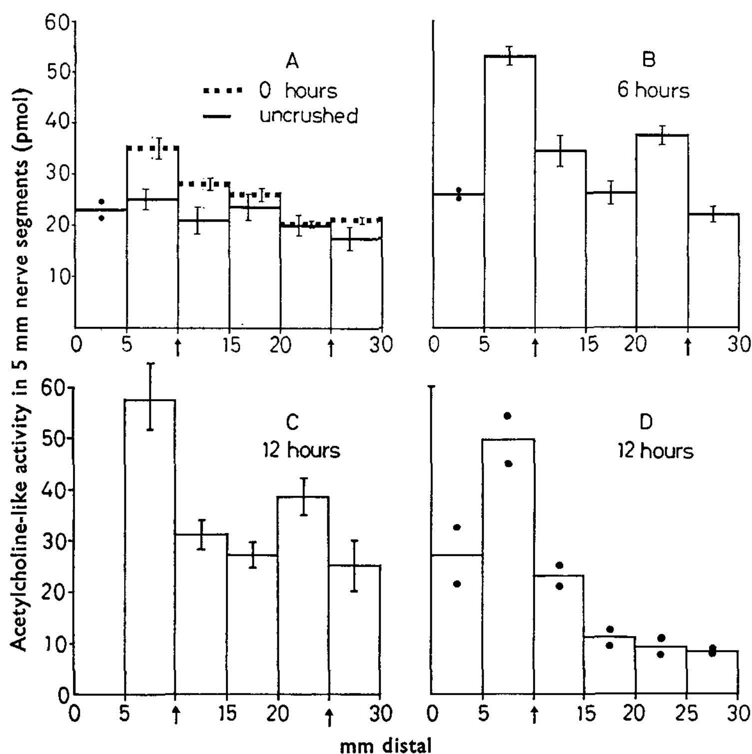

FIG. 2. The amount of acetylcholine (ACh)-like substance in 5 mm nerve segments of rat sciatic nerves. Means ± one s.e. are given for experiments, where n = 4–6. Where n = 2 the individual values are given. The ordinate shows the amount of ACh-like substance in pmol per 5 mm segments (uncorrected for losses). The abscissa indicates the distance along the nerve from the highest level 8–9 mm above *foramen infrapiriformis*. The arrows show the sites of nerve crush made 0, 6 or 12 h before dissection. In A the solid lines are uncrushed nerves. In A–C the total amount of ACh between the crushes is: A, Oh, Crush 63·2; Uncrushed 73·1; B, 98·0; C, 96·1 pmol.

The extract was inactivated by boiling in alkali or by incubating with cholinesterase. The active principle was thus shown to be "acetylcholine-like" but is referred to as acetylcholine.

The acetylcholine content in the 1 cm part of nerve just proximal to a single crush increased with time after crushing (Fig. 1). During the first 3 h the increase appeared to be greater than in later periods. The maximum value was about thrice normal at the longest time investigated (24 h) and was still rising. Below the crush there was only a slight increase. The increase above the crush was probably restricted to a region within 5 mm of nerve just above the lesion, as indicated by the results from the second experiment (Fig. 2B and D). The level of acetylcholine in the 5 mm of nerve distal to the crush was similar to that of the control, but in the more distal parts it dropped to about 40% of control by 12 h (Fig. 2D).

The increase in acetylcholine above the crush could have resulted from (a) interrupted proximo-distal bulk flow of axoplasm giving a local increase in cholinergic axon volume, (b) interrupted specific transport of acetylcholine or some factor (or both) responsible for its synthesis or storage, or (c) increased local synthesis of acetylcholine following crushing. Regarding (c), Feldberg (1943) has shown that damage to cholinergic axons causes increase in their acetylcholine content. This would be expected to

produce a symmetrical increase about the crush. But the accumulation of acetylcholine was always much greater central to a crush (Figs 1 and 2): thus it seems that damage-induced local synthesis is not an important factor in this accumulation. However, increased local synthesis may make a small contribution since the total amount of acetylcholine between two crushes (Figs 2B and C) was approximately 30% higher than in the same length of control nerve.

The rate of bulk flow of axoplasm has been estimated to be about 1–2 mm/day (Weiss & Hiscoe, 1948). This is far too slow to account for the rapid increase in acetylcholine that we found proximal to a crush. It seems more probable that crushing a nerve interrupts a specific cholinergic transport mechanism, since the peripheral loss of acetylcholine, and its accumulation central to a crush described above, would both be accounted for by a distal movement of acetylcholine of about 20 mm/day. However, this is likely to be an under-estimate for two reasons. Firstly, the rate of accumulation in the first 3 h appeared to be faster than in the subsequent period investigated (Fig. 1) Secondly, a proportion of the acetylcholine appears to be relatively immobile since at 12 h below the crush there was still some 40% of the control amount; also it has been found that even after several days the level does not fall below 20% of control (Evans & Saunders, 1967). Allowing for these factors would give a rate of transport which is probably several mm/h.

The distribution of noradrenaline (Dahlström & Häggendal, 1970) and acetylcholinesterase (Lubińska, Niemierko & others, 1964) have previously been studied in single and double crushed nerves. Qualitatively, noradrenaline, acetylcholinesterase and acetylcholine all behave similarly, and their rates of transport are all appreciably faster than the rate of bulk flow of axoplasm (Dahlström & Häggendal 1970 and Lubińska & Niemierko, 1971). Since redistribution of all three substances occurs *between* two crushes, it seems that their transport could be independent of continuity with the perikarya. Accumulation of intra-axonal organelles has been described after crushing (Martinez & Friede, 1970), but further studies are needed to elucidate the possible correlation between transport of acetylcholine and organelles such as vesicles.

Thus we have found that crushing a nerve produces a rapid accumulation of acetylcholine above a crush. We suggest that this is probably due to interference with a fast transport mechanism.

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Department of Pharmacology,
University of Göteborg,
Göteborg, Sweden.
C. JAN HÄGGENDAL

Department of Physiology,
University College London,
London, U.K.
NORMAN R. SAUNDERS

Institute of Neurobiology,
University of Göteborg,
Göteborg, Sweden.
ANNICA B. DAHLSTRÖM

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A novel thin-layer chromatography system for lysergide (LSD)

A continually increasing demand for the rapid identification of substances suspected of containing lysergide (LSD) prompted an investigation into the possibilities of faster and more reliable forensic methods. Screening tests have already been used which eliminate many "innocent" samples from further investigation. The use of ultraviolet induced fluorescence (Government Chemist, 1969) together with a modified field test (Alliston, Bartlett & others, 1971) can restrict the problem to certain erganes and tryptamines within a few minutes.

Thin-layer chromatography has been widely employed for the final stage of the identification of lysergide. Phillips & Gardiner (1969) investigated a number of systems and recommended that of Genest & Farmilo (1964), which employs development with chloroform–methanol (9 : 1) on NaOH treated silica gel plates, for the separation of a number of natural and synthetic erganes. This is not convenient for commercial silica gel coated polyester sheets such as Eastman 'Chromagram' on which it is necessary to spot 2 μl 0·1N NaOH at the origin. Moreover, with certain common (and licit) preparations of the natural ergot base ergotamine, coextraction of the other ingredients in compound preparations may cause mobility to be modified to such an extent that, in some cases, ergotamine is confusible with lysergide. A favoured alternative—development with chloroform–methanol (1 : 4) on silica plates (Martin & Alexander, 1967)—is not an improvement in practice; low mobility and heavy streaking are observed. Separation can be achieved with 1,1,1-trichloroethane–methanol (96 : 4) on alumina plates (dal Cortivo, Broich & others, 1966) but mobilities are low and "eyebrow" shaped spots (indicating movement on a secondary solvent front) are observed. This reduction in the proportion of the plate available for separation occurs with many other systems, especially where more than two solvents are employed.

In seeking an entirely new system for use with "Chromagram" sheets, incorporation of an organic base into the mobile phase seemed desirable. Development with systems containing varying proportions of diethylamine, aniline, quinoline, morpholine, picoline and ethanolamine in a series of solvents was investigated. In most cases the substances examined did not move in the system or were carried with the solvent front. Where separation did occur, the ergotamine spot had a large tail (even after an equilibrium between the epimers had been established) or, in the case of 5% diethylamine in chloroform, 8β-ergotamine and 8α-lysergide were resolved but 8α-ergotamine and 8β-lysergide moved with the solvent front. Morpholine appears to be the best base and 1 : 9 the optimum admixture with toluene. Morpholine–xylene (1 : 4) or morpholine–benzene (1 : 9) are also suitable developing solvents but the extra drying time required for xylene and the health hazard of benzene would preclude them from routine use.

The following procedure is recommended. Place 15–20 mg of the crushed sample in an ignition tube and add 1–2 drops of reagent grade methanol. Stir with a pointed glass rod, stopper and allow to stand for 5 min. Spot 0·5 μl of the supernatant liquor onto a 8 cm × 4 cm "Chromagram 6060" sheet (silica gel with fluorescent indicator).