A light and electron microscopic study of the accumulation of material in sectioned rat dorsal roots and the effect of demecolcine

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Accumulation of vesicular material has been demonstrated in sectioned, crushed and ligated nerves *in vivo* and *in vitro* (Dahlstrom, 1971). The following experiments were carried out in order to examine the effects of sectioning rat dorsal roots as a basis for the study of the nature, formation and transport of axoplasmic material in sensory neurones.

Rats (250 g) were anaesthetized with urethane and dorsal roots L3-5 were sectioned. Twenty hours later the animals were perfused with glutaraldehyde (2.5%) and the sectioned roots were examined by light and electron microscopy.

The nerve on the ganglionic side of the cut showed numerous unmyelinated profiles at the cut tip, many of them packed with vesicles. It was seen from longitudinal sections that some of these unmyelinated 'sprouts' arose from myelinated axons. In both myelinated and non-myelinated axons there was an accumulation of mitochondria and vesicles (60-100 nm in diam., some dense-core) for a distance of 500 μm behind the tip. With increasing distance from the tip, the proportion of mitochondria increased and the accumulation became confined to the periphery of the axoplasm. The nerve on the side of the cut distant from the ganglion showed no unmyelinated profiles at the cut end. In addition, the myelin was degenerate, the Schwann cells were active, and the axoplasm of both myelinated and non-myelinated axons was packed with debris including some vesicles and many lamellated bodies.

When dorsal root ganglia with a length of root attached were cultured *in vitro* by the method of Trowell (1959) for 20 h in Medium 199 (Flow Laboratories), the appearance of the cut-end of the nerve was similar to that of a nerve sectioned *in vivo*. However, when an equivalent segment of dorsal root without the ganglion attached was cultured for 20 h the appearance of the axoplasm some distance from the cut end was normal but there were no unmyelinated profiles at the tip, nor was there any accumulation of mitochondria or vesicles.

When intact ganglion-nerve preparations were cultured for 20 h in the presence of demecolcine (3×10^{-7} M) no axonal sprouting or accumulation of mitochondria and vesicles was seen.

In conclusion it appears that in sectioned dorsal roots axonal sprouting and accumulation of material occur only in the presence of cell bodies. Demecolcine (3×10^{-7} M), which disrupts neurotubules, prevents this accumulation and sprouting. Further work on this preparation is necessary to determine the role of axonal transport in these processes and their importance in neuronal function.

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Proconvulsant action of folic acid

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Folic acid (FA) is convulsant when injected intracerebroventricularly (i.c.v.) in mice (Baxter, Miller & Webster, 1973). It has been suggested that in epilepsy high localized folate concentrations could form epileptic foci (Hommes & Obbens, 1972). Microiontophoretic FA has been shown to either stimulate cortical cells or to enhance the effect of applied glutamate (Hill, Miller, Straughan & Webster, 1973). As increased FA may therefore predispose to convulsions we have studied its proconvulsant action (i.c.v.) in mice and rats and compared it with other convulsants by the same route.

In threshold electroshock studies using mice, the normal clonic response was intensified to hind limb extension (HLE) by FA pre-treatment. The ED_{50} values for HLE for FA alone and with electroshock 15 min later were 20.5 and 1.5 μg

respectively (ratio = 13.7). Picrotoxin and ouabain also had strong proconvulsant actions (ratios 97 and 91 respectively), maximal at 15 min like FA. Ouabain had a longer duration of action (at least 4 h) than FA and picrotoxin (1 h). Other convulsants tested (glutamate, homocysteic acid, leptazol and strychnine) were only weakly active immediately (5-60 s) after administration and had low ratios (1.4 to 2.7). In the leptazol infusion test (0.3 mg/min i.v.) in mice all drugs tested except ouabain when administered at up to convulsant ED_{50} values significantly reduced the time from clonus to HLE ($P < 0.05$).

The proconvulsant action of sub-convulsive doses of FA in mice to a strong afferent stimulus which was itself not convulsant was examined by subjecting the mice to an auditory stimulus (110dB, 15 s) from a bell suspended at 70 cm in a box 34 cm diameter and 76 cm high. In mice pre-treated (−15 min) with FA, generalized convulsions (ED_{50} = 2.3 μg) and HLE (2.8 μg) were readily induced. Convulsions occurred in only 4% of mice (n = 90) pretreated with control fluid (i.c.v.).

It is known that repetitive electrical stimulation applied locally to the cortex can act as an epileptogenic focus which will produce an after-discharge (AD). ADs were induced in rats chronically implanted with an electrical connector linked to screw electrodes in the skull (Goff, Miller, Smith, Smith & Wheatley, 1975). Stimulation (100 Hz, 1 ms width, 1 s duration) through a frontal screw induced clonic convulsions accompanied by AD of mean duration 12.1 s (n = 9). When tested 90 min later, FA (10 μg intraventricularly) at 15 min before the second stimulation significantly increased AD duration by 90% (P = 0.001: n = 3). Control fluid (intraventricularly) significantly reduced AD duration by 62% (P = 0.05: n = 3) whereas in untreated rats (n = 8), AD duration was unchanged after the second stimulation.

The results suggest that raised folate concentrations can increase cortical activity and therefore increase the tendency to convulsions.

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Further observations on the change in sensitivity to halothane induced by acute administration of central nervous system depressant drugs in the rat

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In a previous communication we outlined the use of repeated determination of halothane-induced sleeping time as a method for studying changes in the excitability of the central nervous system (CNS) occurring with time (Turnbull & Watkins, 1975). We reported that pre-treatment of rats with sodium pentobarbitone (a total dose of 90 mg kg^{-1} over a period of 10 h) or meprobamate (800 mg kg^{-1} 10 h^{-1}) resulted in the development of a hyposensitivity to halothane which was followed by a rebound hypersensitivity to the anaesthetic. However, we could not be certain that the changes in sleeping time were entirely due to changes in the sensitivity of the CNS. We have therefore measured brain halothane concentrations on awakening in saline and drug pretreated rats and have found the same pattern of change in CNS excitability as was indicated by the sleeping time.

First, we have confirmed that repeated exposure to halothane does not induce hyposensitivity. The brain halothane concentration found on awakening from the last of twelve exposures to halothane was the same (111 ± 10 (6) μg g^{-1} ± s.e. mean) as that found in rats which had been anaesthetized only twice during the same 48 h period (120 ± 6 (6) μg g^{-1}). Secondly, we have shown that the diurnal variation in sleeping time is due to an altered sensitivity of the CNS to halothane, since rats awakened with a higher brain halothane content at 0100 h (167 ± 7 (6) μg g^{-1}) than at 1100 h (126 ± 11 (6) μg g^{-1}).

We have also repeated our experiments in which