The effects of an anti-mitotic drug, bleomycin, on glial repair in an insect central nervous system

J.E. Treherne, E.A. Howes, C.A. Leech, and P.J.S. Smith
AFRC Unit, Department of Zoology, Cambridge, U.K.

Summary. The DNA-binding drug, bleomycin, has a profound effect on neural repair following selective glial disruption by ethidium bromide. The contribution of the granule-containing cells (which normally appear in the early stages of repair) is greatly reduced, the restoration of the blood-brain barrier is delayed and the ultrastructural organization of the reorganising perineurium is dramatically changed. The aberrant perineurial structure and function observed in the presence of bleomycin are postulated to result from the effects of the drug on haemocytes which, together with endogenous reactive cells, contribute to the normal process of glial repair.

Key words: Glia – Haemocyte – Central nervous system – Bleomycin – Neural repair – Insect, Periplaneta americana

A primary aim of this paper is to contribute to an understanding of the processes of recruitment and differentiation of the cell types utilized in neural repair, following selective glial disruption. An important current problem in this field concerns the identity and functional potentials of the cellular elements involved in morphogenesis of the repairing central nervous system. Recent studies with cultured vertebrate cells have indicated the retention of the mitotic capability by differentiated neuroglia (Latov et al. 1979) as well as by progenitor cells which give rise to more than one class of glial cell (Raff et al. 1983). In vivo work concentrating on anatomical or labelling experiments has demonstrated glial proliferation after lesioning (Morgese et al. 1983; Ludwin 1984) and a participation of blood monocytes in neural repair has also been indicated (Adrian and Schelper 1981). In amphibian limb regeneration, de-differentiation of Schwann cells seems to be important in the early stages of blastema formation (Brockes 1984).

In this investigation we have used the central nervous system of an insect, the cockroach (Periplaneta americana), as a model system to study the repair of the neuroglia following its selective disruption by a DNA intercalating agent, ethidium bromide (Nelson and Tinoco 1984), previously used to demyelinate mammalian spinal cords (Yajima and Suzuki 1979; Blakemore 1982). This compound, which is taken up from low external concentrations into the cockroach nerve cord (Leech 1984), is locally-applied, in vivo. As only central nervous connectives are exposed to the toxin, the neuronal nuclei (which are confined to the ganglia) are unaffected and normal axonal excitability is maintained, with no detectable damage to the neural lamella or the underlying intercellular matrix, despite extensive glial disruption (Smith et al. 1984).

In contrast to the effects of surgical lesioning, which causes extensive axonal damage, glial proliferation and changes in the extracellular matrix (Treherne et al. 1984), selective glial disruption results in a swift and ordered restoration of glial structure and function (Smith et al. 1984). A consistent feature of the early stages of repair is the prominent involvement of granule-containing cells which appear at the outer surface of the neural lamella and penetrate into the disrupted perineurial glia. Initially, these cells are involved in phagocytosis of perineurial cell fragments. However, after 48 h they begin to form ordered layers at the periphery of the connectives with no obvious signs of phagocytotic activity and morphological characteristics similar to those of the normal perineurium are assumed in 4 to 6 days. The cytology of these superficial repairing cells changes progressively as the blood-brain barrier is re-established and by 28 days they are indistinguishable from those of the normal perineurium.

The non-phagocytic, granule-containing cells share a number of cytological characteristics with haemocytes (cf. Scharrer 1972). This suggests that superficial repair could be achieved by exogenous (haemocytic) cells (Smith et al. 1984). It is not clear, however, to what extent endogenous (glial) cells are also involved in repair as might be predicted, for example, from recent work on amphibian limb regeneration in which de-differentiated glia have been postulated to play a critical role (Brockes 1984).

In this investigation we have used a DNA-binding drug, bleomycin, to determine its effects on the pattern of neural repair following selective glial disruption with ethidium bromide. Bleomycin is a basic, water-soluble, glycopeptide which acts by simultaneously binding to DNA and to a metal ion (Fe II) with degradation of DNA resulting from oxidation of the metal ion in its proximity (Sausville and Horwitz 1979). Damage occurs selectively at those regions of the DNA strand which are actively expressing informa-
tion (Kuo 1981). We have shown that this drug dramatically reduces the contribution of granule-containing cells and produces aberrant repair of the superficial neuroglia. These observations suggest that normal glial repair is achieved by the combined action of exogenous and endogenous cells, the degree of their interaction possibly depending upon the location in the nervous system.

Materials and methods

Adult male cockroaches (Periplaneta americana L.) were used in all experiments. To expose the abdominal connectives to ethidium bromide, the animals were immobilised by immersion in water for 4 min before being restrained, ventral surface uppermost, on a Sylgard plate. As previously described in more detail (Smith et al. 1984), a flap of cuticle was lifted to allow access to the connectives, between the 4th and 5th abdominal ganglia, which were then cleared of loose fat tissue. A small stainless steel platform, mounted on a micromanipulator, was then inserted beneath the connectives, a grease well (made with Apiezon N grease) and a 25 mM solution of ethidium bromide (in cockroach saline) was applied. After 10 min the ethidium bromide solution was removed and the exposed length of connective (1 to 2 mm in length) was rinsed with normal saline. The steel platform was then removed, the cuticle flap closed and sealed with dental wax.

Bleomycin (Nippon Kayaku Co. Ltd., Tokyo) was dissolved in saline and injected (10 μl), with an Agla microsyringe, at concentrations to give doses of 6.0 or 12.0 × 10⁻⁸ g per animal immediately after the operation. In some experiments further injections of bleomycin were carried out (at a dosage 12.0 × 10⁻⁸ g) at two-day intervals.

The operated animals were subsequently kept at a temperature of 28°C and fed ad lib. with free access to water. For subsequent electrophysiological and electron-microscopical examination the animals were killed by decapitation and the abdominal nerve cord removed.

The electrophysiological properties of ethidium-treated cords, and those from animals which had also been injected with bleomycin, were investigated using intracellular recording techniques (3M KCl-filled microelectrodes), as previously described (Smith et al. 1984). The integrity of the superficial blood-brain barrier was tested by measuring the effects of exposure to high-potassium saline on the intracellularly recorded action potentials. The high-potassium saline (80 mM K⁺) was produced by substituting K for Na in normal cockroach saline.

The connectives used for electron-microscopic examination were processed by conventional methods as previously described (Smith et al. 1984).

The concentrations of circulating haemocytes in normal and experimentally treated animals were estimated from haematocrit measurements. Heat-fixed blood (60°C) was placed in haematocrit tubes and centrifuged for 15 min at 1700 g (Wheeler 1963). Cockroach saline had the following composition: 157 mM NaCl; 3 mM KOH; 2 mM CaCl₂; 2 mM MgCl₂; 5 mM trehalose; 8.6 mM HEPES buffer (pH 7.2). The osmolarity and pH of the saline were not appreciably altered by the addition of ethidium bromide.

Results

Electrophysiological experiments

As previously demonstrated (Smith et al. 1984) selective disruption of the neuroglia greatly increases the accessibility