MEMBRANE JUNCTIONS IN THE MYELIN SHEATH OF GOLDFISH LATERAL NERVE

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SUMMARY

The myelin sheath of the goldfish lateral line nerve has been studied by means of the freeze-etch technique. Fracture faces of the myelin lamellae reveal linear structural elements which in some respects resemble those of tight junctions. In unfixed myelin rows of particles on the ef face are matched by narrow grooves on the pf face. Fixation with glutaraldehyde produces a partitioning of junctional particles between the 2 fracture faces. These elements are observed most frequently in the outer layers of the sheath where their arrangement is predominantly more or less parallel and longitudinal, merging in places into a more reticulate organization. They also occur at other levels in the myelin and in association with Schmidt-Lantermann clefts. Orientation in these cases appears to be predominantly longitudinal, though circumferentially running elements have been observed. The possible functional significance of these structures and their implications for tissue development, maintenance and growth are discussed.

INTRODUCTION

Recent studies by Dermietzel (1974) in the cat and Reale, Luciano & Spitznas (1975) in the rabbit have used freeze-fracture techniques to demonstrate the presence in the myelin sheath of the mammalian central nervous system (CNS) of structures resembling tight junctions. These were found at the conjunction of the outer loop of the glial cell membrane with the outer layer of the myelin spiral. Similar structures, lying parallel to those in the outer layer, occurred in the underlying layers of the myelin. The arrangement of these structures correlated well with that of the radial component of the CNS myelin described by Peters (1961, 1964). Mugniani & Schnapp (1974) and Schnapp & Mugniani (1975) have also reported freeze-fracture observations of tight junctions in the myelin of both peripheral and central nerves, and discussed their possible significance in preventing potential self-antigens among the myelin proteins from triggering auto-immune reactions. The present paper describes the appearance of tight junctions in the myelin of the goldfish lateral nerve.

MATERIALS AND METHODS

Goldfish (Carassius auratus) about 5 cm in length were beheaded and placed either in fixative (2 % glutaraldehyde in M/15 phosphate buffer at pH 7) or in 30 % glycerol in Ringer solution. A strip of tissue 1 to 2 mm in cross-section containing the lateral nerve was quickly removed and transferred to fresh fixative or glycerol-Ringer. After 1 h it was cut into lengths of about 1 mm, and fixed tissue was immersed in 30 % glycerol in fixative for a further 1 h.
Freeze etching was performed according to the methods of Moor & Muhlethaler (1963) in a Balzers 500 unit at —100 °C. Specimens were shadowed with platinum/carbon and the replicas cleaned in 40% chromic acid prior to examination in a Philips EM200.

RESULTS

Fig. 1 shows an area of membrane from a freeze-fractured, glutaraldehyde-fixed lateral nerve fibre. Consideration of surrounding features permits identification of the exposed membrane faces, which will be labelled according to the nomenclature proposed by Branton et al. (1975). In particular it can be seen that the membrane surfaces are convex, and that at their lower margin they disappear into a cross-fracture of the Schwann cell cytoplasm. Below this again appears extracellular space containing collagen fibres. Because of these relationships, the upper-lying of the 2 exposed membrane faces can be identified as the fracture face of the extracellular half (ef face) of the Schwann cell membrane forming the mesaxon and the lower as the fracture face of the protoplasmic half (pf face) of the apposed membrane.

Numerous rows of irregularly spaced particles traverse the membrane faces exposed in Fig. 1, and because of their evident continuity across both faces, these are taken to be junctional lines. On the ef face the particles lie in shallow valleys, the membrane face swelling into a slight convexity between the rows. On the pf face the particles appear on average to be rather more widely separated and the rows run along crests between shallow valleys. Fig. 1 is typical of the appearance of these junctional elements in glutaraldehyde-fixed material.

In myelin glycerinated in Ringer solution without fixation their appearance is somewhat different. Thus, on the ef face in unfixed material (Figs. 2, 4), the particles are more closely spaced in their rows and sometimes appear as compact rods rather than discrete particles, though still lying in shallow valleys. On the pf face in unfixed myelin (Figs. 3, 4), junctional elements appear as narrow grooves devoid of particles, which frequently run along the crests of ridges.

Perhaps because the occurrence of favourable fractures is more frequent in this region, the junctional elements are most commonly observed in the outer layers of the myelin sheath, particularly in the vicinity of the external mesaxon where they also appear to form the most extensive arrays. However, small areas of membrane faces are sometimes exposed in deeper layers and on many of these short lengths of junctional lines are exposed (Figs. 4-7). Thus in Fig. 5, which shows an oblique
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fracture of myelinated axon with its associated Schwann cell and external mesaxon, junctional elements can be seen both on the $ef$ face of the inner Schwann cell membrane and on 2 other $ef$ faces close to the axon surface, while indications of other junctional elements can be seen at the broken edges of some of the intervening layers. Similarly, in Fig. 6, which shows an oblique fracture of a myelin sheath, a number of junctional elements can be seen on the fracture faces of the membrane layers traversing a Schmidt-Lantermann incisure. In Fig. 7 the exposed myelin faces are concave and junctional elements can be identified both on the upper $pf$ face and at the broken edges of several other layers. Since the fibre axis runs from left to right in this micrograph, these junctional elements lie circumferentially to the fibre axis.

In general, the direction taken by the junctional elements is substantially longitudinal, though wide variations in direction can occur. Areas, similar to Fig. 1, of the outermost layers have been observed where the exposed semi-cylindrical surface has carried up to 15 lines running more or less parallel, with only occasional anastomoses. In other areas (Figs. 3, 4), this arrangement merges into more reticulate patterns. In these regions, however, the strands seem frequently to stop just short of anastomosing, so that many of the corners of the meshes are not completely closed. In deeper layers, groups of up to 7 lines have been observed, in predominantly longitudinal parallel arrangement (Figs. 4, 5). There are also at least some junctional elements which run in a circumferential direction (Fig. 7).

**DISCUSSION**

In their generally longitudinal orientation the junctional elements of goldfish lateral nerve myelin resembled those described by Dermietzel (1974) in cat CNS, by Reale et al. (1975) in rabbit CNS and by Schnapp & Mugniani (1975) in unspecified central and peripheral nerves. Anastomoses occurred occasionally between the longitudinal strands, a feature also noted by Reale et al. (1975) in their material. Areas of more mesh-like organization, observed at times in the outermost layers of the myelin, resembled to some extent the arrangement found in tight junctions of epithelia (Staehelin, Mukherjee & Williams, 1969; Chalcroft & Bullivant, 1970; Goodenough & Revel, 1970; Friend & Gilula, 1972; Claude & Goodenough, 1973; Staehelin, 1973; Pitelka, Hamamoto, Duafala & Nemanic, 1973; Tice, Wollman & Carter, 1975), though incomplete loops occurred frequently. Overall, however, these regions with their flexuous junctional strands possibly showed a greater similarity with the endo-

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Fig. 5. Fixed. Oblique fracture showing axon, myelin and Schwann cell including nucleus (n). The external mesaxon (m) is cross-fractured. $ef$ face exposures of junctional elements on 3 levels. Indications of several others (arrows) at intermediate levels. $\times 50000$.

Fig. 6. Unfixed. Oblique fracture of myelin showing junction elements in Schmidt-Lantermann incisure. c, extracellular collagen; s, Schwann cell cytoplasm. $\times 50000$.

Fig. 7. Fixed. Longitudinal fracture exposing concave myelin faces. Fibre axis runs horizontally. Circumferential junction elements exposed on the upper $pf$ face, with smaller exposures at several other levels (arrows). $\times 50000$. 
thelial occluding junctions of arteries and veins described by Simionescu, Simionescu & Palade (1976).

Staehelin (1973) has shown that tight junctions in unfixed epithelium of the mouse intestine sometimes exhibit the same fracturing behaviour as do the unfixed goldfish myelin junctions, particles on the ef face being matched by narrow grooves on the pf face. In fixed goldfish nerve, however, a partitioning of junctional particles appeared to take place between the 2 fracture faces. This contrasts with the stabilization of junctional elements on to the pf face characteristic of fixed epithelia, and also with the opposite behaviour shown by Simionescu et al. (1976) to occur in fixed endothelia. These differences in fracturing characteristics presumably reflect variations in the chemistry and structural organization of junctions in the various tissues.

Tight junctions between epithelial cells have the effect of reducing the permeability of the intercellular spaces. In a comparative study of the freeze-fractured appearance of zonulae occludentes from a number of epithelia of differing paracellular permeability, Claude & Goodenough (1973) were able to establish a qualitative correlation between permeability (as measured by transepithelial resistance) and the number of strands making up the junctional complex. On the basis of these results, the junctions of the outer layers of goldfish lateral nerve myelin might be expected to have high resistance, i.e. very low permeability. However, as Martinez-Palomo & Erlij (1975) have shown, other factors as yet undefined also influence the permeability of tight junctions.

The presence of a radial component of CNS myelin was demonstrated by Peters (1961, 1964) in permanganate-stained sections. Dermietzel (1974) has pointed out the correlation between Peter’s observation and the presence of linear junctional elements in freeze-etch replicas of CNS myelin, lying one below another in successive lamellae beneath the outer tongue of glial cytoplasm. Though no correlate of the CNS radial component has yet been reported from sectioning studies of peripheral myelin, it is interesting to note that in goldfish lateral nerve also, junctional elements can be found at multiple levels in the myelin sheath, and that when as in Fig. 5 the fracture is such as to expose both the outer layer junction and one or more inner layer junctions, they tend to lie in a more or less radial arrangement.

The frequent occurrence of multiple longitudinal junctions, together with the occasional finding of circumferential junctions such as those in Fig. 7, suggests considerable subdivision of the intraperiod gap in goldfish lateral nerve myelin. Functionally, such an arrangement might be of significance in a number of ways; in stabilizing the structure mechanically, in isolating the intraperiod gap or portions thereof from the tissue extracellular space, in delineating extracellular channels within the intraperiod gap, and, as Mugniani & Schnapp (1974) have suggested, in defining regions of the myelin lamellae with differing composition. There is evidence that some parts of the myelin spiral of peripheral nerves are mechanically more stable than others. In particular, Hall & Williams (1970) showed this to be the case for the Schmidt-Lantermann incisures of mouse sciatic nerve myelin. In a further study, Hall & Williams (1971) also demonstrated the continuity of the incisural intraperiod gap with extracellular space and the presence of a barrier preventing the lateral diffusion
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of lanthanum tracer into the surrounding myelin. In the light of the present results, and those reported by Schnapp & Mugniani (1975), these observations on the response of the Schmidt-Lantermann clefts to experimental procedures may possibly be a reflexion of the presence of tight junction-like elements.

Webster (1971) pointed out that though early stages of myelination can be explained on the basis of extension growth of Schwann or glial cell membranes, the later growth of compact myelin sheaths in length, internal circumference and number of layers is as yet poorly understood. Geometrical considerations suggest (Hirano & Dembitzer, 1967) that in CNS fibres, increase in the number of lamellae can only occur by edge extension at the inner cytoplasmic fold. If this were the only point of growth in the circumferential direction, it would be necessary to postulate shearing on the intermediate line to account for increase in sheath calibre to accommodate growth in thickness of the axon. Friede & Miyagishi (1972) have shown that such slippage can occur in experimentally induced swelling of developing sciatic nerve. However, Pinto da Silva & Miller (1975) have reported the presence of extensive arrays of membrane particles in freeze-fractured myelin from both peripheral and central nerves and have shown that the distribution of these arrays exhibits close correspondence over many successive lamellae. They point out that such a layer-to-layer correspondence of structures appears to impose restrictions on the slippage theory of myelin expansion.

A further constraint on relative movement of myelin and axon would seem to be provided by the glial-axonal junction (Livingston, Pfenninger, Moor & Akert, 1973). In the case of CNS fibres and the goldfish lateral nerve, because of the presence of tight junctions throughout the sheath it seems unlikely that increase in calibre is achieved by slip on the intermediate line. It therefore seems to be necessary to postulate that each lamella of the myelin sheath can increase in area by interpolation of fresh material as the axon increases in diameter. Such a process requires that there should be a transport mechanism for membrane precursors throughout the sheath. While a route via the aqueous phase of the intraperiod gap is a possibility, the extensive partitioning of this space by junctional elements demonstrated by Dermietzel (1974), Reale et al. (1975) and the present study must reduce its permeability to precursor molecules considerably. In the case of peripheral fibres a route via the patent extracellular and/or intracellular channels (Singer, Krishnan & Fyfe, 1972) of the Schmidt-Lantermann incisures is a second possibility, while a third alternative is a process of active trans-membrane transport. Rawlins (1973) reviewed previous studies suggesting that whatever route or combination of routes is used in the process, transmyelin transport and intra-myelinic metabolic activity does occur, and demonstrated rapid in vivo movement of tritiated cholesterol throughout the sheath of peripheral nerves from both the Schwann cell and the periaxonal surface. The possibility that protein-containing membrane particles might be involved in functional shortcuts across the myelin sheath has been discussed by Pinto da Silva & Miller (1975).

Widespread occurrence of multiple tight junctional elements in peripheral myelin would have considerable implications for theories of its development, maintenance and function. However, apart from the present study, so far only Mugniani & Schnapp (1974) and Schnapp & Mugniani (1975) have published micrographs showing
junctional elements in freeze-fracture preparations of peripheral nerves. Dermietzel (1974) was unable to find evidence for them in rat sciatic nerve. Further investigation will be necessary to define the range of occurrence of these structures in myelin.

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REFERENCES


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