Part I

Physiology and pathophysiology of nerve fibres

Ion channels and their roles in nerve are becoming more widely studied as new techniques are developed, the number of identified channel types increases, and evidence for the involvement of channels in disease processes accumulates. The first seven chapters deal with ion channels and ion exchange mechanisms from widely differing viewpoints. Ritchie reviews the distribution of Na^+ and K^+ channels, both along axons and between axons and supporting cells, while Waxman focuses on the node of Ranvier, and other ion movements that are important there, in addition to the sodium influx responsible for action potentials. Baker and Konishi consider different K^+ channels in Schwann cells that may be involved in maintaining the immediate environment of the internodal axolemma. This comprises the vast majority of the axon membrane and, as the following chapter by Reid indicates, is very far from the passive cable once assumed. Species differences are important for some aspects of nerve and channel function: the lack of fast K^+ channels at mammalian, as against frog nodes (Ritchie) is the best known, but there is also a surprising difference between K^+ channels reported in mouse and rabbit Schwann cells, the latter lacking inward rectification (Baker, Konishi). Konishi's elucidation of the factors affecting expression of this conductance may be relevant to the species difference. Ultimately we may have to study channel behaviour in human axons directly to be sure that the work on animal preparations is relevant to human physiology and disease, and pioneering results with this approach are described by Reid. Pathological alterations of channels are described by Grafe in a new model of diabetic neuropathy, and by Newsom-Davis in diseases affecting the neuromuscular junction.

The last five chapters in this section deal with different aspects of demyelinating diseases, mostly related directly or indirectly to multiple sclerosis (MS). The pathophysiology of demyelination is reviewed in the human peripheral nervous system by Feasby, in central nerve fibres by Smith, and by Pender in experimental autoimmune encephalomyelitis (normally regarded as the best available animal model of MS), in which the lesions can be primarily

central or peripheral, depending on the immune challenge. MS itself is discussed in the chapters by Smith and McDonald. Finally, the use of glial transplants to overcome demyelination or amyelination is reviewed by Rosenbluth.

1

Ion channels in normal and pathophysiological mammalian peripheral myelinated nerve

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The ionic basis of the nerve impulse was well established over four decades ago by Hodgkin & Huxley (1952); and its application to myelinated nerve soon followed (Frankenhaeuser & Huxley, 1964). Huxley & Stämpfli (1949) had already provided clear evidence that conduction in peripheral myelinated nerve fibres was saltatory; and Rushton (1951) in a seminal theoretical analysis had made general predictions about the properties of myelinated fibres, particularly how these change as the fibre diameter changes. These predictions correspond extremely well with the situation that prevails in real axons.

One question remained unanswered, namely the nature of the axolemma under the myelin. Was the internodal axolemma similar to the nodal axolemma, i.e. capable of conducting but not doing so because of the insulating myelin sheath; or were the internodal electrophysiological properties quite different? The earliest study on conduction in single demyelinated fibres (Rasminsky & Sears, 1972) failed to resolve the question of whether demyelinated axons can conduct impulses in a continuous (as opposed to saltatory) manner. However, with improvements in technique, Bostock & Sears (1978) showed clearly that single undissected myelinated fibres in perfused ventral roots of normal rats treated with diptheria toxin to produce demyelination could indeed conduct impulses – but in a continuous manner, at less than one-twentieth of the velocity expected for normal stretches of the same fibre. This provided unequivocal evidence for the presence of Na^+ channels in the now demyelinated internodal axon. However, the question whether these channels were present *normally* remained unresolved. And the fact remains that extensive demyelination produced experimentally, particularly in the acute stage, is commonly associated with conduction block.

Nodal and internodal sodium channels

The essential question raised by the demyelination experiments was whether these Na^+ channels underlying the continuous conduction resulted from a remodelling of the internodal axon, or whether they were already in place.

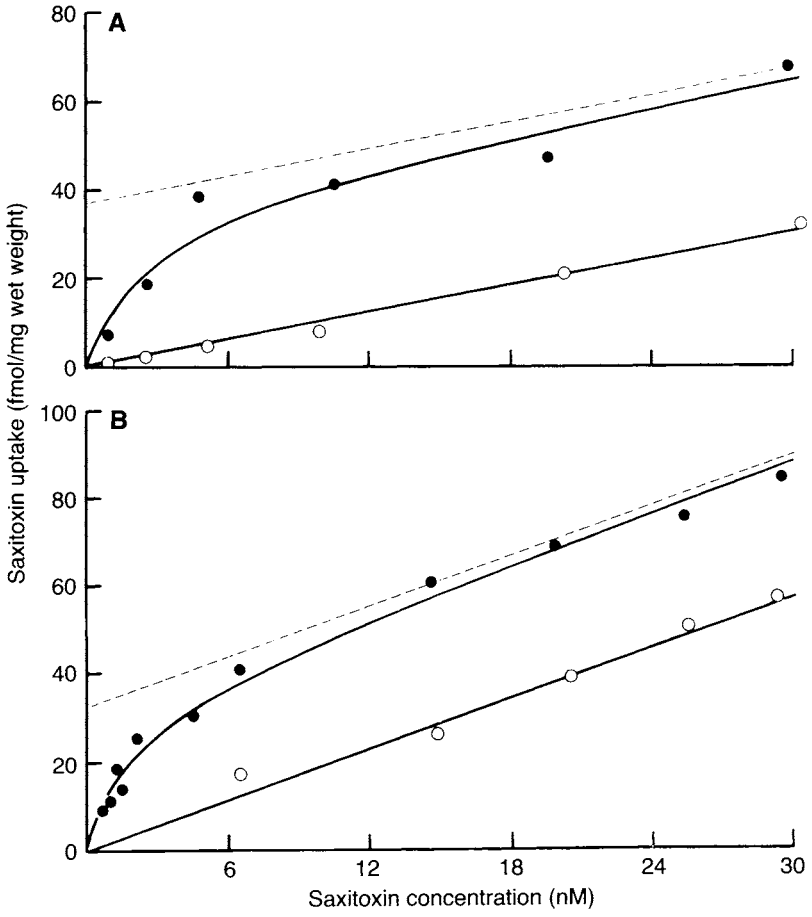

Fig. 1.1. Total (filled circles) and linear (open circles) components of the uptake of [^{3}H] saxitoxin by the myelinated fibres of desheathed rabbit sciatic nerve; either intact (A) or after homogenization (B). From Ritchie (1986), with permission.

An answer was sought experimentally by Ritchie & Rogart (1977) using radiolabelled saxitoxin as a specific marker for Na^+ channels. Arguing that extracellular saxitoxin would gain access only to the nodal axolemma and not to the internodal axolemma under the myelin, they compared the saturable uptake of saxitoxin in normal and homogenized rabbit sciatic nerve (Fig. 1.1). The answer was clear. In spite of the fact that in the homogenized preparation the axolemma exposed to the saxitoxin was now increased by 2–3 orders of magnitude, there was no statistically significant increase in the saturable binding of saxitoxin. Two conclusions were drawn from this experiment. The first was that the nodal density of Na^+ channels was extraordinarily high, being apparently about 10 000/μm^2 (but see later). The second (based

on a statistical analysis of the 150 observations embodied in Fig. 1.1) was that if Na^+ channels were indeed present in the internodal axolemma, their density had to be less than 25/μm^2. This latter value for the upper limit has recently been confirmed electrophysiologically (Chiu & Schwarz, 1987; see also Ritchie, 1988).

Nodal and internodal potassium channels

That the non-uniform distribution of Na^+ channels described above might be accompanied by a non-uniform distribution of voltage-dependent K^+ channels was first suggested by the observation that K^+ channel blocking agents, such as tetraethylammonium (TEA) ions and 4-aminopyridine (4-AP), have little or no effect on the mammalian A-fibre action potential yet greatly prolong the action potential in demyelinated fibres (Bostock, Sherratt & Sears, 1978; Sears, Bostock & Sherratt, 1978; Sherratt, Bostock & Sears, 1980). Voltage-clamp experiments confirmed that there is an inhomogeneous distribution of the fast delayed rectifier channel that in non-myelinated nerve, and in *non-mammalian* myelinated nerve, is involved in the rapid repolarization

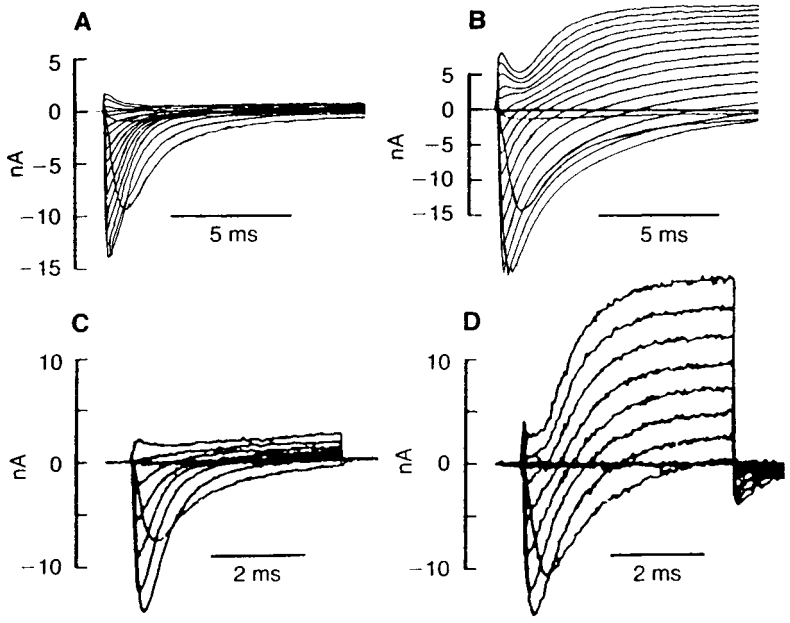

Fig. 1.2. Ionic currents in response to step depolarizations of voltage-clamped nodes of Ranvier from the sciatic nerve. Upper panel: comparison of the normal currents in rabbit (A) and frog (B) nodes. Lower panel: comparison of currents taken before (C) and 90 minutes after (D) a rabbit node had been exposed to collagenase. Data from Chiu *et al.* (1979) and Chiu & Ritchie (1980), replotted.

phase of the action potential. Thus, Chiu *et al.* (1979) showed that in rabbit myelinated nerve this phase of outward current is virtually absent (Fig. 1.2A), unlike the case of frog myelinated nerve where the inward Na^+ currents on voltage-clamp are followed by an outward delayed K^+ current (Fig. 1.2B) that can be blocked by TEA. This latter observation had originally been made by Horackova, Nonner & Stämpfli (1968) but not subsequently investigated by them in detail.

That fast delayed rectifier currents are, however, present normally in the paranodal membrane was shown in experiments where paranodal demyelination was brought about *acutely* by one of a variety of procedures (Chiu & Ritchie, 1981). When the paranodal seal between the myelin and axolemma is suddenly broken, there is an abrupt appearance of outward K^+ current (compare Fig. 1.2C and D). Fast delayed rectifiers are thus clearly present in the mammalian paranodal region. Subsequent experiments showed that these currents are also found in frog and rabbit internodal axolemma (Chiu & Ritchie, 1981, 1982; Ritchie & Chiu, 1981).

Function of the inhomogeneous distribution of axonal sodium and potassium channels

In the mammalian myelinated nerve fibre the experiments described above showed that there is a complementary distribution of Na^+ and fast delayed rectifier K^+ channels. Na^+ channels are plentiful in the nodal axolemma, where they are needed; and they are relatively sparse in the internodal axolemma, where they normally can perform no known electrophysiological function. By comparison, fast delayed rectifier channels are absent from the mammalian node of Ranvier; but they are present in relatively high density in the internodal axolemma. It should be noted that K^+ channels other than the fast delayed rectifier are also present in myelinated fibres. Their distribution does not necessarily conform with that just described; and this issue will be dealt with elsewhere in this volume (see Chapters 2 and 5).

The inhomogeneous distribution of K^+ channels may well subserve several functions. First, the demarcation between the high density of Na^+ channels in the node and the low density in the internodal membrane in the paranode is unlikely to be sharp. Some Na^+ channels may well be present in the paranode but at reduced density; and these may be the locus of a depolarization that is delayed and slowly developing because of the high access resistance in the region of the paranodal seal. Computer simulation studies indeed show that such a response could long outlast the nodal action potential, placing the nodal membrane at risk of being re-excited. The presence of K^+ channels in the paranodal axolemma, by preventing or reducing the extent of this slow depolarization, would prevent such ectopic generation of impulses and minimize repetitive firing. More importantly, these K^+ channels, together with the

other K^+ conductances present in the internodal axolemma, also control the internodal resting potential. This is important because it is now clear that the maintenance of an adequate *nodal* membrane potential requires that the *internode* also has a substantial resting potential (Bohm & Straub, 1961; Barrett & Barrett, 1982; Chiu & Ritchie, 1984).

Given that there are advantages for the presence of internodal K^+ channels, what are the possible advantages of their absence in the node? The first answer is that they will permit a higher frequency of firing in the axon, which may be advantageous in the mammal. This is because the presence of a delayed potassium conductance necessarily means that for some time after the impulse, the persisting high potassium conductance makes it more difficult to set up a subsequent impulse due to the prolonged refractoriness. A second advantage is that the presence of nodal K^+ channels increases the energetic cost of an action potential (Ritchie, 1985). This is because the presence of K^+ channels leads to a faster repolarization of the membrane so that during the later stages of the action potential, larger inward Na^+ currents flow through the still incompletely inactivated Na^+ channels; more Na^+ ions have therefore to be actively extruded during recovery. For this reason, the absence of nodal K^+ channels, together with the fact that in the mammal there is a speeding up of inactivation kinetics by a factor of 2–3 (Chiu *et al.*, 1979), the metabolic cost of an action potential in a mammalian fibre might be as little as 15% of the corresponding cost in a frog myelinated fibre of the same diameter and at the same temperature.

Although it thus became established that the internodal axolemma contained both Na^+ channels (but at low density) and K^+ channels, it remains unclear to what extent continuous conduction in demyelinated fibres relies only on the channels present in the now exposed axolemma, and to what extent there is a remodelling (insertion of new Na^+ channels, spreading of erstwhile nodal channels). But whatever happens in the acute phase of demyelination, it is clear that some remodelling does occur during the recovery phase. Thus, fibres that have remyelinated following demyelination (and fibres that have regenerated following nerve section) exhibit a 2- to 3-fold increase in saturable saxitoxin binding, indicating an increase in the number of Na^+ channels per unit length of axon (Ritchie, Rang & Pellegrino, 1981; Ritchie, 1982). The increase in the number of Na^+ channels probably does not reflect an increase in nodal channel density, merely the fact that remyelinated and regenerated fibres have about 3 times as many nodes per unit length as they do normally.

In addition to this involvement of Na^+ channels, for the first several months of recovery after demyelination or crush, the action potential is considerably broadened by 4-AP, indicating a contribution of K^+ currents in the repolarization phase of the action potential. As recovery proceeds, this sensitivity to 4-AP disappears (Ritchie, 1982). The sensitivity to 4-AP need not signify

the insertion of *nodal* K^+ channels, but merely that the paranodal seal, normally denying access to the internodal K^+ channels, is not perfect (as is also the case in developing myelinated axons).

Extraneuronal sodium and potassium channels

Completely unexpected was the finding that Wallerian degeneration of the sciatic nerve was not accompanied by the expected complete loss of saxitoxin binding. In the rat, about 7% of the binding remained; but in the rabbit sciatic nerve, following an initial fall after nerve section, there was a maintained 2- to 3-fold increase in the number of Na^+ channels in the degenerated stump (determined by saxitoxin binding), which was clearly axon-free (Ritchie & Rang, 1983). Patch-clamp experiments on rabbit cultured Schwann cells showed that Schwann cells, which proliferate during Wallerian degeneration, express Hodgkin–Huxley type Na^+ (Fig. 1.3) and delayed rectifier K^+ channels (Chiu, Shrager & Ritchie, 1984; Shrager, Chiu & Ritchie, 1985). Subsequent experiments (Bevan *et al.*, 1985) showed that cultured rat astrocytes similarly express both Na^+ and K^+ channels; and both Schwann cells and astrocytes express voltage-gated Cl^- channels (Bevan *et al.*, 1985; Howe & Ritchie, 1988). The species difference in the extent of Schwann cell expression of Na^+ channels (rabbit versus rat) remains unexplained.

At least three types of delayed rectifier K^+ channel are expressed by rabbit Schwann cells. The fastest activating (type 1) is blocked by 4-AP and by α-dendrotoxin. A slower channel (type 2) is blocked by 4-AP but not by α-dendrotoxin. A third channel that under normal conditions activates only at very positive potentials is blocked by neither 4-AP nor

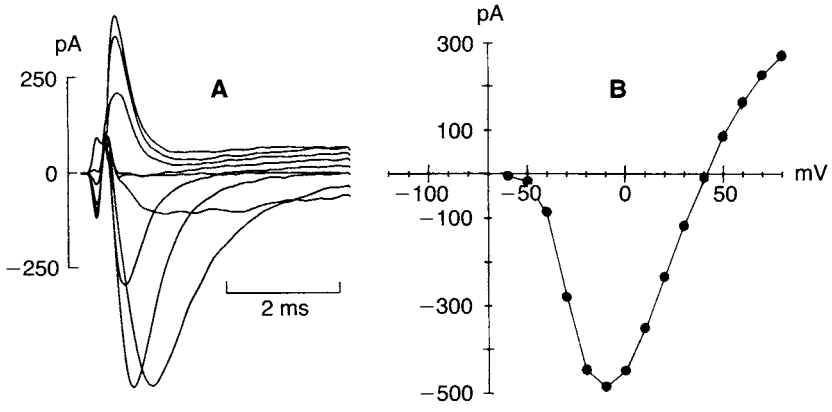

Fig. 1.3. (A) Na^+ currents in a voltage-clamped rabbit cultured Schwann cell. (B) Current–voltage relationship obtained from the records in (A). From Howe & Ritchie (1989), with permission.

α-dendrotoxin. All three are blocked by TEA (Baker, Howe & Ritchie, 1993; Howe & Ritchie, 1988). These channels are described in more detail by Baker (see Chapter 3).

Whereas K^+ channels in a variety of tissues have been implicated in a diversity of physiological functions, the presence of Na^+ channels in satellite cells is less easy to account for. It is clear, however, that their presence was the main confounding factor in the calculation of nodal Na^+ channel density mentioned earlier, which gave much too high a value (Ritchie & Rogart, 1977). Indeed, if the Na^+ channel density at the node is 1000–2000/μm^2, the saxitoxin binding experiments can now be reinterpreted to mean that about half the Na^+ channels present in a normal rabbit sciatic nerve trunk are extraneuronal, i.e. in Schwann cells. In cultured Schwann cells, the density is about 30/μm^2, but their density *in vivo*, as well as their distribution along the Schwann cell plasmalemma, are unknown. It is clear, however, that Schwann cell Na^+ channels are not an artifact of cell culture. Not only were they first described as a result of Wallerian degeneration *in vivo* (Ritchie & Rang, 1983) but immunostaining with antibodies to the Na^+ channel clearly reveals their presence *in situ* in Schwann cells of normal mammalian myelinated nerve – particularly in the region of the cell body and in the microvilli that the Schwann cell sends down to the axolemma in the nodal regions (Ritchie *et al.*, 1990).

These voltage-dependent Na^+ and K^+ channels show a considerable plasticity depending on the method of dissociation of the cells and on their developmental age. Thus, when examined under similar conditions of cell culture, the electrophysiological behaviour of Schwann cells cultured from a predominantly myelinated (sciatic) and from a predominantly non-myelinated (vagus) mammalian nerve, from either neonatal or adult tissue, is similar (Howe & Ritchie, 1990). However, Chiu (1988), looking at explants and acutely dissociated Schwann cells, has suggested that voltage-gated Na^+ currents are found only in Schwann cells associated with non-myelinated axons. Furthermore, Ritchie (1988) has shown that all Na^+ currents are abolished for several days after treatment with proteolytic enzymes. Similarly, in astrocytes Barres *et al.* (1990) have shown that replacement of their normal dissociation procedure by a 'tissue print' method radically changes the expression of different kinds of ion channel in cultured type 1 astrocytes. Finally, Sontheimer and his colleagues have shown that expression of Na^+ and K^+ channels by astrocytes is dramatically influenced by the presence or absence of neurones; and furthermore, large changes in the electrophysiological and pharmacological (tetrodotoxin sensitivity) parameters occur with developmental changes in astrocytes maintained in culture for several days or obtained at different times postnatally (Sontheimer & Ritchie, 1994).

The functional significance of these voltage-gated channels in the satellite cell of the nervous system remains unclear. Some of the kinds of K^+ channel

(five have been identified so far) may be involved in mitosis: for K^+ channel blocking agents (such as TEA, 4-AP or quinidine) interfere with Schwann cell proliferation (Chiu & Wilson, 1989) as they are indeed known to do in lymphocytes (Chandy *et al.*, 1984) and fibroblasts (Gray *et al.*, 1986). They may also be involved in the maintenance of the extracellular ionic concentrations, especially of K^+ ions (Bevan *et al.*, 1985). But the role of the satellite cell Na^+ channels remains mysterious. One suggestion for the Na^+ channels (Gray & Ritchie, 1985), which could equally apply to the K^+ channels, is that they are transferred from the Schwann cell system of microvilli or at the corresponding system at the astrocytic node (perhaps both normally and during remodelling after demyelination). This suggestion is attractive, at least from the point of view of economy of supply (Ritchie, 1988); but even nearly a decade after it was first suggested it remains just a speculation.

Acknowledgements

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