NERVE REGENERATION AND EXTERNALLY APPLIED ELECTRIC FIELD

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ABSTRACT

Ample body of recent evidence exists that regenerative processes can be influenced by an externally applied electric field. However, the question remains, how the cell perceives and translates the electrical signal into a functional response. To reveal the possible mechanisms the relevant current knowledge was united with our assumptions into a simplified qualitative model of peripheral nerve regeneration after crush lesion. Some of our recent results are presented demonstrating some beneficial influences of electric currents applied across the nerve crush on muscle function recovery.

KEY WORDS: nerve regeneration, qualitative model, electric stimulation, muscle function

INTRODUCTION

In vitro and *in vivo* experiments demonstrated that the normal process of nerve regeneration and functional recovery may be enhanced by externally applied electric fields (EAEF) of various strength and duration, but have yet to determine and prove the underlying mechanism or mechanisms [Borgens et al. 1987; Kerns and Freeman 1988; McDevitt et al. 1987; Nix and Hopf 1983, Nix 1988a; Pockett and Gavin 1985, Pockett and Philip 1988; Politis et al. 1988; Pomeranz et al. 1984; Roederer et al. 1983; Roman et al. 1987; Sisken and Smith 1975]. The experiments were performed on diverse experimental models with different protocols making it difficult to compare and to integrate the obtained results. One of the most fundamental questions about the effects of EAEF on cell behaviour is to comprehend how the cell translates the electrical signal into a functional answer [Hinsenkamp et al. 1985]. To facilitate our understanding and future studies of the complex changes during in vivo nerve regeneration and possible beneficial effects of EAEF, we united the relevant current knowledge and our own assumptions into a simplified model (figure 1).

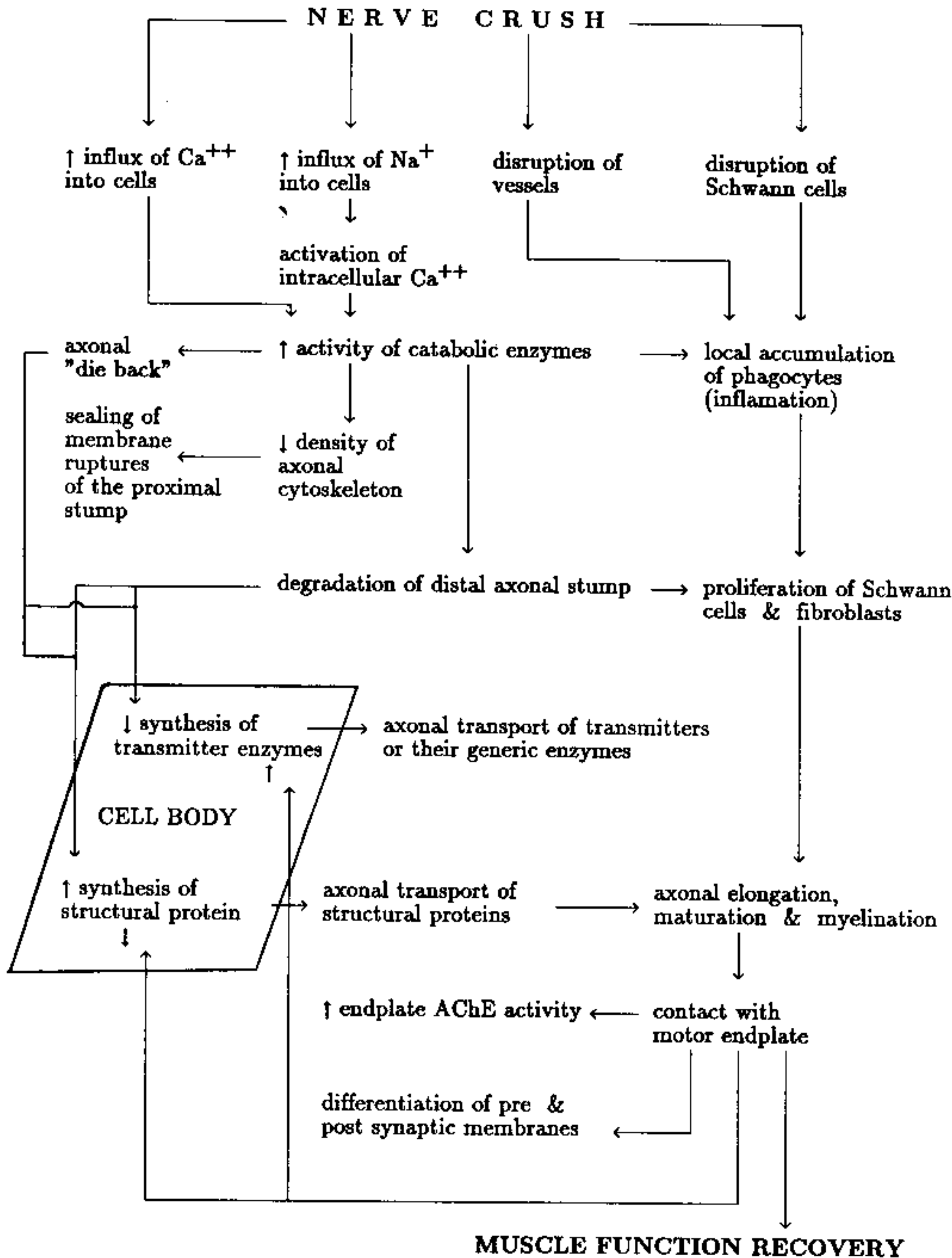

Figure 1. A simplified presentation of degenerative and regenerative processes in the peripheral nerve after crush lesion.

ENDOGENOUS INJURY CURRENTS

Nerve crush or cut abolishes the selective permeability across the axon membrane. Due to the differences in Na^+ and Ca^{2+} ion concentrations in intracellular and extracellular medium, membrane disruption causes the flow of electric currents into the cell - also called endogenous injury currents (EIC) [Borgens 1982]. Borgens has suggested that this flow of current is involved in the process of cell membrane regeneration [Borgens et al. 1980]. Immediately after transection of the lamprey larvae spinal cord he and his coworkers measured current densities from 0.5 to 0.8 mA/cm^2 entering the cut surface of the spinal cord. After 48 hours the current density has stabilized at a considerably lower level of about 4 A/cm^2 [Borgens et al. 1981]. The bulk of incurrent is most probably carried by Na^+ and Ca^{2+} ions [Borgens et al. 1980; Meiri et al. 1981]. Small increases in intracellular Na^+ release internally stored free Ca^{2+} from the mitochondria [Carafoli and Crompton 1976] additionally increasing the concentration of free intracellular Ca_{2+}. In vitro, high intracellular free Ca^{2+} causes dissolution of contractile and tubular elements in the axoplasm of cultured peripheral nerves [Schlaepfer 1974, 1977]. On the other hand, Wallerian degeneration of a distal nerve segment can be delayed with Ca^{2+} free media [Schlaepfer and Bunge 1973, Schla..epfer and Hasler 1979]. Increased Ca^{2+} cell membrane conductance was found at the terminal ends of regenerating axons [Meiri et al. 1981], suggesting that increased Ca^{2+} membrane conductance is a feature of regenerating and differentiating neurons [Spitzer 1979]. Numerous experiments imply that changes in intracellular free Ca^{2+} have a universal role in remodelling cell architecture [Jaffe et al. 1975, Jaffe 1979; Nuccitelli et al. 1977; Tilney 1979]. The increase of free intracellular Ca^{2+} leads to a net breakdown of structural proteins with a decreased density of axonal cytoskeleton. This could promote sealing of membrane ruptures, the first step towards the recovery of cell homeostasis [Borgens 1982].

THE IMPORTANCE OF STIMULATING-ELECTRODE POSITIONING

The positioning of stimulating electrodes at the nerve injury site determines the effects of EAEF on nerve regeneration. The die-back during the first 5 days post-transection of the giant reticulospinal axons of the lamprey spinal cord was significantly reduced by application of a 10 A direct current across the site of transection, with the cathode distal to the lesion. Reversing the polarity of the applied current (cathode proximal to the lesion) increases the extent of axonal die back relative to the sham treated control [Roederer et al. 1983]. On the basis of these results Roederer and co-workers offered the following explanation. The net inward current through the site of transection (i.e., EIC) causes the die-back. In the sham treated controls, the direct current (DC) entering the severed axons is only the EIC causing axonal die-back. In the cathode proximal group, the applied direct current (DC) enhances the EIC, increasing the total inward current entering the cut axon face with respect to the control group. This could explain the observed increase in the extent of die-back seen in this group as compared to the controls. In the cathode-distal group the applied current opposes the EIC to produce a decrease in the net inward current, reducing the extent of axonal die-back [Roederer et al. 1983]. One might speculate that from the standpoint of cell homeostasis there exists an optimal injury current of Ca^2 promoting the sealing of membrane ruptures on one hand and causing minimal axonal die back on the other. This might be achieved by placing the cathode stimulating electrode distally to the

lesion site. The importance of positioning of the stimulating electrodes was independently confirmed by different researchers [McDevitt et al. 1987; Politis et al. 1988; Roman et al. 1987].

AXONAL TRANSPORT

There may also be other beneficial effects of EAEF during the early phases of nerve regeneration. The breakdown of axonal transport following axon interruption leads to a build-up of transported substances proximal to the lesion site [Brimijoin 1982; Fahim et al. 1982; Smith 1980; Tsukita and Ishikawa 1980]. Some researchers speculate that these local accumulations cause a temporary surge of retrograde axonal transport (RAT), thus transmitting the information of axon damage to the cell body [Bisby 1984]. The cell body responds by changing the priority of synthesis to a relative increase in structural protein enzymes and a concomitant decrease in neurotransmitter enzymes synthesis [Hoffman and Lasek 1980; Jones and Lavelle 1986; Kreutzberg 1982; Sinicropi et al. 1982; Wujek and Lasek 1983]. EAEF might have a beneficial influence on nerve regeneration by amplifying the information on cell damage, shortening the delay of nerve cell body response to injury, thus leading to an earlier start of nerve regrowth. There is not much data concerning the possibility that EAEF act on nerve cell bodies to speed up axon regeneration. Pockett and Philip [1988] reasoned that if stimulation was acting on nerve cell bodies the growth accelerating effect should be seen if both stimulating electrodes were proximal to the crush lesion but not if they were both distal to it. Regeneration was evaluated by assessing the toe-spreading reflex. Their results showed that electrical stimulation enhanced the recovery of the toe spread reflex only when both electrodes were distal to the crush. They concluded that the effect of EAEF is largely a local one rather than an effect on cell bodies [Pockett and Philip 1988]. One could argue that quantifying protein synthesis in the nerve cell body during EAEF would be more appropriate method for studying the effects of EAEF on the nerve cell body function.

As mentioned, the disruption of the cell membrane produces a steady current entering the intracellular space. The flow of ions through the cell membrane defect produces a voltage gradient within the axon, which could contribute to local accumulation of cellular organelles (according to their net charge) at the membrane defect by means of axoplasmic electrophoresis [Borgens et al. 1980; Jaffe 1977; Meiri et al. 1981; Woodruff and Telfer 1980]. EAEF might maintain and enhance the normally transient axoplasmic electrophoresis, speeding up the anterograde axonal transport of structural components thus promoting nerve elongation. Furthermore, the electrophoretic effect exerted by EAEF might enhance the information of established contact between regenerating nerve endings and target organ (travelling via retrograde axonal transport?), inducing the cell body to revert its synthesis priority sooner (e.g., the nerve content of transmitter enzymes, acetylcholinesterase and choline acetyltransferase might normalize at a faster rate).

MEMBRANE RECEPTORS

The effect of EAEF is not only limited to the axon transport. It has been demonstrated that different membrane receptors (including acetylcholine receptors)

moved along the plasma membranes of various cells towards the cathode [Poo and Robinson 1977; Robinson 1977; Poo et al. 1978; Orida and Poo 1978]. Nerve growth factor (NGF) acts primarily on the growing end of the neurite in vitro rather than the cell body [Campenot 1977]. Furthermore, the outgrowth of neurite from chick ganglia embedded within collagen gels was denser on the side facing the tips of NGF filled capillary tubes inserted into gels [Ebendal and Jacobson 1977]. In vitro, the direction of axonal growth was shown to follow the concentration gradient of NGF [Gundersen and Berrett 1979, Gundersen 1980, 1985]. Faster growth of neurite towards the cathode than the anode in a steady electrical field was explained by the movement of the receptors for NGF along the membrane of the neurite towards the growing tips [Jaffe and Poo 1979].

NONNEURONAL CELLS

When studying the influences of EAEF on nerve regeneration in vivo, one must not ignore the role of the Schwann cells (SC). Loss of axonal continuity is followed by an intense proliferation of SC in the distal nerve segment, peaking 3 days after axonal severance and continuing with decreasing intensity for about 2 weeks after injury [Aguayo et al. 1976, Aguayo and Bray 1980; Bradley and Ausbury 1970]. Some researchers believe that SC proliferation in the distal segment is associated with production of diffusible substances that may attract the regenerating axons [Ramon y Cajal 1928; Muller and Grubel 1981; Skene and Shooter 1983; Skene 1984]. Chemical inhibition of protein synthesis in a degenerating distal nerve segment inhibits axonal growth in rat sciatic nerve [Kanje et al. 1986]. Unfortunately, the exact role and relevance of SC in guiding of the regenerating axons towards their target organs is far from being clear. Their sacred role in regard to in vivo peripheral nerve regeneration is not supported by recent findings. In their experiments, Sketelj and co-workers demonstrated rapid growth of regenerating axons across the segments of sciatic nerve devoid of SC. Their results suggest that non-interrupted acellular basal laminae of SC provide good, although not optimal conditions for elongation of regenerating sensory and motor axons. If biochemical or structural integrity of the basal lamina is destroyed, the regenerating axons must be accompanied or preceded by viable SC and axon elongation is significantly reduced [Sketelj et al. 1989]. It would be interesting to evaluate the influences of EAEF on SC proliferation, phagocytic activity and secretion of various neurite promoting substances and how the latter reflect on nerve regeneration in vivo.

FUNCTIONAL RECOVERY OF THE REINNERVATED MUSCLE

The fact that peripheral nerves regenerate does not necessarily imply successful functional recovery. More likely, functional recovery is suboptimal and at least part of this deficiency is caused by the lack of guidance of some nerve fibers to their corresponding target organs [DeMedinaceli et al. 1982, 1983; Fields and Ellisman 1986a,b; Kerns et al. 1987]. The extent of functional recovery after nerve lesion is therefore the cumulative result of initial degenerative and subsequent regenerative events of the injured nerves and successful reestablishment of nerve target organ connections. There is some evidence that applying EAEF to the regenerating peripheral nerve improves muscle function recovery [Kerns and Freeman 1988; McDevitt et al.

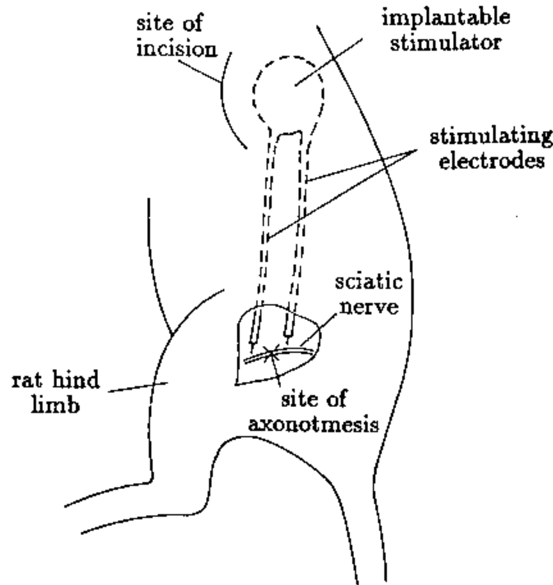

Figure 2. Implantation of a stimulator

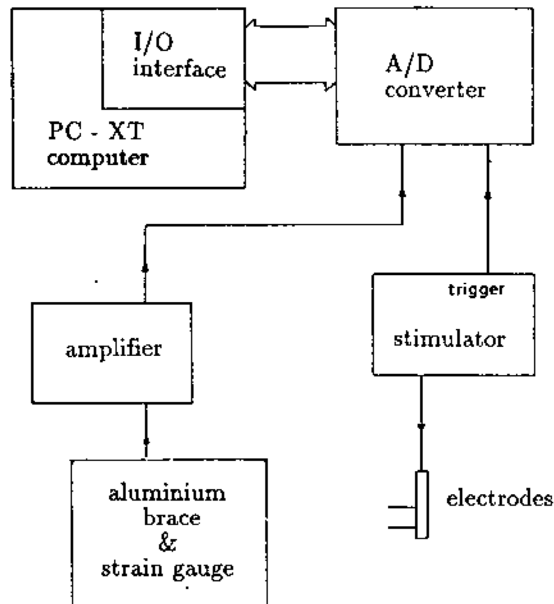

Figure 3. Measurement of muscle force.

1987; Nix and Hopf 1983, Nix 1988a; Pockett and Gavin 1985; Pomeranz 1987]. When the electrical stimulus to the regenerating nerve is supramaximal (electing action potentials) the mechanism of muscle function recovery might be understood in terms of indirect muscle stimulation [Nix and Hopf 1983, Nix 1988a]. But how to explain improved functional recovery after stimulating the regenerating axons with subthreshold, low intensity direct current (LIDC) producing no measurable action potentials [Borgens et al. 1987; Kerns and Freeman 1988; McDevitt et al. 1987; Pomeranz 1987, Stefanovska et al 1989]? Several mechanisms have been suggested but none of them unequivocal [Pomeranz et al. 1984].

In vitro experiments (on cultured rat extensor digitorum longus muscles) have demonstrated the presence of substances which have an innervation-like effect on the cholinesterase activity and acetylcholine sensitivity. They are transported by axonal transport, released from the nerve by stimulation and are present in innervated muscles but apparently absent from denervated muscle [Younkin et al. 1978]. Crude preparations of protein from rat sciatic nerves have been shown to prevent the disuse atrophy of the rats extensor digitorum longus muscle when injected into the denervated muscle daily for 1 week [Davis and Heinicke 1984]. The active factor or factors in such preparations were shown to be glycoproteins, with a molecular weight of approximately 100.000 [Davis et al. 1985].

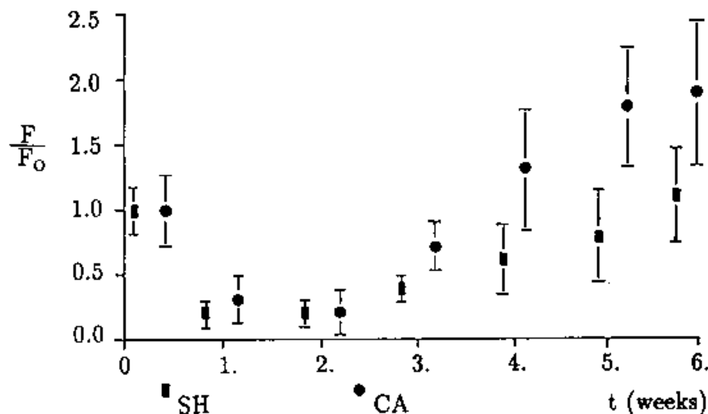

Figure 4. The influence of LIDC on muscle force recovery after crush lesion.

Recent results of our study of muscle function recovery after peripheral nerve crush lesion point to the possible enhancement of the transport of such muscle trophic factor or factors due to LIDC stimulation. The animals were randomly divided into groups CA (cathode distally to the site of axonotmesis) and SH (with sham implants, identical in size, shape and weight to the real one, battery replaced by a piece of noncorrosive metal). LIDC of 1 A was continuously applied (figure 2). Isometric contraction of plantar flexors was recorded during reinnervation (figure 3), as a measure of muscle function state. The details of the measuring method can be found elsewhere [Stefanovska et al. 1987]. The force of tetanic contraction was assessed in

a period of six consecutive weeks, once weekly. Muscle force returned to the control value during the fourth week in the CA group and in the fifth week in the SH groups (figure 4). The differences were statistically significant (two tailed, unpaired Student t test). In the CA group muscle force steadily increased up to the sixth week. One could argue that the increase in force may be due to the changes in muscle contraction properties only. The effects of denervation on force output, contraction speed and fatigue sensitivity have already been shown [Finol et al. 1981; Gundersen K 1985; Gutmann et al. 1972; Kotsias and Muchnik 1987; Lewis 1971]. After chronic cathode stimulation triceps surae muscle (particularly the slow soleus) might undergo changes in fiber phenotype being transformed into the faster one and thus being able to produce stronger contractions [Young 1984]. This could be accomplished through the transport of target organ trophic factors, synthesized in the nerve cell [Buller et al. 1960], enhanced by the applied LIDC to the nerve. To test this possibility we intend to study muscle contractile properties during the time of reinnervation. It has already been shown that different frequency patterns of directly applied electrical stimuli can induce changes in contractile properties of denervated muscles [Hennig and Lomo 1987; Nix 1988b]. However, no results have been published on the effects of LIDC stimulation of the crushed peripheral nerve on contractile properties and fatigue sensitivity of denervated muscle.

The review of existing data shows that there is insufficient knowledge of precise mechanisms by which EAEF effect nerve regeneration and muscle function recovery but also calls attention to the growing body of evidence in favour of beneficial effects of EAEF on these processes. For its practical application in rehabilitation of human nerve injuries further experimental work towards the optimisation of LIDC parameters is also necessary.

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