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INFLUENCE OF ELECTRICAL STIMULATION ON REGENERATION OF THE RADIAL NERVE IN DOGS

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The effects of biphasic electric fields on nerve regeneration that follows injury to the left radial nerve were studied in dogs by electromyography (EMG). Left and right radial nerves were crushed with a serrated haemostat. Stimulating electrodes were positioned proximally and distally to the site of the injury. The left nerves received rectangular, biphasic and current pulses (30 µA, 0.5 Hz) through the injury for two months. The right radial nerves were treated as controls and regenerated without electrical stimulation. EMG activities were recorded intramuscularly from the left and right musculus extensor digitalis communis (MEDC). Results obtained at the end of the two-month stimulation period showed a significant difference in EMG activity between the left (stimulated) and the right (non-stimulated) MEDC, suggesting that electrical treatment enhanced nerve regeneration.

Key words: Electrical stimulation, electrode, nerve regeneration

Mammalian peripheral nerves regenerate if crushed or severed and re aligned (Lundborg, 1987). Severe compression may result in loss of axonal continuity at the level of the lesion, however, still with intact endoneurial tubes. The lesion is called axonotmesis (Sunderland, 1968). The axons degenerate but the prognosis is good because of the preserved endoneurial tubes as the regenerating axons grow down through these tubes (Lundborg, 1987; MacKinnon and Dellon, 1988). The healing of an injured nerve is a unique biological problem. When a peripheral nerve fibre is crushed or transected, fibres distal to the injury undergo Wallerian degeneration (Lundborg, 1987; Hall, 1989). Within the first days after crushing the nerve trunk, axonal sprouting occurs at the end of the proximal stump. The sprouts grow across the injury into the distal stump where the regenerating axons enter Schwann-cell-rich bands where they continue to grow towards the periphery to re-innervate organs (Lundborg, 1987; Hall, 1989).

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The effects of electric fields on nerve cells have been known for almost a century (Verworn, 1889). A notation that an imposed electrical field can enhance or direct nerve growth dates back to Sven Ingvar in 1920 (Ingvar, 1920). An imposed direct current (DC) has an effect on nerve growth in tissue culture (Borgens et al., 1977; Jaffe and Poo, 1979). In tissue culture, neuronal cell processes orient along the lines of a DC field (Marsh and Beams, 1946). Alternating current (AC) stimulation also enhances the progress of nerve regeneration (Sebille and Bondoux-Jahan, 1980; Nix and Hopf, 1983). Both DC and AC stimulation can enhance the terminal sprouting of intact nerves into denervated zones of the extre mity.

The recovery of muscle function after the nerve is crushed is a complex process involving axon regeneration (Kerns et al., 1991; Ribarič et al., 1991; Ribarič et al., 1994) and the re-establishment of nerve-muscle connections (Borgens et al., 1981; Román et al., 1987) with the recovery of nerve transmission and muscle contractions. The purpose of this work was to investigate whether continuous stimulation with rectangular, biphasic pulses of low current amplitude across the lesion in a dog's crushed radial nerve accelerates the reconnection of motor axons with the musculus extensor digitalis communis (MEDC).

Materials and methods

A totally implantable stimulator (Fig. 1) delivering rectangular, biphasic, stimulating current pulses with fixed amplitude of 30 μA and a frequency of 0.5 Hz has been developed (Kelih et al., 1986). The bulk body of a stimulator is a 10 mm-high cylinder with a diameter of 29 mm. It consists of electronic circuitry and a battery, hermetically encapsulated in a low stainless steel cylinder covered with epoxy resin. Stimulating electrodes are made of a 10 cm long biomedical wire (Cooner Wire AS 631) with 1.5 cm de-insulated ends (Fig. 2). At each end, two loops with a diameter of 1 mm are made. The length of the de-insulated part between the loops is equal to the circumference of the nerve enabling encircling the nerve trunk with the de-insulated part.

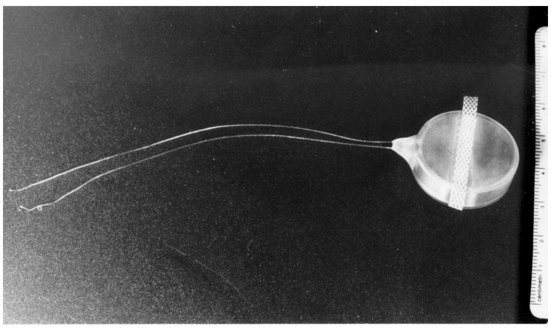


Fig. 1. Totally implantable electrical stimulator

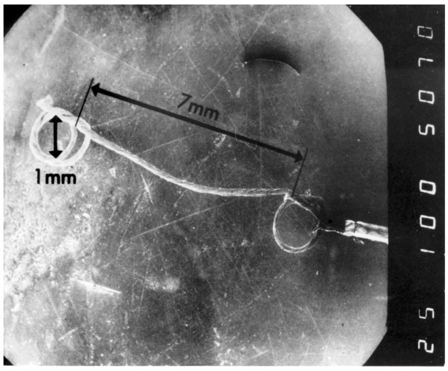


Fig. 2. Magnified view of stimulating electrode

The research was performed on four healthy adult Beagle dogs. They were premedicated with medetomidine 40 μg/kg i.m. (Domitor, Orion Corp.) and methadone 0.2 mg/kg s.c. (Heptanon, Pliva). Induction was performed with propofol 1.0 to 2.0 mg/kg i.v. (Diprivan, Zeneca Pharamaceuticals Ltd.). General anaesthesia was maintained with isoflurane 0.8 to 1.5 vol. % (Forane, Abbott) in 100% O_2. Analgesia during surgery was sustained with ketamine 0.5 to 2.0 mg/kg i.v. (Ketamine, Veyx-Pharma GmbH) when necessary. Antibiotics (cefazolin 20 mg/kg i.v.; Cefamezin, Krka) were administered perioperatively. Analgesia during the early recovery period was provided with methadone 0.3 to 0.5 mg/kg s.c. TID. Tramadol 8.0 mg/kg s.c. TID (Tramal, Grünenthal GmbH) was administered for additional two days.

Left and right radial nerves were exposed via 4–5 cm incisions made on the lateral distal humeral region of the front legs. The left radial nerves were tested for the conduction of action potentials when intact and across the lesion when crushed. Two wire electrodes were inserted into the muscle belly 2 cm apart, and a common electrode for recording EMG activity in the MEDC was inserted in the tissue beneath the skin. The left radial nerve was lifted and placed on a pair of testing, bipolar stimulating electrodes (Fig. 3, A). Biphasic, charge-balanced pulses with a frequency of 30 Hz and amplitude of 2 mA were employed to the left radial nerves to stimulate the contraction of the MEDC. The right radial nerves were treated as controls and were left to regenerate without the electrical stimulation. Pulses delivered through the testing electrode pair attached on both sides of the nerve lesion elicited action potentials that propagated to the target muscle. EMG activity in the MEDC elicited through stimulation of nerve fibres was recorded differentially. It was amplified and displayed on a Goldstar 3040 oscilloscope. Left and right radial nerves were crushed with a 3 mm wide serrated haemostat for 3 sec 1 cm above the bifurcation of radial nerve and about 1 cm distal to the site where the axonal conductivity in intact nerve was tested (Fig. 3). After the injury a pair of testing, bipolar stimulating electrodes was attached distally to the lesion and EMG activity was recorded again (Fig. 3, B).

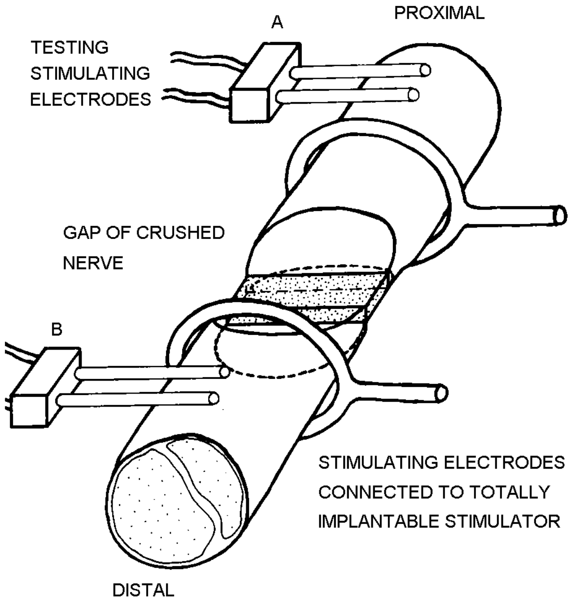


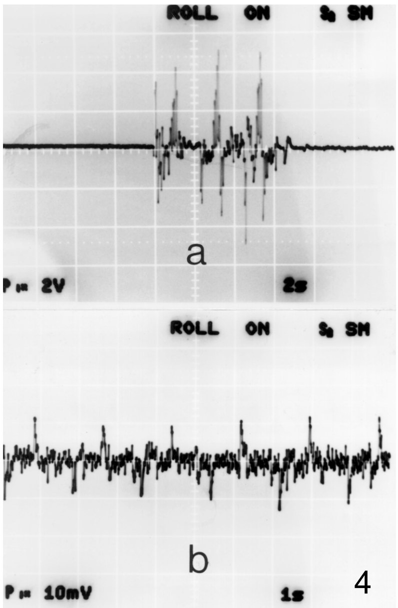
Fig. 3. Schematic diagram of a crushed radial nerve and the position of testing and stimulating electrodes

Afterwards, electrodes of the totally implantable stimulator were installed encircling the nerve with the deinsulated part of biomedical wire. They were fixed by suturing the two loops together, thus forming a ring around the nerve. One of the two electrodes was positioned proximally and one distally to the lesion (Fig. 3). Therefore the current from the stimulator passed through the crushed neural tissue between the two electrodes. Finally, the stimulator was placed beneath the skin of the leg about 8 cm above the lesion.

Two months later the dogs were anaesthetised again. All nerves were exposed and visually inspected, and the stimulating procedure was repeated. EMG activities of left and right MEDC elicited by stimulation of the left and right radial nerves proximally and distally to the lesion were recorded and compared to the activities recorded immediately after the nerve crush.

Results

EMG activity of the MEDC elicited with stimulation of the radial nerve distally to the nerve lesion was almost the same after the surgery as in intact nerves (Fig. 4a). When the pair of stimulating electrodes was positioned proximally to the nerve lesion, EMG activity of the MEDC was much lower than that of the intact and injured radial nerve distal to the lesion (Fig. 4b).



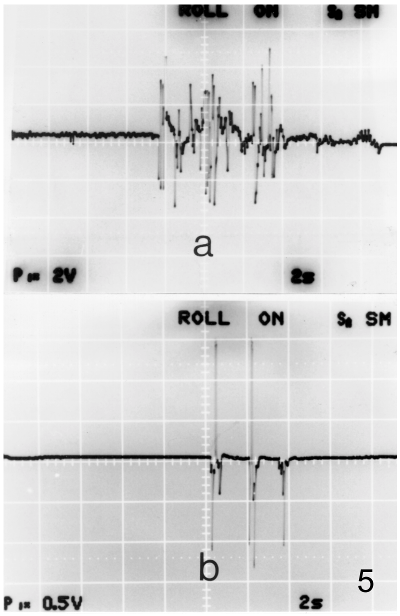


Fig. 4. (a) EMG activity of the MEDC elicited by stimulation of the injured radial nerve distally to the lesion, and (b) EMG activity of the MEDC elicited by stimulation of the injured radial nerve proximally to the lesion

Fig. 5. (a) EMG activity of the MEDC elicited by stimulation of the left radial nerve proximally to the lesion after two months of electrical stimulation, and (b) EMG activity of the MEDC elicited by stimulation of the right control radial nerve that did not receive electrical stimulation

A small number of action potentials crossed the nerve lesion, and the loss of axonal continuity in the crushed radial nerve was confirmed. In all dogs, EMG activity of the non-stimulated MEDC was much lower than that of the stimulated MEDC after two months of electrical stimulation. The EMG activity of the MEDC evoked by electrical stimulation of the left radial nerve proximally to the regenerated lesion after two months of electrical stimulation is shown in Fig. 5a, and the EMG activity of the MEDC evoked by electrical stimulation of the right (control) radial nerve proximally to the regenerated lesion after two months of regeneration without electrical stimulation is shown in Fig. 5b.

Clinical and neurological examination of the dogs after the implantation revealed mild paresis and mild proprioceptive deficit of the front legs. Postural reactions were slightly depressed. The deep pain sensation and the flexor reflex were normal. All neurologic signs disappeared after two weeks in all dogs. The skin on the dorsal sides of both front legs beneath the elbow was not sensible for a period of one week. After two months of electrical stimulation of both nerves there were no visible changes and no reactions of the tissue around the implant.

Discussion

The effects of biphasic electrical stimulation on crushed canine radial nerve were evaluated by electromyography. Our method of two-month continuous electrical stimulation with totally implantable stimulator enhanced the process of regeneration of crushed radial nerve in a dog. Consistently with the previous studies (Sebille and Bondoux-Jahan, 1980; Nix and Hopf, 1983; Kerns et al., 1991; Ribarič et al., 1991; Ribarič et al., 1994) we found out that electrical stimulation with biphasic current pulses enhances the progress of the nerve regeneration. Our study is also the first one evaluating the regeneration of crushed radial nerve in a dog enhanced by a totally implantable electrical nerve stimulator. This approach could also be used for enhancing the regeneration of other frequently injured peripheral nerves in small animals, such as the n. radialis, n. ischiadicus and n. peroneus. Further work should be done on testing the aforementioned methodology in a larger number of cases including different peripheral nerves, and evaluation of the enhancement of nerve regeneration should be supported by histological studies.

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