Compression Induced Damage on In-Situ Severed and Intact Nerves

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ABSTRACT: The effect of rapid as well as sustained compressive forces applied to the surface of intact and severed peroneal nerves of rabbits was studied. Considerable effort was taken to ensure a quantitative and consistent experimental paradigm. Stimuli were delivered to the sciatic nerve, and the compound action potential was recorded in the peroneal nerve, with compressive forces applied more proximally on the peroneal nerve. It was found that the conduction of action potentials on the larger nerve fibers was more sensitive to compressive force than that of the smaller nerve fibers, although all nerve fibers stopped conducting when sufficient compression was applied to the nerve. The effect on the conduction of action potentials on the nerve fibers appeared to be determined both by Laplace's law (as previously reported by others) and the viscoelastic properties of the entire nerve. Relatively low compressive forces (20 gm applied over approximately 7 sq mm) were found to decrease the neutral conduction of the larger nerve fibers for at least two hours, whereas stagnation of blood circulation was not found to affect measurably the neural conduction of all the nerve fibers for up to two hours.

Introduction

Our previous work has shown that electrode units implanted around the distal ends of severed sciatic and peroneal nerves in rabbits have successfully recorded neuroelectric activity associated with volitional intentions of the animals for periods up to 142 days. However, the neuroelectric signals recorded from the electrode units have exhibited degradation during the implant period. To improve signal quality and prolong the period for successful recording of neuroelectric activity, several preliminary experiments were conducted on rabbits and subhuman primates. These indicated that a possible cause for the decrease in the amplitude of the neuroelectric signal recorded from the implanted electrodes was due mainly to the compressive forces generated by the musculature surrounding the implant. Application of an external pressure cuff inflated to 300 mmHg around the limb of the animal for a period of 1 min at the site of the implant produced a similar decrease in signal amplitude.

The effect of compressive forces on severed nerves is of practical interest to surgeons who deal with this issue on a regular basis during operative procedures. Concerns arise over the amount of manipulation to which severed nerves should be subjected and the effect of subsequent compression by the adjacent contracting muscle tissue. Also of interest is the nature and temporal perseverance of nerve compressions unwillingly self-induced by patients, such as: Saturday night palsy, cast pressure on the peroneal nerve, crutch pressure on the axilla, carpal tunnel syndrome, etc.

The current literature is controversial concerning the factors influencing neuroelectric signals during compression. Loss of axonal structural integrity has been reported by Strain and Olson and others; while Gelfan and Tarlov and others have reported anoxia of the nerve fiber as the main cause of reduction of
neuroelectric signals during compression. Other factors such as ischemia have also been considered. This controversy appears to be due in part to a lack of strict quantitative methods when applying compressive forces to the nerve. The present study was undertaken to verify this indication in intact and severed nerves.

Materials and Methods

Fifteen New Zealand white rabbits weighing 3.5 kg to 4 kg and ranging in age from 1 year to 2 years were chosen for the experiments. The animals were initially anesthetized with ethyl carbamate (10 cc IP) and were sustained with ether. A heating pad maintained the preparation at a constant temperature (37°C) throughout the experiment. The rear legs were placed in a positioning fixture; an incision was made in the upper thigh exposing the sciatic nerve, and the connective tissue binding the nerve in place was carefully removed. The nerve was supported on a hook-type platinum wire stimulation electrode having an inter-electrode spacing of 2 mm (Fig. 1). A second incision was made in the popliteal fossa exposing the peroneal, tibial, and sural branches of the sciatic nerve. A cuff-type electrode unit with three differential recording channels described in previous publications was installed on a section of the peroneal nerve which had been carefully freed of binding connective tissue. The recording electrode unit consisted of two 4 mm exposed lengths of stranded 90% platinum, 10% iridium wire woven on the inner surface of a 15 mm length of Dacron fabric tube. The contact surfaces were oriented parallel to the axis of the tube with an inter-electrode spacing of 5 mm. The diameter of the electrode unit was selected to match the diameter of the peroneal nerve.

In one series of experiments, the peroneal nerve was severed near the distal end of the cuff-type recording electrode unit; whereas, in another series of experiments the nerve was left intact. In both series, the recording electrode unit was located approximately 5 cm distal to the stimulation electrode. To prevent foot movement and to minimize EMG artifact, the tibial nerve was crushed at the beginning of each experiment. An effort was made to minimize the amount of dissection and to maintain nerves and blood vessels in their original location throughout the experiments. After dissection, the exposed nerve was carefully covered by the supporting soft tissue, muscles, and the skin flap. This kept the nerve from becoming dry or exposed to the external atmosphere. Temperature in the vicinity of the nerve was monitored at random and found to be constant throughout the period of the experiment.

The sciatic nerve was stimulated supramaximally with 0.05 ms monopolar square wave pulse at 30 pulses per second. The resulting compound action potentials (CAP) of the peroneal nerve from each channel of the electrode cuff were detected and amplified with a bandwidth of 10 Hz to 10 kHz. They were simultaneously observed on an oscilloscope and recorded on a multi-channel FM tape recorder for subsequent analysis. During stimulation, a controlled compressive force was applied to a 3 mm length of the exposed nerve approximately 1 cm proximal to the implanted recording electrode unit. The rate and the duration of the applied compressive forces were controlled with a specially designed apparatus (Fig. 1) which incrementally compressed the nerve trunk with gram weights. This nerve compression apparatus was specifically designed so that angulation, stretching, or distortion of the nerve could be avoided during nerve compression. Throughout this article, we refrain from expressing the compression in terms of pressure because the surface of the nerve continuously deformed during compression.

The following sets of experiments were performed with severed and intact nerves. A) In the first paradigm, forces were rapidly applied to the nerve in increments of 10 gm every 5 s until no observable
CAP was present. This procedure was performed on two intact nerves and three severed nerves. B) In the second paradigm, a sustained force was applied for a two hour period and the decrease in the CAP amplitude was monitored. This procedure was performed on two intact and two severed nerves with a 20 gm force; and on one intact and one severed nerve with a 10 gm force. C) In the third paradigm, no external force was applied to the nerve and the CAP was monitored for a two hour period. This control experiment was performed on an intact and a severed nerve in both a live and a sacrificed animal.

Data obtained from the different experiments were digitized via a PDP 11/34 computer at an effective sampling rate of 160 x 10^3 samples per sec. This sampling rate was achieved by playing the data at one-fourth of the recording speed and sampling it at 40 kHz. Experiments in which compressive forces were rapidly applied were sampled during the middle of each incremental force application with an average of 30 CAPs taken for each sample (1 s sample time). Experiments with sustained 10 gm or 20 gm forces were sampled at 60 s intervals with an average of 150 CAPs taken for each sample (5 s sample time). With the described stimulation and recording arrangement, the resulting average CAPs always displayed two positive peaks and, at times, three positive peaks (Fig. 2). The amplitudes of the two positive peaks were measured from the base line (dc level) of the CAP. The propagation times from the stimulus pulse to the first and second positive peaks were taken for each average sample. The conduction velocity was calculated using the propagation time and the distance between the stimulation and recording sites. The amplitude of the CAP and conduction velocity, during application of compression, was normalized to the maximal amplitude and conduction velocity before compression.

Results

In the control experiments, when no force was applied to the nerve, the evoked CAP remained consistent in amplitude and shape during the two hour duration of the experimental procedure (Fig. 2A). This indicated that the animal preparation used in this study did provide a stable, reproducible method of obtaining the required measurements. Direct compressive forces on the nerve produced similar changes in the wave-shape of the CAP with both rapid and sustained force applications. The initial positive phase of the signal decreased in amplitude as a function of compressive force. The other phases of the signal were less sensitive to the effects of the compressive force (Fig. 2B, C). A corresponding increase in the propagation time measured from the stimulus artifact to the initial positive peak of the CAP was noted.

The results were categorized into three sections: A) experiments with rapid force application; B) experiments with sustained constant force application; C) experiments with no force applied to the nerve for control purposes.
Fig. 3: The normalized amplitude and conduction velocity of the compound action potential during rapid compressive force application (10 gm every 5 s) to the surface of the nerve. Note that initially the amplitude does not change, but it is subsequently followed by a sharp decline. Refer to the text for details appertaining to the severance of the nerve.

Rapid Force Application

In the experiments involving intact nerves, the amplitude of the CAP remained relatively constant up to a force threshold value of 20 gm to 40 gm. (The force threshold is the minimal force required to initiate a noticeable change in the wave-shape of the CAP.) Further increases in force above this value caused a rapid decrease in the amplitude of the CAP. The average slope of the decrease was approximately 25 μV/gm. The signal decreased to 50% of the initial value after application of 85 gm to 95 gm. Signals from experiments in which the nerve was severed distally exhibited a threshold value almost equal to those of intact nerves while the slope of the decrease was approximately 29 μV/gm. Furthermore, the CAP in the severed nerves decreased to a 50% initial value of 55 gm to 70 gm of compressive force (Fig. 3).

The conduction velocity of the CAP decreased during the incremental force application exhibiting a less defined threshold point and a more linear rate of decrease (Fig. 3B). The rate of decrease in CAP amplitude and conduction velocity was more pronounced in severed nerves.

Sustained Constant Force Application

Based on the above results from rapid force application, forces at minimal-threshold level (20 gm) and sub-threshold level (10 gm) were selected for experiments in which a sustained constant force was applied to the nerve. These two values were selected to illustrate the corresponding effect of application-time with force levels which had previously demonstrated no observable change in the CAP during short periods. The observed changes in wave-shape during sustained threshold and sub-threshold force applications occurred more gradually than when rapid force application was applied over a time span of 20 min to 80 min (Fig. 4). In this figure, the normalized amplitude and conduction velocity of the CAP in individual experiments were plotted versus time of force application. During sustained application of sub-threshold force (10 gm) to intact nerves, the CAP decreased in amplitude to a 50% initial value in 45 min, whereas signals recorded from experiments in which the nerve was severed decreased more rapidly (30 min) to a 50% value. Conduction velocity measurements indicated similar decreases with less distinction between intact and severed nerves. CAPs recorded during sustained application of a 20 gm force (Fig. 4) decreased to 50% of the initial value in 10 min to 15 min (a rate of 3 to 4 times faster than those recorded during 10 gm sustained force application). CAPs from experiments in which the nerve was severed decreased more rapidly (30 min) to a 50% value. A parallel reduction in the normalized conduction velocity was also noticed with application of a 20 gm sustained force.

Control Series

Experiments in which no compressive forces were applied for purposes of control exhibited a maximal 15% reduction in amplitude of the CAP during the two hour recording period. This was true for both the
larger diameter nerve fibers (Fig. 5) and the smaller diameter nerve fibers (Fig. 6). The relative size of the fiber diameter was determined by the value of their conduction velocity which was calculated from the latency between the stimulus and CAP. The latter measurements were obtained by measuring the conduction time of the second positive peak of the CAP. Measurements of the amplitude of the second positive peak are not presented because the limitations of the experimental paradigm render the value of this parameter inappropriate for consideration. The spacing between the stimulation and recording electrodes was not sufficient to provide a distinctive temporal separation of the action potentials of the smaller diameter category. The variation in normalized conduction velocity was less than 10%. Recordings from intact and severed nerves using live and sacrificed animals produced comparable results. In the results of the sacrificed animals, t=0 corresponds to cessation of heart pulsation.

**Discussion**

The wave-shape of the CAPs recorded in these experiments were similar to those reported in the literature. In the present experiments, the first pulse of the CAPs represents the activity of the faster conducting, large diameter, nerve fibers of the peroneal nerve, most probably what has been termed the \( \alpha \) and \( \beta \) components. The conduction velocity of the \( \alpha \) and \( \beta \) population of nerve fibers were not sufficiently different to contribute consistently distinguishable pulses to the CAP. This was due to the limited distance between the stimulus and the recording electrodes. The \( \beta \) component was at times seen as a notch occurring on the negative slope of the \( \alpha \) pulse (Fig. 2A), and at other times as a separate pulse following the initial \( \alpha \) pulse (Fig. 2B). The conduction velocity of the \( \alpha \) component was found to be 72 m/s; that of the \( \beta \) component was approximately 56 m/s. The last pulse in the CAP represents activity of the myelinated small diameter nerve fibers, possibly of the \( \gamma \) population in the peroneal nerve, and was found to have a conduction velocity of 35 m/s. All of these measurements are in agreement with those reported by Matthews and others. Larger diameter nerve axons of approximately 12 \( \mu \)m to 18 \( \mu \)m such as the Ia and \( \alpha \) motor axons most likely contribute to the first pulse of the CAP (72 m/s). Smaller diameter nerve axons of approximately 4 \( \mu \)m to 6 \( \mu \)m such as the \( \gamma \) fibers and small sensory fibers contribute to the last pulse (35 m/s). Boyd and Davey have reported that the fiber population of the peroneal nerve in cats also contains two distinct measurable populations similar to those clearly identified by the two pulses recorded in the present experiments.

When the compressive forces were applied to the nerve, the CAP changed its shape. The first pulse decreased in amplitude and increased in latency as seen in figure 2. This may be explained by the occurrence of structural damage to the faster conducting, larger diameter nerve fibers. The relatively greater
insensitivity of the subsequent pulses (Fig. 2B) to such compressive forces implies that the slower conducting, smaller diameter nerve fibers are relatively less susceptible to damage by external forces. These results are in agreement with those reported by Strain and Olson who found that large diameter nerve fibers of rat sciatic nerve were compressed disproportionately. This selective susceptibility of the larger diameter fibers to damage by compression has been noticed not only in the peripheral nerves but also in the dorsal root and dorsal column of the spinal cord. In fact, the selective susceptibility of larger diameter fibers may be explained by a purely mechanical process described by Laplace's law as pointed out by Strain and Olson.

When the compressive forces were rapidly applied to the nerve (Fig. 3), the presence of a threshold value followed by a rapid decrease of the CAP amplitude supported the concept of the occurrence of a relatively abrupt structural dislocation of the nerve fibers and its surrounding connective tissues. A dislocation of the epineurium, perineurium, and possibly the endoneurium, must occur for the protected nerve fibers to be disturbed structurally. The presence of a threshold value may demonstrate the necessity of providing sufficient compressive pressure to overcome the elevated (greater than atmospheric) endoneurial fluid pressure. This elevated endoneurial pressure has been measured directly in the sciatic nerve of normal rats. If an abrupt change in the diameter of the axons occurs, it would cause a correspondingly abrupt change in the amplitude of the CAP. Subsequent changes in the axon diameter are noticeable in an accentuated manner in the decrease of the CAP amplitude. Rosenfalk has shown that the amplitude of an action potential is proportional to the diameter of a fiber raised to the power of 1.7. On the other hand, the conduction velocity of nerve fibers is approximately linearly related to the diameter. In fact, the near-linear decrease of the conduction velocity (after the threshold value), with increasing compressive force shown in figure 3, implies that the diameter of the relatively large axons is being disrupted in a near-linear fashion. Hence, the curves of figure 3 appear to demonstrate the radial compliance of the nerve (nerve fibers and connective tissue) during compression. This observation concurs with that of Baba et al who reported a reduction in maximal motor conduction velocity as well as reduction in axonal and total fiber diameter of large diameter axons after continued ligation to the tibial branch of the rabbit’s sciatic nerve.

When minimal-threshold and sub-threshold compressive force was constantly applied to the nerve, the amplitude of the CAP and the conduction velocity of the nerve fibers also decreased as a function of time (Fig. 4). This behavior may be explained by a time-dependent distortion of the nerve; that is, the radial viscosity of the nerve fascicles (nerve fibers and connective tissue).

The data in figures 3 and 4 indicate that the effect of the compressive force on the efficiency of a nerve to conduct action potentials is a time-force dependent process with characteristics which can be explained by the viscoelastic properties of the nerve. The observed variation and disparity in the results of the various experiments point to the likely variability in the viscoelastic properties of different nerves. These inter-experimental variations were obtained despite a serious attempt to maintain experimental consistency among the different experiments.

Several degrees of physical distortions have been reported to occur during nerve compression which could in part account for the observed changes in the CAP. Strain and Olson reported possible physical interruption in the continuity of the axon and/or its neurilemmal sheath. Rydevik and Lundborg noted damage to the epineurium along with intrafascicular edema formation on the edges of the site of force.
application. It was suggested that this edema formation, which was instantly noticed after the compression was removed, produced secondary compression on the endoneurial vessels with possible secondary axonal damage. 16,17

Compression of the nerve also can cause localized ischemia to the nerve if the forces are sufficient to collapse the longitudinal and collateral circulation of the nerve. Rydevik et al 16 reported that interference in the epineurial blood flow was noticed at a pressure of 20 mmHg to 30 mmHg; intra-fascicular capillary flow at about 40 mmHg to 50 mmHg; whereas, complete interruption of blood flow to the nerve segment was observed at a pressure of 60 mmHg to 80 mmHg. The resulting diminution of nutrient supply could impair the action potential transmission. (These pressures were approximately one-half those applied in this study.) In fact, several investigators 5,18 have reported that anoxia is the primary cause of inactivation of the smaller diameter (γ population) nerve fibers.

In this study it was found that the conduction velocity of the α, β, and γ components of the CAP were only moderately diminished over a two hour period when the animal was sacrificed and had no systemic blood flow. Our results indicate a lack of preferential effect on the nerve fiber population due to arrested blood circulation during the two hours of the experimental period. Therefore, it appears that lack of oxygen, as a result of arrested blood circulation, is not the primary factor in the preferential injury of nerve fibers. This observation concurs with that of Smith 19 who reported that a large portion of nerve fibers could function as a free graft after separation from its mesentry until blood supply is restored by the growth of new blood vessels. Results from the present study are also in agreement with those of Lundborg 20 who reported that six hours of ischemia failed to induce structural damage to rabbits' peripheral nerves. It remains to be pointed out that a more recent study by Fowler and Gilliatt 21 demonstrated varying degrees of nerve degeneration and conduction block after regional ischemia in the sciatic nerve of rabbits. In that same study, however, 35% of the animals showed little, if any, nerve degeneration indicating that local ischemia is not a primary cause of the decrease in amplitude and conduction velocity of the CAP.

It was generally noticed in the present results that the severed nerves were more susceptible to compressive forces than intact nerves, a finding which is not well understood. Similar observations were reported previously by Gilliatt and Wilson 22 and Fullerton 23 who showed that injured nerve fibers are more susceptible to induced ischemia than normal nerve fibers. This was also noticed in our previous work, where the amplitude of the CAP recorded from chronic implants around a severed nerve decreased with time. 3 The accentuation of the effects of compressive forces on the severed nerves may be due to the increased radial compliance and the reduction of axonal fluid at the severed end.

External compressive forces (greater than 10 gm weight) applied over a 3 mm length of a peripheral nerve affect the conduction mechanism of all nerve fibers. Larger diameter axons are more susceptible than smaller diameter axons. The extent of the restriction to neural conduction within the two hour period of the present study appears to be regulated by the viscoelastic properties of the nerve (nerve fibers and connective tissue). Brief applications of compressive forces (20 gm or more over a 3 mm length) cause decreases in conduction velocity which do not revert to normal value within two hours. Severed nerves were found to be more sensitive to compressive force than intact nerves. This indicates that caution should be exercised when handling peripheral nerves during surgery or experimentation. Ischemia lasting two hours was not noted to affect the neural conduction of either the larger or smaller diameter nerve fibers.

References

9. Low PA, Dyck PJ: Increased endoneurial fluid pressure in


