Action potentials conducted along a nerve fiber leave in their wake consistent alterations in excitability, including the absolute and relative refractory periods, a supernormal period (SNP) and a late phase of subnormality. We describe an automated technique for reliably determining the recovery cycle of human sensory nerve fibers by delivering series of paired stimuli and precisely measuring the latencies (to within 0.5 μsec) of the compound action potentials. The recovery cycle can be compiled from the differences in latency between the two responses of a pair. Consistent changes in conduction velocity are demonstrated during each phase of altered excitability. Possible physiological mechanisms underlying the recovery cycle are discussed, and the effects of cold, ischemia, prior tetanization and subcutaneous lidocaine are presented. This technique may prove to be a useful and more sensitive tool for the study of certain disorders of peripheral nerves.
Key words: recovery cycle • supernormal period • paired stimulation • excitability • nerve conduction

MUSCLE & NERVE 13:750–758 1990

AN AUTOMATED TECHNIQUE FOR MEASURING THE RECOVERY CYCLE OF HUMAN NERVES

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Following the passage of a nerve impulse, an axon undergoes a series of excitability changes before returning to its resting state.[1] First there is the absolute refractory period, during which propagated action potentials cannot be generated, regardless of the strength of the stimulus. This is followed by the relative refractory period during which action potentials can be generated but a stronger stimulus is required. This is followed by a supernormal period (SNP), which is, in turn followed by a longer lasting phase of subnormality. This recovery cycle has been observed in both myelinated[8,9] and unmyelinated[6] fibers in both the central[11] and peripheral[9] nervous systems in a variety of species.[8,10,11,19] Although the mechanisms responsible for both refractory periods are fairly well known (inactivation of sodium channels and residual potassium conductance),[14] those of the supernormal phase are the subject of continuing investigation.[2,4,9] The late subnormality following activation is even less well understood.

There are several ways in which these changes in excitability following the passage of a nerve impulse can be demonstrated. (1) By using paired stimuli at varying interstimulus intervals and adjusting the intensity of the second, it is possible to compare the stimulus currents required to make the second compound nerve action potential equal to the first or to bring a single axon to threshold a second time.[3,20] (2) Using paired stimuli of equal intensity, it is possible to compare the number of fibers activated (as estimated from the amplitude of the compound action potential)[12,24] by the first and second stimulus. (3) Using paired stimuli of equal intensity, it is possible to compare the latencies of the single fiber or compound nerve action potentials in response to the first and second stimulus.[8,15] This estimate is based on the close relationship between nerve fiber excitability and impulse conduction velocity.[6,7,10,15]

In man, it is difficult to study the excitability of single axons as this requires either intraneural recordings or recordings from single motor units[3] (in which case the excitability of the neuromuscular junction and muscle fiber are included). The measurement of threshold is tedious and difficult to automate. This article describes a method that allows rapid and accurate measurement of sensory nerve fiber excitability changes following activa-

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Accepted for publication October 26, 1989.

CCC 0148-639X/90/080750-09 $04.00

tion by automating the measurements of distal latency of nerve compound action potentials.

MATERIALS AND METHODS

Sensory fibers of the human median nerve were stimulated with ring electrodes on the third finger. The cathode was applied at the base, and the anode 3 cm distally. Compound sensory action potentials were recorded at the wrist with 0.5-cm diameter disc surface electrodes placed 3 cm apart along the length of the nerve, the active electrode being 13 cm proximal to the stimulating cathode. A ground electrode was applied to the dorsum of the wrist. Rectangular pulses of 100 μsec duration were delivered from a constant current stimulator, and the stimulation current was adjusted to 50% above that required to elicit a maximal compound sensory action potential at the wrist. A probe was fixed to the palm to monitor any changes in temperature, and the hand was wrapped in a towel to minimize heat loss during a study. The recorded signals were amplified and bandpass filtered at 500 Hz and 2 kHz with a Neuromatic 2000 EMG machine, and the signal was further bandpass filtered at 400 Hz and 1.7 kHz at −12 dB/octave to decrease the jitter in latency measurements. Custom circuitry was built to allow detection of positive threshold crossings by the rising phase of the compound sensory action potential. The analog threshold was set under software control and could be adjusted to any point on the waveform. A variable time lockout feature was incorporated to prevent inappropriate triggering from noise or stimulus artifact. The latency was measured to within 0.5 μsec by a hardware timer chip and was defined as the interval from the stimulus onset to the time at which the rising phase of the compound sensory action potential crossed the predefined threshold. Each response was also digitized and stored on disk for later inspection and analysis. Data were acquired and analyzed automatically using an IBM PC-AT microcomputer.

Figure 1 explains the terms used throughout

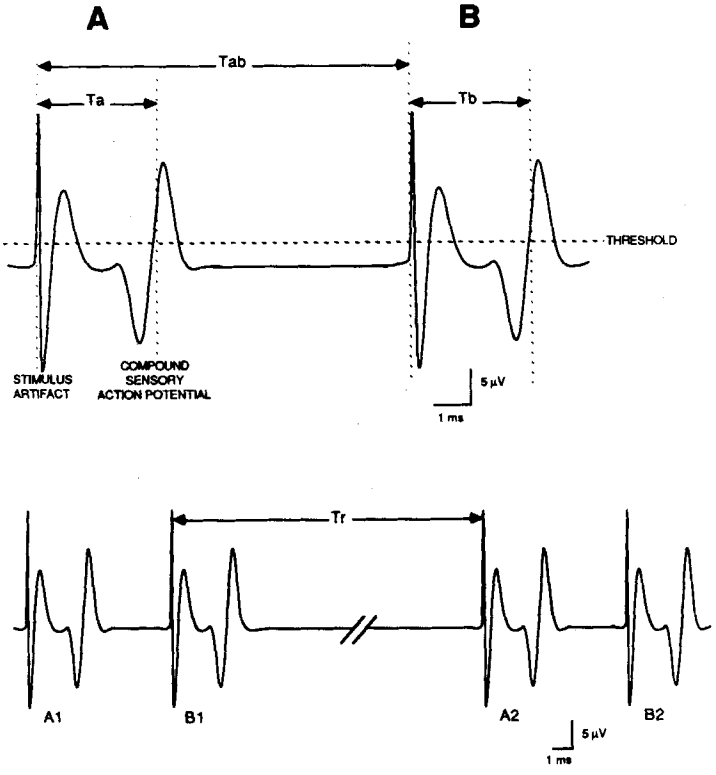

FIGURE 1. Schematic representation of a pair of stimuli **(A)** and **(B)** with their respective responses. Ta and Tb are the distal latencies of the first and second response, respectively, at the point where the compound sensory action potential crosses threshold. Tab is the interval between stimuli. Tr is the relaxation time allowed between successive pairs. Calibration marks are approximate. The triggering level ('THRESHOLD', dashed line) was typically set to 50% of the peak-to-peak amplitude of the nerve action potential.

this article to denote the various intervals. Pairs of stimuli were delivered with the interstimulus interval (designated as Tab) varying between 3 and 200 msec. The shortest Tab that could be used (without the second stimulus artifact becoming superimposed on the first response) was limited by the distal latency of the median nerve (about 3 msec) plus a 300 μs software delay. The longest Tab used was 200 msec as recovery was usually complete by 110 msec. The sequence of Tab intervals used within a study was randomized to avoid the effects of slow trends. A series of 30 to 50 pairs of stimuli were given at each Tab, with a one-second relaxation period (Tr) allowed between each pair to reduce the likelihood of cumulative effects. The distal latencies of the first, unconditioned response (Ta) and of the second, conditioned response (Tb) were measured with the circuitry described above. The 'normal' (unconditioned) distal latency was taken as the median of all Ta's for each series. The absolute shift in latency between the second response of the pair (B) and the first (A) for each individual Tab was determined by calculating the difference in latencies (Tb-Ta) for each pair, then computing the median of these differences. Any result differing by more than 3% from the collective mean was assumed to be contaminated by noise and was omitted from the calculations (typically less than 5 pairs were rejected at any given Tab). Responses were obtained in normal subjects between the ages of 16 and 49 years.

Effects of ischemia were studied by inflating a blood pressure cuff around the upper arm to 30 mm Hg above systolic pressure and maintaining for 10 minutes before starting a study. The hand was cooled for several experiments by either packing the forearm with ice (which resulted in a drop of skin temperature of only 2–3°C at the palm) or applying ice packs directly around the hand (which cooled the skin of the palm by about 10°C or more). The effects of tetanic stimulation on the recovery curve were examined by delivering a 20-second train at a frequency of 200 Hz (4000 shocks) immediately before each series of paired stimuli. Since the effects of high frequency stimulation were found to subside with time, and since a 30- to 50-second recording period was required to gather data at each interstimulus interval, the tetanus was repeated prior to each Tab.

RESULTS

The mean recovery curve (±2 standard deviations) obtained by pooling the measurements from 20 normal subjects is shown in Figure 2A. Surface temperatures measured in the palm were within the range of 32–36°C. The interstimulus interval (Tab) is plotted along the x-axis and the absolute shifts in latency in microseconds on the y-axis. Negative latency shift values denote that the second response was conducted more rapidly than the first, ie, supernormality, and positive values indicate slowed conduction of the second response of a pair, ie, subnormality. There was an initial short period of subnormality ending at around 3.5 msec, corresponding to the relative refractory period. This was followed by a supernormal period extending from about 3.5 to 18 msec, being maximal at 6 msec. A longer and less pronounced period of subnormality followed, and slowly decayed so that by 110 msec both responses had the same latency. Figure 2B shows eight separate curves obtained from the same subject on different occasions to test the repeatability of the method.

How are the recovery curves affected by altering the stimulating and recording conditions? Figure 3 shows the effects of varying the distance between stimulating and recording electrodes on the recovery curve. The difference in latency in the supernormal period increased as the distance increased from 13 to 15 cm, suggesting that the phenomenon responsible for the increase in conduction velocity is distributed along the length of the nerve. No consistent increase in latency could be demonstrated for the subnormal phase although the absolute changes are smaller and may have been obscured by noise. The effects of different separations between the recording electrodes were tested by placing the active electrode at a constant location, and moving the indifferent electrode from 1 to 6 cm proximal to it. No appreciable change in the shape of the recovery curve was detected. Nor was the triggering level (Figure 1) an important variable. Recovery curves on subjects obtained with three different settings of the triggering level (approximately 25%, 50%, and 75% of the peak-to-peak amplitude of the compound sensory action potential) were similar. The intensity of the stimulus current was also not critical, provided that it was set at or above that required to elicit a maximal response. Stimuli just sufficient to produce maximal sensory action potentials and stimuli 50% stronger than this produced similar recovery curves. With a weaker stimulus that evoked a compound sensory action potential whose amplitude was half of maximum, the shift in latency in the supernormal phase was greater.

The effect of temperature was examined in 6

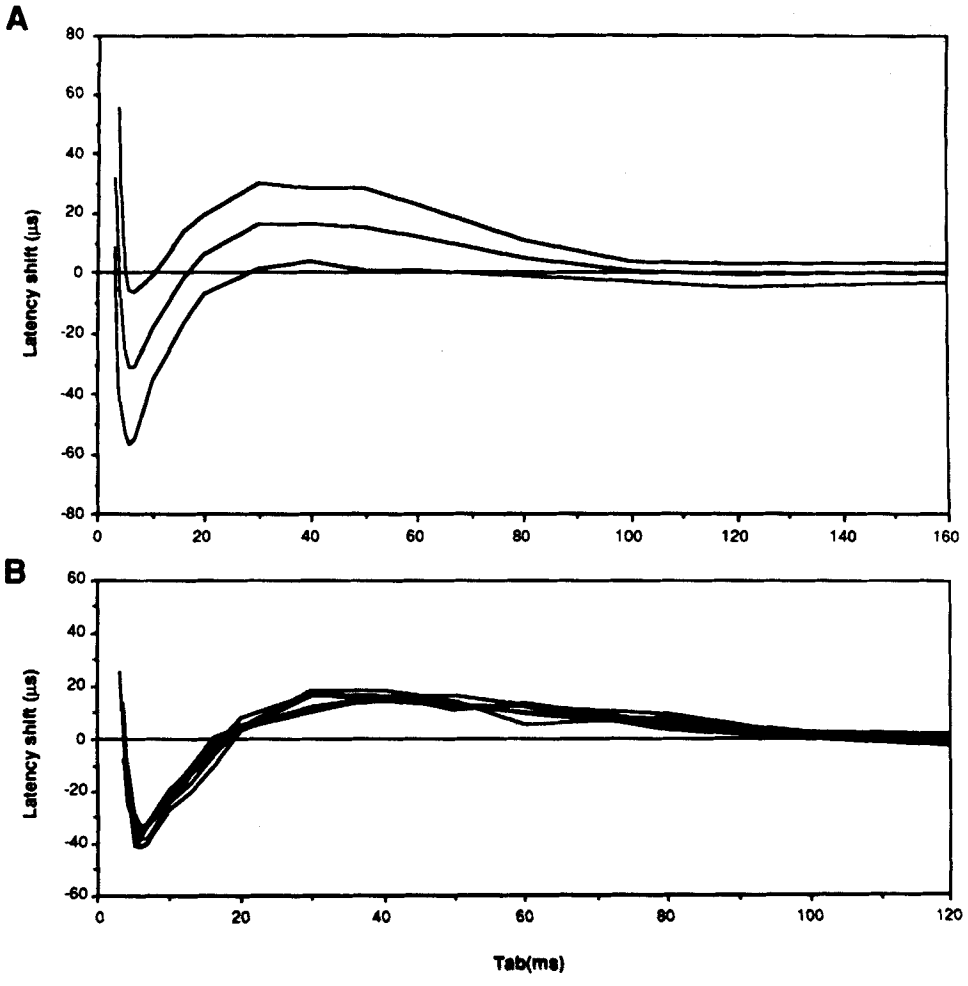

FIGURE 2. (A) Graph of the mean recovery curve (±2 SD) of the median nerves of 20 normal volunteers. The latency difference (in µsec) of the second response compared to the first is plotted against the interstimulus interval (Tab) in milliseconds. Negative values denote supernormality of conduction velocity, and positive values represent subnormality. The initial, steep subnormal phase reflecting the relative refractory period is followed by a supernormal period from 3 to 18 msec, and a prolonged subnormal phase lasting from 18 msec to approximately 110 msec. **(B)** Eight separate studies performed on the same subject on different occasions showing the repeatability of the recovery curve.

subjects. An example is shown in Figure 4. With decreasing skin temperature from 34.2 to 25°C, the relative refractory period became longer, and both the duration and the magnitude of the SNP increased. The subnormal period also widened and increased slightly in amplitude.

The effects of tetanic stimulation on the recovery curve were tested in two subjects. The SNP was markedly enhanced in both duration and depth (Figure 5A), and the subnormal phase was prolonged. Note that, in contrast to the effects of cooling, the duration of the relative refractory period remained unchanged.

Ischemia (3 studies in 2 subjects) also produced significant alterations in the recovery curve, as seen in Figure 5B. During the period of ischemia, the relative refractory period increased in duration and merged directly into the subnormal period. No supernormality was detected. The temperature varied by no more than 1.4°C and was not enough to account for these findings. With reperfusion, the relative refractory period shortened, and the SNP increased in amplitude and duration. Note the similarity between the effects of tetanization and those following ischemia. Subjects reported paresthesiae at the time when the SNP was enhanced, both following ischemia, and to a lesser extent following tetanization.

The effects of local infiltration with lidocaine around the nerve (1 subject) are shown in Figure

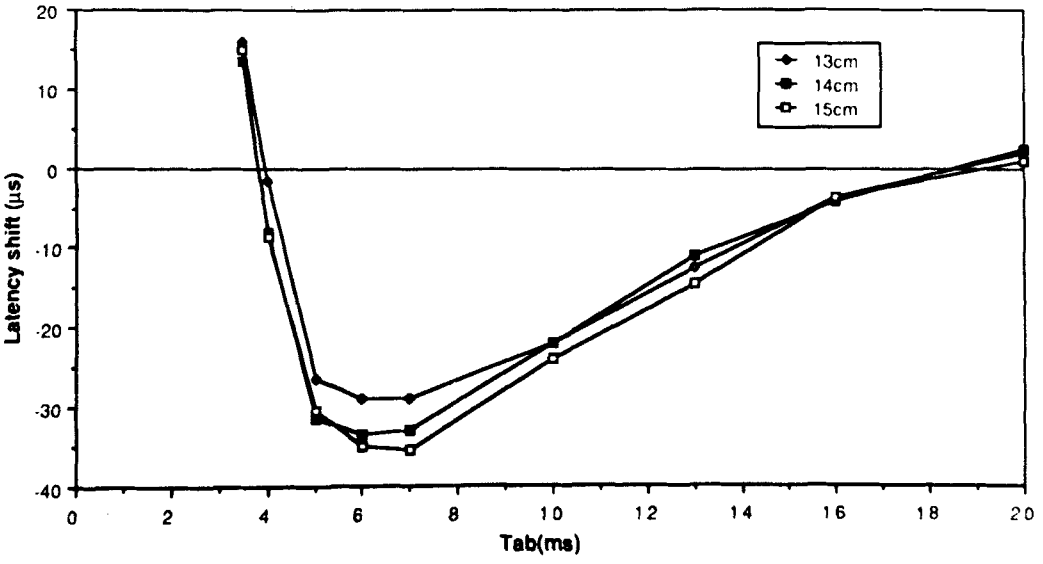

FIGURE 3. Plots of recovery curves in a single subject as a function of distance between stimulating and recording electrodes. At the trough of the supernormal period (6–7 msec), a small but consistent increase in the latency shift can be seen with increasing distance, indicating that the effect is distributed along the length of the nerve.

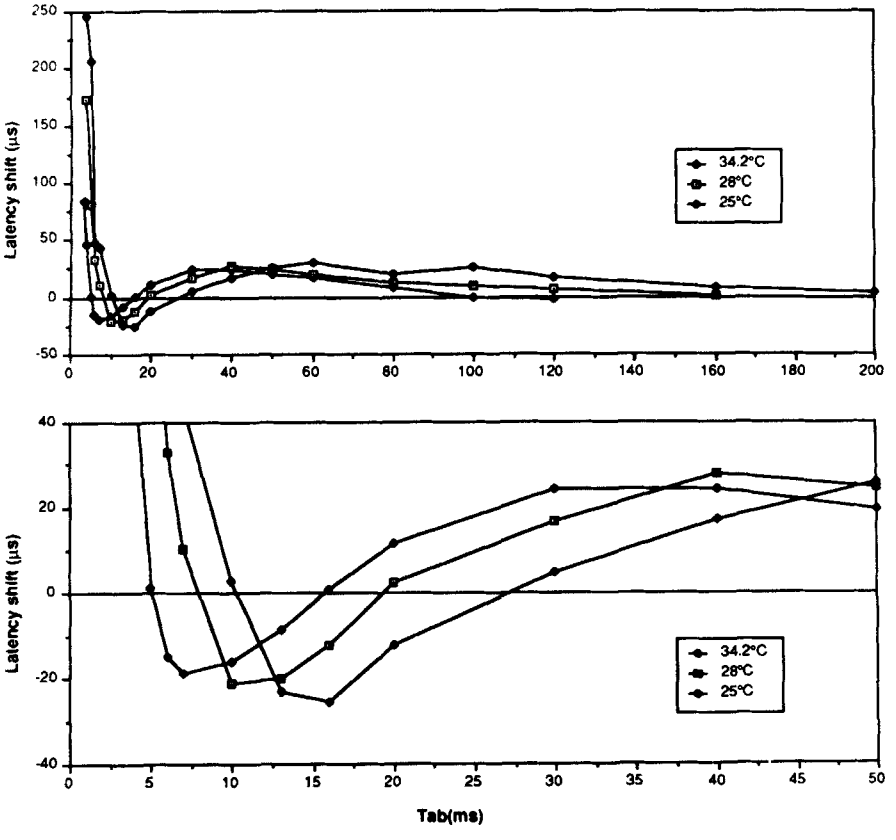

FIGURE 4. Effects of changes in temperature on nerve recovery. Curves are shown for three different temperatures measured on the surface of the palm. The relative refractory period becomes prolonged lasting up to 10 msec at 25°C, with very large latency shifts compared to normal. The supernormal period widens and deepens. The same is true for the subnormal phase, which lasts until 200 msec at 25°C and increases slightly in amplitude. Curves at 34.2°C and 25°C are from the same subject, whereas data at 28°C is from another volunteer. The bottom figure is an expanded view emphasizing the supernormal period.

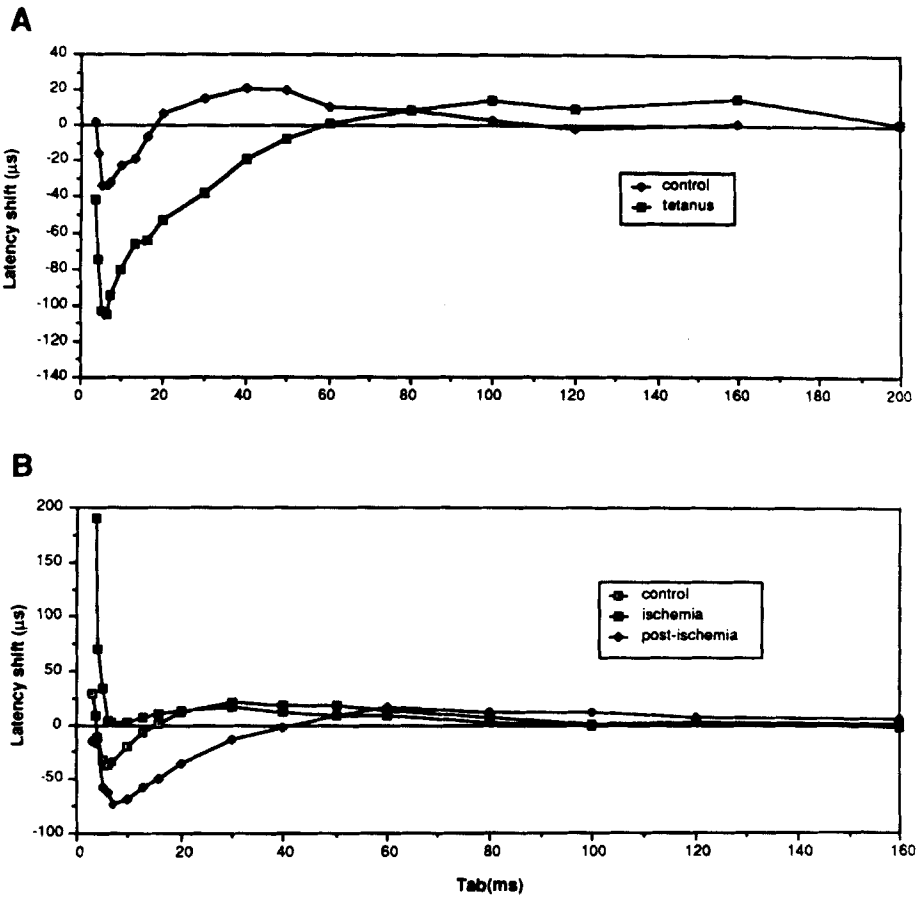

FIGURE 5. (A) Effects of prior tetanization. A high frequency train (200 Hz for 20 sec) was delivered immediately before each Tab. The depth of the supernormal period increases threefold and its time course is very prolonged, extending to 60 msec. The subnormal period is delayed; its time course also is prolonged (to 200 msec), although the amplitude is unaffected. **(B)** Effects of ischemia. The solid squares represent the curve obtained after 10 minutes of complete ischemia. The relative refractory period becomes longer, and the supernormal period disappears. With re-perfusion (diamonds), the SNP is re-established, and at double the amplitude of control. The time course is also prolonged. Note how this curve resembles the effects of tetanization.

6. The SNP gradually diminished in magnitude as the anesthetic took effect. The duration of the relative refractory period remained unchanged, and the subnormal period appeared sooner, as it was unmasked by the smaller SNP. The absolute latency changed less than 1.5% even though the compound sensory action potential amplitude fell to 50% of control at the peak of anesthesia. The reduction in amplitude of the action potential alone does not explain the change in the SNP.

DISCUSSION

There is a predictable relationship between nerve fiber excitability and conduction velocity.[8,19,20,23] Thus, measuring very small (0.5 μsec) shifts in conduction velocity during the various periods of super- and subnormality will disclose changes in basic electrical properties of the nerve fibers under study. The recovery of excitability of a peripheral nerve can be determined by using paired stimulation. Very reproducible recovery curves are obtained provided that stimuli supramaximal for the large sensory afferents contributing to the nerve action potential are used, and that the distance between stimulating and recording electrodes and the temperature of the limb are standardized. The subject must have compound action potentials greater than 5 μV and be capable of relaxing in order to obtain good recordings. In other respects, the method is shown to be quite tolerant of minor changes in technique. While others have had mixed results,[22,24] our data clearly show that there are consistent fluctuations

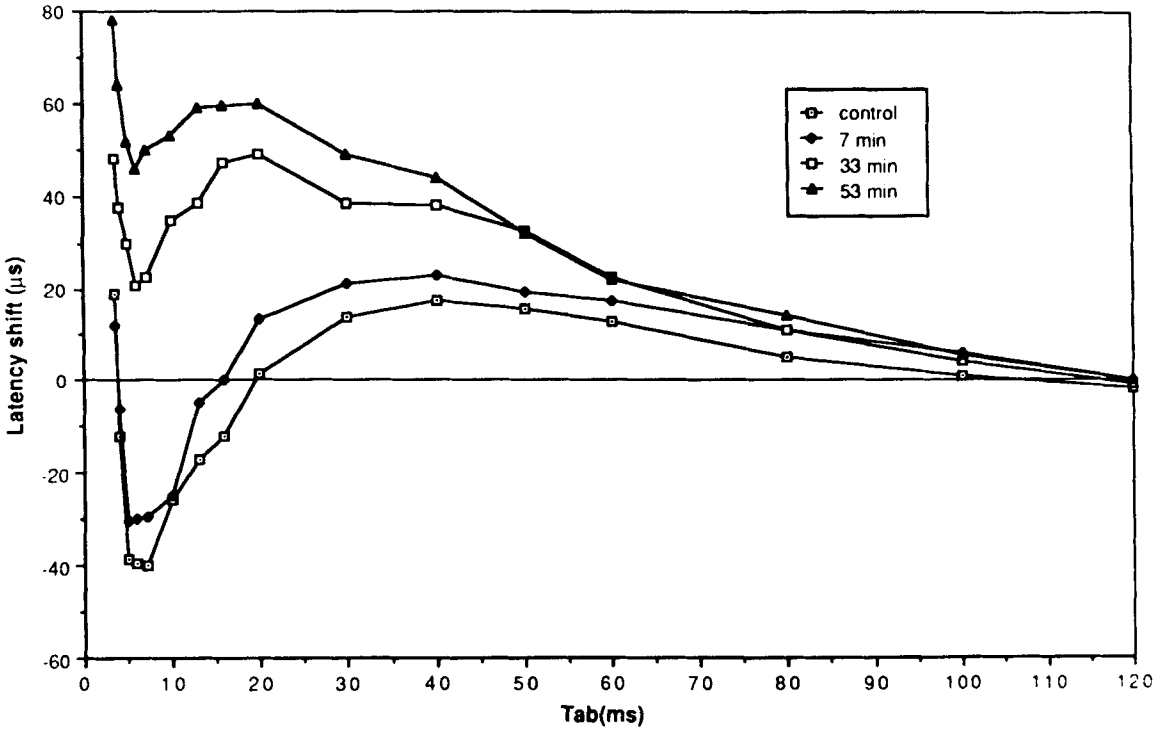

FIGURE 6. Effects of lidocaine on nerve recovery. A control curve is plotted along with three response curves 7, 33 and 53 minutes after infiltration around the median nerve at the wrist with 1.5 mL of lidocaine 2%. As the anesthetic takes effect, the supernormal period (SNP) is gradually reduced in magnitude. The relative refractory period is unchanged in duration, and the subnormal period appears sooner. At 53 minutes, the peak-to-peak amplitude of the compound sensory action potential fell to approximately 50% of control.

in latency during both phases of super- and subnormality.

The recovery of nerve excitability included an absolute and relative refractory period, followed by a supernormal period (from 3.5 to 18 msec, maximum at about 6 msec), and a final subnormal period (from 18 to about 110 msec) as described previously for human nerves.[3,12,13,15] The time course of these latency shifts is almost identical to activity-dependent changes in excitability obtained by other means.[24] The shortening of latency associated with the SNP is greater with increasing length of nerve studied (Figure 3) suggesting that the mechanism responsible is distributed along the axon, and is physiological rather than just occurring at the stimulation site. With a 50% supramaximal stimulus intensity used in our study, it is unlikely that additional fast-conducting fibers are recruited,[24] and therefore the SNP reflects a true increase in conduction velocity of the same axons previously activated by the conditioning volley.

The absolute and relative refractory periods have been attributed to inactivation of sodium channels and to the residual conductance of potassium channels following an action potential.[14] These refractory periods are known to be prolonged by cooling and ischemia[12] and this was confirmed in our study. Slowed channel kinetics at lower temperatures accounts for the prolongation of refractoriness. It is unclear why prolonged refractoriness should occur with ischemia. The recovery curve is likely a complex summation of events (refractoriness, supernormality and subnormality), each with its own time course. If ischemia abolishes the SNP (possible reasons for this are discussed below), it is possible that an underlying refractoriness is unmasked and appears longer than normal in the absence of the counteracting influence of the SNP. Therefore, what is commonly referred to as the refractory period may in fact be composed of several distinct phenomena such as channel inactivation and the early stages of the SNP.

The SNP has been described in a variety of preparations including unmyelinated cerebellar parallel fibers in the rat[18] and cat,[11] myelinated rabbit callosal axons,[26] turtle olfactory nerve,[10] myelinated rat spinal cord axons[4] and peripheral axons in frog, rat[9] and lizard.[2] It has been

suggested[9] that this supernormality may be a consequence of the depolarizing afterpotential (DAP) which immediately follows the action potential for the following reasons: (1) there is a close temporal association between the DAP and SNP; (2) axons exhibiting a DAP also exhibit a SNP; (3) brief, subthreshold depolarizing pulses applied intraaxonally result in a passive, slowly decaying depolarization and a parallel period of increased excitability without the generation of an action potential.

The DAP may have more than one mechanism. In unmyelinated fibers, it may result from a transient extracellular accumulation of potassium that follows the passage of an action potential, depolarizing the fiber and bringing it closer to threshold.[10,17] In myelinated fibers, on the other hand, extracellular potassium accumulation appears to play a minor role. Barrett and Barrett[2] have argued that the DAP represents a passive potential resulting from the capacitive charging of the internodal axolemma by the action potential, and the subsequent discharge through low resistance pathways either through or under the myelin sheath. Consequently, action potentials that are either of greater amplitude (for example those induced in a hyperpolarized axon) or wider (as a consequence of reduced temperature) result in a greater DAP. Conversely, a smaller DAP is seen when the amplitude of the action potential has been reduced by the sodium channel blocker tetrodotoxin.[2] There is also the possibility that a slowly inactivating inward sodium current may contribute to the late afterdepolarization.[17]

Intracellular recordings in myelinated mammalian spinal cord axons have shown that the DAP (and consequently the SNP) has a later onset and is of greater amplitude at lower temperatures.[5] We found that when the nerve was cooled, the onset of the SNP was delayed, the duration prolonged and the absolute magnitude increased (Figure 4), consistent with in vitro studies.[4]

Hyperpolarizing an axon by injecting current into it increases the DAP[2,5] (by increasing the peak amplitude of the action potential), and would therefore be expected to enhance the SNP. Tetanizing a fiber stimulates the sodium-potassium pump and results in fiber hyperpolarization and a secondary increase in the amplitude of the DAP.[8] The correlate in this study is an increased SNP as seen in Figure 5A. Under ischemic conditions, the nerve fibers depolarize and the peak action potential amplitude diminishes,[21] resulting in a reduced DAP[2,5] and smaller SNP. In the present study ischemia resulted in a reduced SNP (Figure 5B). After ischemia the axon hyperpolarizes[21] as a result of the electrogenic Na pump[16] producing an increased SNP (Figure 5B).

The intense paresthesiae experienced with reperfusion after ischemia may be a reflection of the greatly increased SNP. A distinct "afterglow" of tingling was reported by subjects following each stimulus, as if the fibers oscillated for a brief time in response to the shocks. The firing frequency of such discharges would be expected to coincide with the peak of the SNP (ie, a period of approximately 6 msec). Ligature-induced injury of peripheral nerve in rats results in repetitive firing in response to a single stimulus with a period of 4–5 msec when bathed in the potassium channel blocker 4-aminopyridine.[27] This is not far from our expected firing frequency and may reflect inherent differences in the rat fiber, or the effects of 4-aminopyridine.

The local anesthetic lidocaine is known to block sodium conductance in an activity-dependent fashion[25] and would be expected to reduce the DAP and consequently the SNP. In our studies, lidocaine infiltrated around the nerve did result in a reduction of the amplitude of the action potential (reflected by a smaller compound sensory action potential) and a reduction of the SNP (Figure 6), as expected.

The technique we describe provides an easily automated method for recording the changes in excitability of human sensory nerves by precisely measuring latency changes in response to stimulation. Latency measurements yield very reproducible results and may be more reliable than changes in amplitude or threshold. To our knowledge, no thorough study has been performed on the changes in recovery curves in various neuropathies, and this technique may prove to be a useful and more sensitive tool for the study of certain disorders of peripheral nerves. Furthermore, with a better understanding of the fundamental mechanisms underlying the different phases of altered excitability following stimulation, changes in recovery curves in various disease states may offer important clues about the basic pathophysiology of human neuropathies.

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