OBSERVATIONS ON ANODAL POLARIZATION OF CUTANEOUS NERVE

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INTRODUCTION

The selective blocking of large-diameter peripheral nerve fibers by anodal polarization provides a potentially useful technique for studying, in isolation, the central effects of smaller-diameter afferents[4]. Unlike the earlier techniques for blocking larger fibers, anodal polarization holds the promise of being selective, reversible, and more easily controlled.

In the studies using this method thus far, the compound action potential has been used to indicate the onset and degree of block. Paintal[5], however, has shown that, in cooled peripheral nerve, increased dispersion of the afferent volley may eliminate a compound potential although individual fibers continue conducting. More subtle changes in the temporal dispersion of the volley could further complicate the interpretation of results obtained with this blocking technique. The purpose of this study was to examine the effects of anodal polarization on mammalian peripheral nerve and to evaluate the reliability of the compound action potential as an indicator of preferential large-fiber block during polarization. Computer simulation of the compound potential has been used to aid in understanding the experimental results.

METHODS

The sural nerve or dissected strands of superficial radial nerve of pentobarbital-anesthetized cats were placed on silver–silver chloride stimulating and recording electrodes and immersed in mineral oil warmed to 35–32° C. Cutaneous needle electrodes were used to stimulate within receptive fields during single fiber recording. Polarizing current (0.05–1.50 mA) was passed from a constant current stimulator through 2 silver–silver chloride troughs 4 mm wide and 3–4 mm apart with the anode proximal; the nerve rested on saline-soaked cotton pads. Both stimulating and recording electrodes were at least 8 mm from the polarizing electrodes in all experiments. The conduction velocities of single fibers were estimated from the latency and conduction distance; in a few instances, additional estimates were made by using the

'collision technique'[3]. Monopolar (killed end) recording was employed except in those experiments in which the central effects of the afferent volley were being examined. For the latter purpose, extracellular microelectrode recordings were taken from the medial medullary reticular formation of decerebrate, cerebellectomized cats in which the dorsal columns had been sectioned at a high cervical level.

Computer simulation of changes in the compound action potential was performed on an IBM 360/67 digital computer.

RESULTS

Changes in the compound action potential

As others have observed[4], it is possible to eliminate all trace of the A and A-delta compound action potentials while the C fibers continue to conduct (Fig. 1). As

Fig. 1. Changes in the compound action potential during anodal polarization. Superficial radial nerve. Upper traces, C wave. Middle traces, A and A-delta waves. Lower traces, calibration pulse; 50 μV and 10 msec for top trace, 1 mV and 2 msec for middle trace. Conduction distance: 39 mm. A, Before polarization. B, C, D, 200, 400 and 600 μA polarization. Shock artifacts connected by lines, peaks of responses by arrows, to show latency shifts.

the polarizing current was increased over a 3–10-sec period in order to achieve this block, several changes in the compound action potential were consistently observed. First, the amplitude of the large A wave increased to 120–130% of control value during the early phase of polarization (Fig. 7); changes in wave duration, if any, were too small to be measured accurately. Second, both A and A-delta waves increased in

latency and progressively decreased in amplitude, the A-delta wave, with one exception, being the first to disappear. Changes in the form or duration of the A-delta wave could not be accurately assessed, as the decreased amplitude also decreased the signal to noise ratio. Finally, the remaining C wave was usually slightly smaller in amplitude and increased in duration and latency (Fig. 1). At this point, neither A nor A-delta waves could be detected even at high gain.

The above changes in the compound action potential suggested that anodal polarization altered the conduction velocities of fibers prior to block and, within the A and A-delta group, blocked different sized fibers at the same level of applied polarization. Single fiber recordings were therefore used to gain information about the changes in conduction velocity and the temporal order of blocking of A and A-delta fibers. The rest of this study is confined to fibers conducting within the A–A-delta range in cutaneous nerve. Fibers conducting at 24 m/sec or below are classed as A-delta fibers; all those with higher conduction velocities are placed in the A (alpha-beta) category.
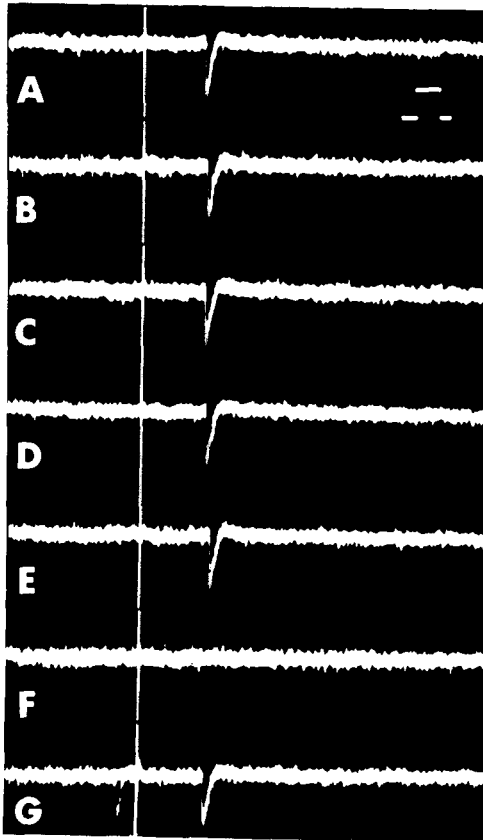

Fig. 2. Serial conduction velocity decreases prior to polarization block. Superficial radial, single fiber. Calibration: 30 μV, 1 msec. Polarization of 100 μA starting at B and increasing in 100 μA steps through F. A and G were recorded immediately before (A) and after (G) polarization. Conduction velocities (m/sec): A, 37.8; B, C, G, 35.1; D, 33.9; E, 32.3; F, blocked by 500 μA polarization.

Single fiber recordings

Recordings from 36 single fibers during anodal polarization revealed varying degrees of conduction velocity decrease prior to block in all but 9 fibers. Ten of these fibers also showed 2–4 latency increases, often of different size, before blocking took place (Fig. 2). When the polarizing current was stopped, conduction velocity returned to normal, usually after a delay of 2–5 sec. No changes in action potential amplitude were observed during polarization.

Simultaneous recordings from 2 fibers of widely different conduction velocities showed that, within the A–A-delta range, the temporal order of blocking was not strictly a function of initial conduction velocity (Fig. 3). In 4 of the 6 instances in which such recordings were obtained, the fiber conducting in the A-delta range was the first to be blocked.
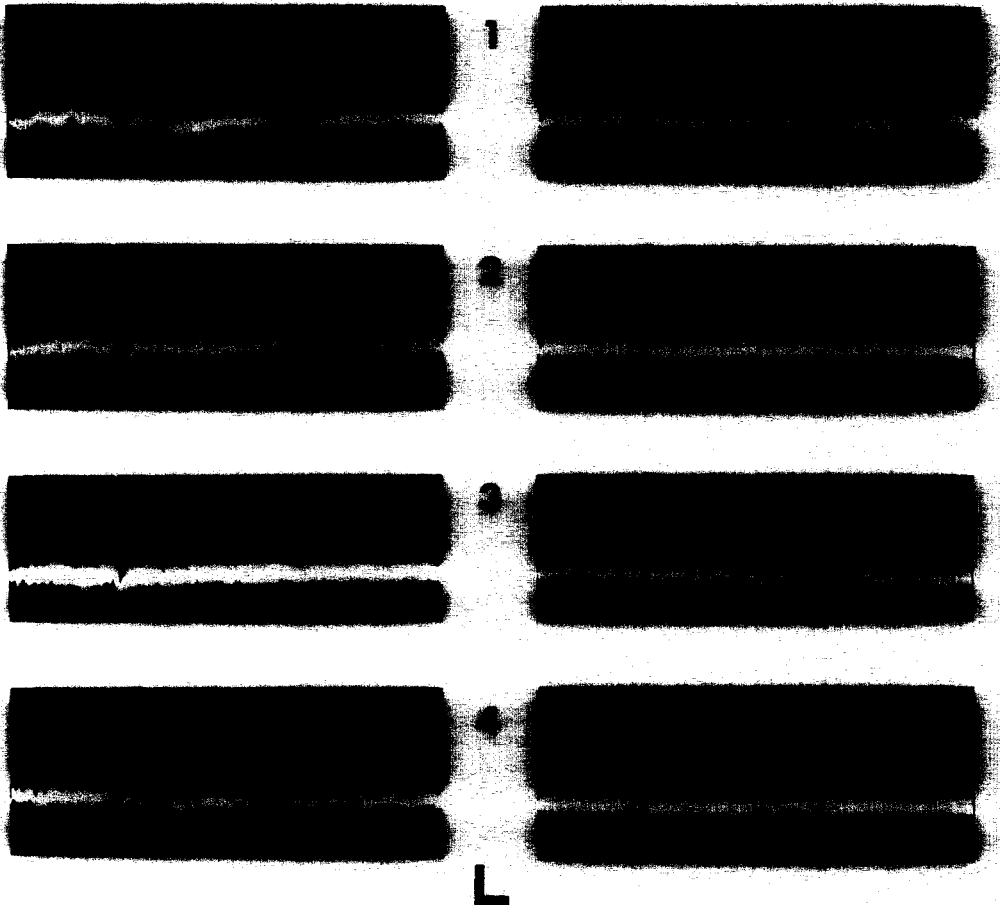

Fig. 3. Simultaneous recording of small and large fibers, showing blocking sequence. Sural nerve. Calibration: 50 μV, 2 msec. 1, before polarization; 2, 100 μA; 3, 200 μA; 4, immediately after polarization. On left, large fiber (35 m/sec) blocked before small fiber (8.7 m/sec). On right, small fiber (9.6 m/sec) blocked before large (32.6 m/sec).

Six experiments were performed with the cutaneous nerve split for recording the compound action potential from one strand and single fibers from the other while polarizing the common trunk. Single fibers conducting above 30.2 m/sec were always blocked before the large compound A wave disappeared, but 4 of the 9 fibers conducting below 24.2 m/sec continued conducting, at slower velocities, even though all trace of the compound A-delta potential had been eliminated by anodal polarization of the common strand (Fig. 4).
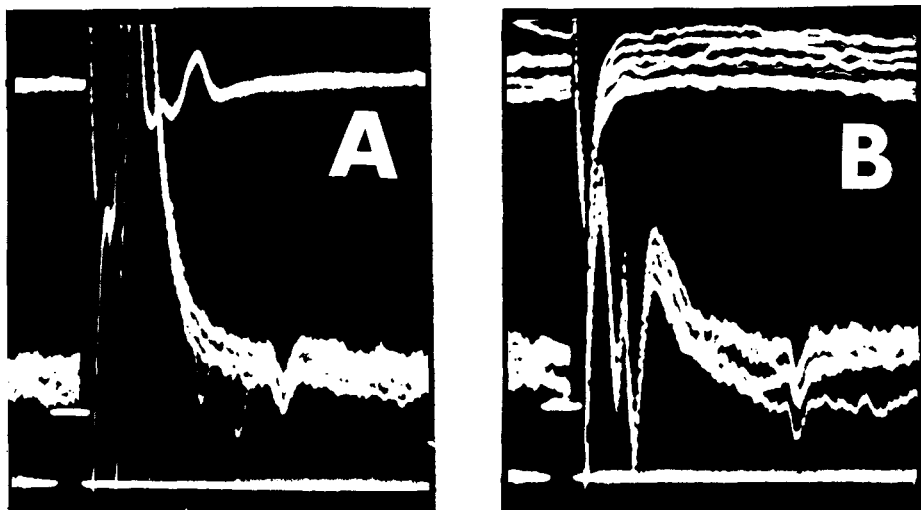

Fig. 4. Simultaneous recording of compound potential and single fiber. Superficial radial nerve strand split for recording the A-delta compound potential (top traces) and single fiber (middle traces). Lower trace shows calibration: 100 μV, 1 msec. A, Conduction velocity of A-delta wave peak is 17 m/sec; single fiber conducts at 21.2 m/sec. B, After applying 260 μA polarization to the common strand, the compound potential has disappeared; the single fiber continues conducting at the reduced velocity of 19.2 m/sec. Several sweeps are recorded in each trace.

A comparison of velocity decreases of 21 fibers with different initial conduction velocities is shown in Fig. 5. Although the accuracy of such a comparison is limited by the possible errors in measuring the latency shift of the faster-conducting fibers, the correlation ($r = 0.76$) between initial velocity and change in velocity indicates that the magnitude of velocity decrease is related to fiber diameter. The mean conduction velocity decrease of these fibers is 10.54 ± 5.13 (S.D.) % of the initial velocity (99% confidence limits of μ: 6.39–14.69%).

Computer analysis of changes in the compound action potential

The decreased amplitudes and increased latencies of the A and A-delta compound potentials might be attributed to non-preferential blocking of different sized fibers or to increased temporal dispersion of the volleys resulting from the decreased conduction velocity. While single fiber recording reveals that both phenomena occur during polarization, it is not apparent which of these factors contributes most effec-

tively to the amplitude changes in the compound potentials. Moreover, there is no indication of the percentage of A-delta fibers which might continue conducting in the absence of a compound potential. Finally, the experimental data did not show amplitude increases in the faster conducting single fibers which might explain the increase in A wave amplitude during the initial stages of polarization. We wondered if this latter phenomenon could be explained by an early shift in the conduction velocity of the largest fibers before conduction block was established.
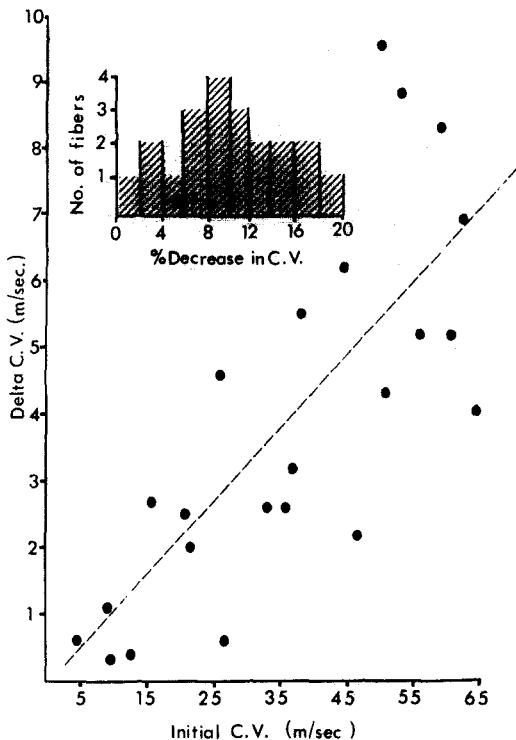

Fig. 5. Change in conduction velocity (delta C.V.) of different-sized fibers. Dashed line is the regression line. Correlation coefficient r = 0.76. Insert shows distribution of percentage changes in conduction velocity. Mean % decrease in C.V. is 10.54 ± 5.13 (S.D.) % of initial velocity.

Accordingly, the compound action potential was modeled on a digital computer, using a modification of the method of Gasser and Grundfest[1]. The model is limited to the A and A-delta fibers and assumes that, in whole-nerve recording, each fiber action potential contributes to the compound potential a triangle of 0.4 msec duration with an amplitude proportional to the product of fiber diameter and number of fibers. At a given conduction distance, the position of each unit triangle was established by assuming that a conduction velocity constant of 8.8 m/sec · μ relates conduction velocity to axon diameter[1,2]. The compound potential was generated by algebraic summation of the unit triangles at each calculated position, using the histological data of Gasser and Grundfest[1] (Fig. 5) for the saphenous nerve of cat. The results of the computation are shown in Fig. 6A.

The computed effect of volley dispersion alone was tested by decreasing the conduction velocity of each fiber by a percentage of its initial conduction velocity, as suggested by the experimental observations. The maximum velocity change tested
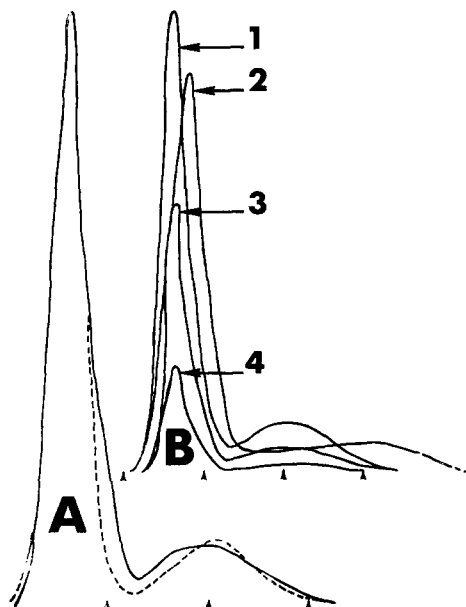

Fig. 6. Computer simulation of compound action potential. A, Simulated potential (solid line) compared with potential recorded from superficial radial nerve strand at 40 mm conduction distance (dashed line). B, 1,3, 4 are simulated potentials with 100%, 59.0% and 25.3% of the original fiber population conducting at normal velocity. In 2, all fibers are conducting, but the conduction velocity of each fiber has been decreased by 19.3% of the original velocity. Conduction distance, 40 mm. Time marks, 1 msec. Hand tracings of plotted digital output.

(19.3%) is slightly more than the maximum percent decrease observed experimentally (19.0%). To achieve this shift, the conduction velocity constant was decreased from 8.8 to 7.1 m/sec · μ. At a conduction distance of 40 mm, for example, this shift would produce latency increases of 0.1 and 1.0 msec in fibers with initial conduction velocities of 60 and 9 m/sec, respectively. The computed effect of blocking alone was accomplished by successively and uniformly dropping 10% of the remaining fiber population from each computation.

The results show (Fig. 6B) that neither velocity decrease nor blocking alone produced the series of changes observed experimentally. While uniform blocking of both large and small fibers does lead to an early loss of the A-delta wave, the latency increase is absent; decreasing the velocities as a percentage of initial velocity, within the experimental range, has very little effect on wave amplitude but does yield latency shifts. As expected, neither procedure leads to an early increase in A wave amplitude.

A better approximation to the experimental results was obtained by assuming that the largest fibers were the first to undergo velocity decreases during polarization; the resulting synchronization of the initial volley would then be expected to increase
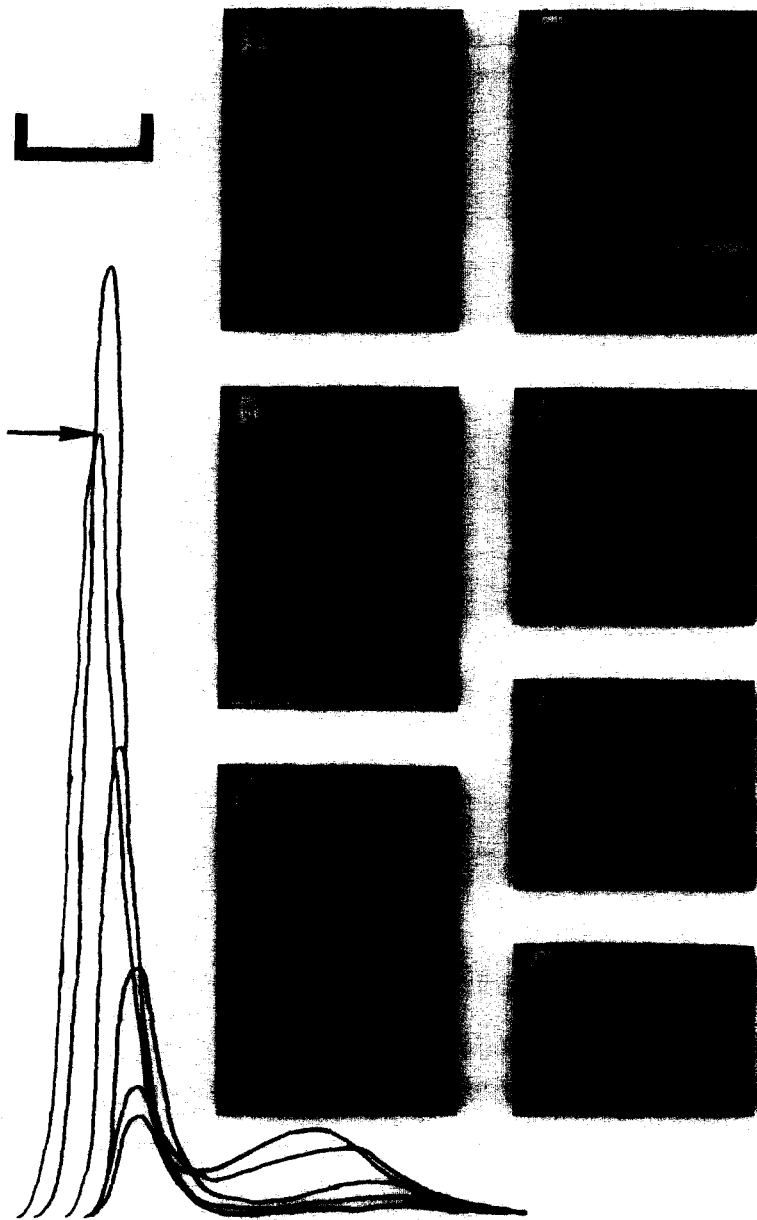
Fig. 7. Simulated and recorded changes in compound potential during polarization. Arrow (on left) points to normal simulated potential (40 mm conduction distance). Increased amplitude of initial A wave (to 120% of control) simulated by a 17% decrease in the conduction velocity of all fibers with outside diameters greater than or equal to 8.5 μ. In the 4 subsequent computations, 10% of the remaining population was 'blocked' on each run and the conduction velocity of all fibers progressively decreased to 19.3% of original. A–G: A, control record from superficial radial nerve strand at 38 mm conduction distance; B–G, taken at 2 sec intervals with 300 μA polarization. Time calibrations, 1 msec. Note the possibility of confusing the small, delayed initial A wave in G with an isolated A-delta wave.

the A wave amplitude. The program called for a decrease in the conduction velocity constant (from 8.8 to 7.3 m/sec · μ) for each group of fibers, starting with the largest-diameter axons, until the resulting synchronization produced a 20% increase in A wave amplitude. Thereafter, these largest fibers were 'blocked' and both the number and conduction velocity constant of all the remaining fibers were decreased in steps as
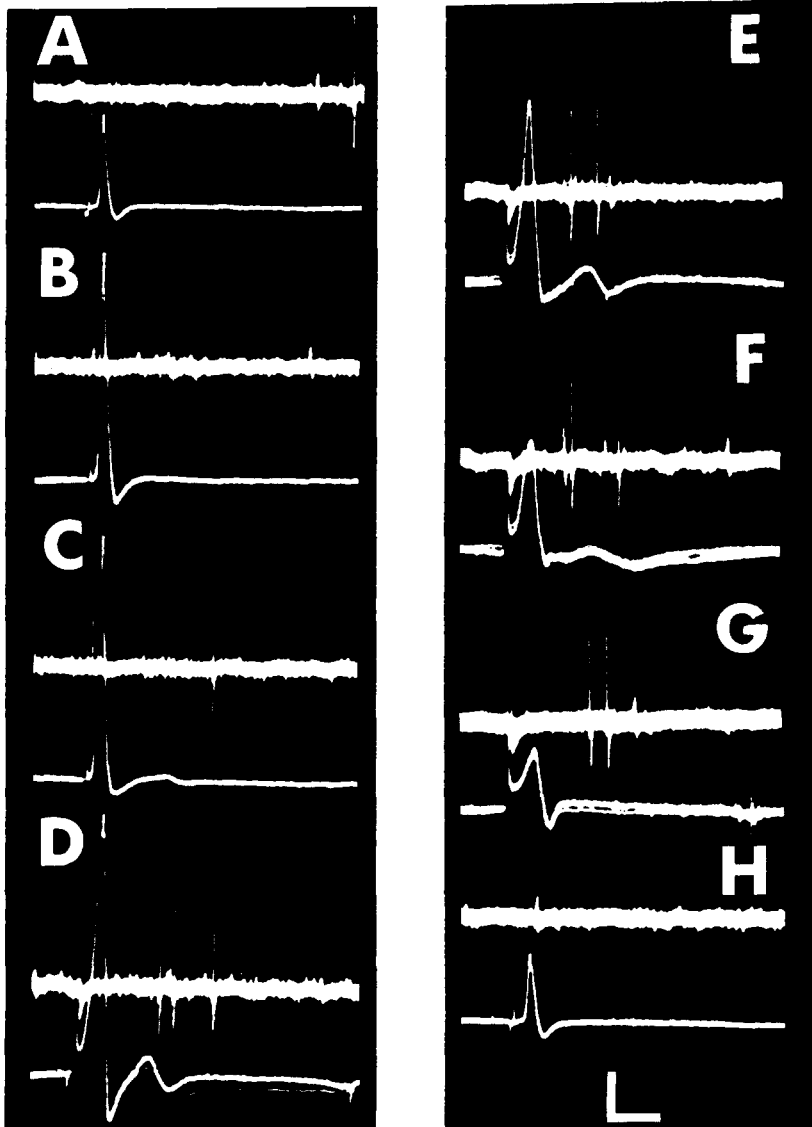

Fig. 8. Central effects of polarized nerve volley. Top traces, unit recorded from medial medullary reticular formation. Bottom traces, diphasic record from superficial radial nerve strand. Calibration, 200 μV, 20 msec (top traces) and 2 msec (bottom traces). A–D, Progressive increase in stimulus strength evokes unit discharge as A-delta wave appears. E–G, With stimulus as in D, progressive increase in polarization (to 300 μA) eliminates A-delta wave and reduces initial A wave amplitude; unit continues to respond. H, Immediately after polarization, small or large (as in B) initial A wave fails to evoke unit activity.

in the previous computations. A similar series of events could be responsible for the experimentally observed phenomena (Fig. 7). The computed increased A wave amplitude resulted from a 17 % decrease in the conduction velocity of all fibers with outside diameters greater than or equal to 8.5 μ. When the A-delta wave has essentially disappeared, a fiber count on the final computation, corresponding to G of Fig. 7, shows that 25.2 % of the original A-delta and 24.3 % of the A fiber population is still conducting. Thus, it is possible that over 20 % of the A-delta population continues conducting even though the compound potential fails to indicate activity in that part of the fiber spectrum.

Central effects of the polarized volley

To determine the central effect of an afferent volley in which polarization had eliminated the A-delta wave, unit activity was recorded from the medial medullary reticular formation of decerebrate cats in which the cerebellum had been removed and the dorsal columns sectioned between C1 and C2. Work now in progress (Casey, unpublished observations) has revealed, in the region of nucleus gigantccellularis, a neural population which responds only to afferent volleys containing A-delta activity. In these experiments, the nerve volley was recorded by the less sensitive diphasic method. The recording and stimulating electrodes were, however, never separated by more than 35 mm, thus limiting the dispersion of the A-delta volley at the recording electrodes. The sensitivity of this recording procedure is usually sufficient to detect the effective A-delta volley since many of the medullary reticular units studied thus far are driven only when the A-delta wave is seen in the diphasic record.

Fig. 8 shows an example of this phenomenon and the result of increasing polarization until the A-delta wave was eliminated. Although this cell failed to respond to a full A-fiber volley below A-delta threshold, a consistent response was obtained above A-delta but below C fiber threshold after polarization had apparently eliminated all A-delta activity and reduced A wave amplitude to a fraction of its maximum value. Post-stimulus histograms of the response of another neuron during polarization are given in Fig. 9. Apparently, the compound action potential during polarization does not reveal the A-delta component needed to excite these central cells; the hidden unblocked portion of the A-delta population is capable of exerting a detectable physiological influence.

DISCUSSION

The results show that a physiologically significant proportion of small myelinated fibers may continue conducting after anodal polarization has eliminated the A-delta compound potential. Single fiber recording and computer simulation suggests that, within the A and A-delta range, anodal block of different sized fibers at the same level of applied polarization is the major factor responsible for the order of disappearance of these waves. Although decreases in conduction velocity often precede the onset of block, the magnitude of velocity shift and the results of computer simulation argue

against the hypothesis that volley dispersion plays the major role in the early disappearance of the A-delta component. Since an increase in the amplitude of individual action potentials was not observed during polarization, the increased amplitude of the large A wave appears to be best explained as a decrease in the conduction velocities of the largest fibers during the initial stages of polarization. Computer simulation of this event, at any rate, closely approximates the experimental observation and is consistent with the theoretical expectation that the largest fibers would be the first to be affected by polarizing current.
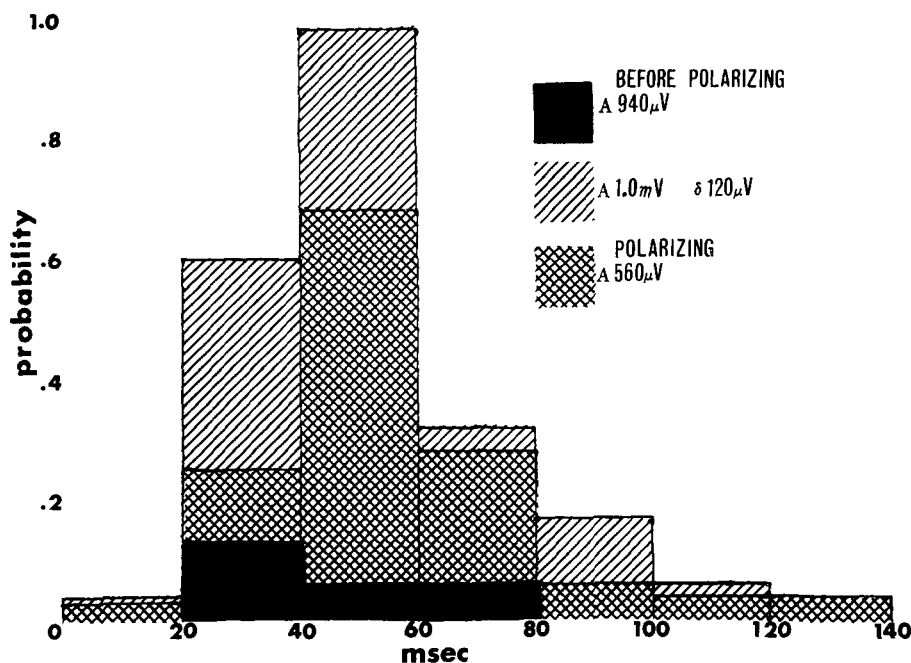

Fig. 9. Post-stimulus histograms of medullary reticular formation unit. Sural nerve stimulation. Amplitude of A and A-delta waves are shown before (solid black and hash marks) and during (cross hatch) 150 μA polarization. Note that the small amplitude A volley during polarization is more effective than the larger amplitude volley before polarization.

The electrophysiological basis for some of the phenomena observed during anodal polarization is not clear. Theoretically, blocking hyperpolarization should occur first across the nodes of Ranvier of larger fibers since the amount of current flow through a fiber is determined, in part, by the square of fiber diameter. Since this selectivity does not always appear to hold within the A and A-delta range, there are undoubtedly other critical factors, such as the distribution of current flow through the nerve, the position of fibers with respect to the polarizing electrodes, and the number of nodes through which current flows. Thus, using Hursh's[2] data on internodal distances, a 5 μ diameter fiber might have 8 nodes lying over the 4 mm polarizing anode while a fiber of 10 μ diameter would have half this number. For nodes with similar resistance to transmembrane current flow, this would tend to offset the usual relation-

ship between intrafiber current flow and fiber diameter. A more accurate prediction of the effect of this variable will require more information about the relationship between node area, resistance, and fiber diameter.

Several factors may also contribute to the rather sizable decreases in conduction velocity prior to block. If a 0.10 msec delay, for example, takes place entirely within the 10 mm polarization zone, then a fiber with a 60 m/sec conduction velocity will have a 38% decrease in velocity through the polarized region. The conduction delay resulting from block at one or more nodes will depend on the electrotonically distributed potential from the nearest active node and the effect of the polarizing current on ion flow, ion distribution, and membrane properties. At the higher polarizing currents, it is possible that current flow through one or two nodes of a fiber could develop transmembrane potentials sufficient to cause dielectric breakdown of the membrane. The observation that an increased latency often persists for several seconds after polarization is stopped may be attributable to electrode polarization or to relatively long-term changes in membrane properties and/or ion distribution within the nerve.

This investigation has been concerned principally with the limitations of anodal polarization as applied to large and small myelinated fibers. An apparently pure C fiber volley might contain few, if any, active large myelinated fibers since fibers with conduction velocities in this range were always blocked before the compound potential was eliminated. In view of the results reported here, however, one could not be certain that a significant number of small myelinated fibers were not active. Since some central cells appear to be especially responsive to this portion of the afferent spectrum, the possibility of a hidden A-delta population should be considered when studying the central effects of polarized nerve volleys.

SUMMARY

A number of findings indicate that a physiologically significant number of small myelinated fibers may continue conducting although the compound A-delta wave has been eliminated by anodal polarization. As anodal polarization was applied to cat cutaneous nerve, the initial A (alpha-beta) wave showed a transient increase in amplitude; the A-delta wave was usually the first to disappear. Both A and A-delta waves showed decreases in conduction velocity prior to block. Single fiber recording revealed that: (1) the amount of velocity decrease is positively correlated with fiber diameter, (2) blocking sequence is not strictly a function of fiber diameter, and (3) single A-delta fibers can be recorded from one strand of a split nerve after the compound A-delta wave of the adjacent strand has been eliminated by polarization of the common trunk.

Computer simulation of the compound potential indicates that blocking of different-sized fibers at the same level of applied polarization is the major factor leading to early elimination of the A-delta wave. Simulation of compound potential changes revealed that over 20% of the A-delta population may continue conducting after loss of the A-delta wave. The physiological effectiveness of a 'hidden' A-delta volley was revealed by recording medullary reticular formation units which were

excited only by volleys containing active A-delta fibers. These results appear relevant to the interpretation of the central effects of polarized nerve volleys.

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