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Differential Margin of Safety of Conduction in Individual Peripheral Axons

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The relation between fiber size and safety of conduction was tested *in vitro* on individual afferent axons of rabbit vagus nerve by lowering the external sodium ion concentration and noting the effect on threshold excitability and conduction velocity. Conduction safety of myelinated axons was found to be independent of fiber size and slightly less than among unmyelinated axons. The results are consistent with previous data from the same model, where blocking concentration and diffusion time of lidocaine to the excitable membrane of individual axons also were independent of myelinated axonal size. The evidence from these single-unit studies implies that the differential blocks of functional modalities observed with spinal and epidural anesthesia probably do not arise from fiber size-related differences in susceptibility to block: possible alternatives are mentioned briefly. (Key words: Anesthetics, local. Ions: sodium. Nerve: differential conduction; differential transmission.

THE TRUE RELATION between axonal size and blocking concentration of local anesthetic is becoming clearer. Earlier ideas were largely based on studies of compound action potentials (CAPs),[1–4] where differential depression of the amplitude of different components had been taken as an indication of differential block. Reduction of the height of the CAP is a measure of the total action of blocking drugs but is subject to the limitation that it does not solely measure conduction block of fibers but may also reflect temporal dispersion without block; thus, ratings of sensitivity of different fiber sizes to local anesthetic block based on the criterion of CAP amplitude are all potentially unreliable and may be misleading. Study of individual fibers in a nerve avoids the factor of temporal dispersion. The potential importance of this factor became apparent when work with teased mammalian fibers[5] *in vivo* failed to find any evidence of absolute differential block among myelinated axons, a finding recently confirmed by work on individual undissected mammalian units *in vitro*.[6] Nevertheless, the idea of size-related differential block has persisted and is still offered as an explanation for clinical differential blocks associated with spinal and epidural anesthesia, where it has been attributed to differences in safety of axonal conduction.[3,7,8]

An axonal action potential produces a sodium current several times greater than the minimum required for active propagation. This excess is referred to as the margin of safety of conduction. Local anesthetic molecules, by occupying sodium channels, encroach on the excess and consequently slow conduction before blocking it. Lowering the external sodium concentration also slows and, if sufficiently extreme, blocks conduction; it was used here to evaluate, for the first time, size-related differences in the margin of safety of conduction on individual mammalian units by measuring the extent of conduction slowing and the incidence of inexcitability.

Method

The preparation consisted of the excised and desheathed inferior ganglion (ganglion nodosum) and cervical vagus nerve of rabbit.[6] The ganglion contains the cell bodies of the myelinated and unmyelinated afferents. The specimen was installed in a chamber comprising two compartments (fig. 1), each holding about 1 ml, separated by a petroleum jelly barrier and separately perfused at a rate of 5–10 $ml \cdot min^{-1}$. The purfusate of the recording chamber consisted of Ringer–bicarbonate–glucose[6] solution equilibrated with 5% CO_2–95% O_2 gas mixture at 37.5° C. The stimulating or nerve compartment was perfused independently with a similar solution, except during hyponatric exposure as specified below. Units were observed individually in the ganglion by means of an extracellular tungsten microelectrode. The nerve rested on two stimulating cathodes 10 mm apart in experiments with 48 mM sodium and 20 mm apart with lower concentrations, enabling precise measurement of the control conduction time (latency or reciprocal of conduction velocity) in a length of the axon of the unit under observation in the ganglion. The study was confined to axons having somata within 150 μm of the surface of the ganglion, in order to avoid the risk of environmental hypoxia of the cell body. The stability of the latency before and after hyponatric exposure, typified by figure 2, indicated that the cell was undamaged. The nerve, whose total length, excluding the ganglion, exceeded 45 mm, was installed so as

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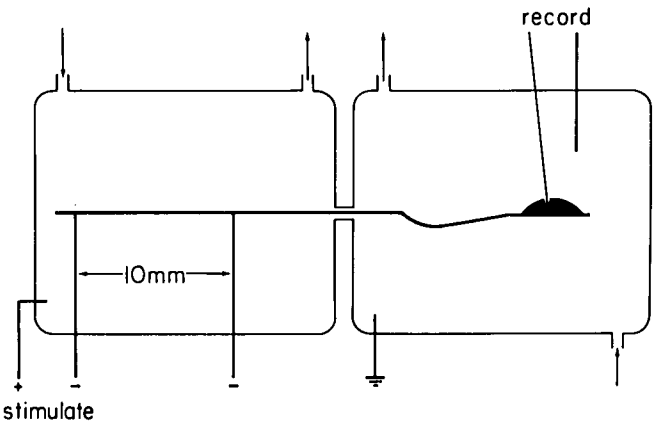

FIG. 1. Diagram of vagus nerve and ganglion in exposure chamber. The nerve was in contact with the bare tips of two insulated platinum wires, one or the other of which served as the stimulating cathode. In the experiments with 48 mM sodium, the intercathode distance was 10 mm, as shown; with other concentrations it was 20 mm.

to leave about 15 mm of nerve in the recording compartment and at least 10 mm from the cut end of the nerve to the nearest cathode; thus, the distance from the cut end to the stimulating electrode was more than five times the length constant of the largest fibers and meant that depolarization at the cut end had a negligible effect at the point of stimulation. The stimulus most likely became effective at the nearest node, at most 400 μm to the left or right of the site of the cathode for the largest fibers of the experiments; this factor produced a maximum total uncertainty of about 8% in the determinations of conduction velocity between the two cathodes, calculated from the intercathodal distance and the difference in conduction times to the ganglion. The uncertainty was less for small fibers and was half of the above in the experiments where the intercathodal distance was 20 mm.

The threshold stimulus strength was determined at each cathode before the beginning and at the end of each hyponatric protocol. The conduction time from the cathode was the time from the start of the shock artifact to the first inflection of the unit potential recorded in the ganglion, when the shock strength was 1.2 times threshold strength. After the determination of conduction time between the two cathodes, computerized threshold "hunting"[9] and measurement of conduction time was instituted with stimuli applied exclusively at the distal cathode, once every 6 s. Conduction time was measured from the distal cathode, first while perfusing the stimulation compartment with Ringer–bicarbonate–glucose solution[6] for at least 20 min, next for up to 60 min during perfusion with solution in which sodium chloride had been partly replaced by an equimolar amount of choline chloride, and finally again with the original solution, throughout at 37.5 ± 0.5 ° C and *p*H 7.35 ± 0.05. Only one low-sodium concentration, 64, 48, 32, 24, 20, or 16 mM, was studied per nerve. An exposure of 20 min is known to approximately suffice for endoneurial diffusional equilibrium in this preparation.[10,11]

The stimulus was held near threshold strength by the computer-controlled "hunt." The first shock applied to the nerve was a threshold strength current, usually 100 μs in duration but sometimes longer for unmyelinated axons. Thereafter the duration of the shock varied in steps of 5% of the control duration: if the ganglion cell responded, the duration of the shock decreased by one step; if the cell did not respond, the duration of the next shock increased by one step. The computer program calculated the fractional change in conduction velocity, with the assumption that control conduction velocity was uniform throughout the observed length of the axon and that the change in conduction time occurred exclusively in the portion between the cathode and the chamber partition. The conduction time and the shock duration measured in microseconds and the fractional change in conduction velocity were typed by the computer print-out; on-line recording was effected with an XY plotter. The ganglion responses were recorded once per minute on magnetic disc with a Nicolet 4094® digital storage oscilloscope.

Statistics

The statistical significance of differences between myelinated and unmyelinated axons was evaluated by unpaired *t* test, and differences between the effects of the various sodium concentrations were evaluated by chi-square test; $P < 0.05$ was regarded as probably significant.

Results

In 48 mM sodium, during exposures lasting 40 or 60 min, all the units examined remained excitable. Figure 2*A* illustrates the time course of latency and threshold changes in a myelinated unit of control conduction velocity 12.6 $m \cdot s^{-1}$. On exposure to the hyponatric solution, the conduction time, which had been stable, increased in two phases. In the first phase, the conduction time increased asymptotically and the threshold increased so rapidly that the programmed rate of increase of stimulus strength sometimes was temporarily insufficient to achieve excitation, as shown by gaps in the ascending portion of the record of conduction time. Within a few minutes this phase began to merge into the second phase, throughout which there was a slight upward drift of the conduction time and threshold remained elevated

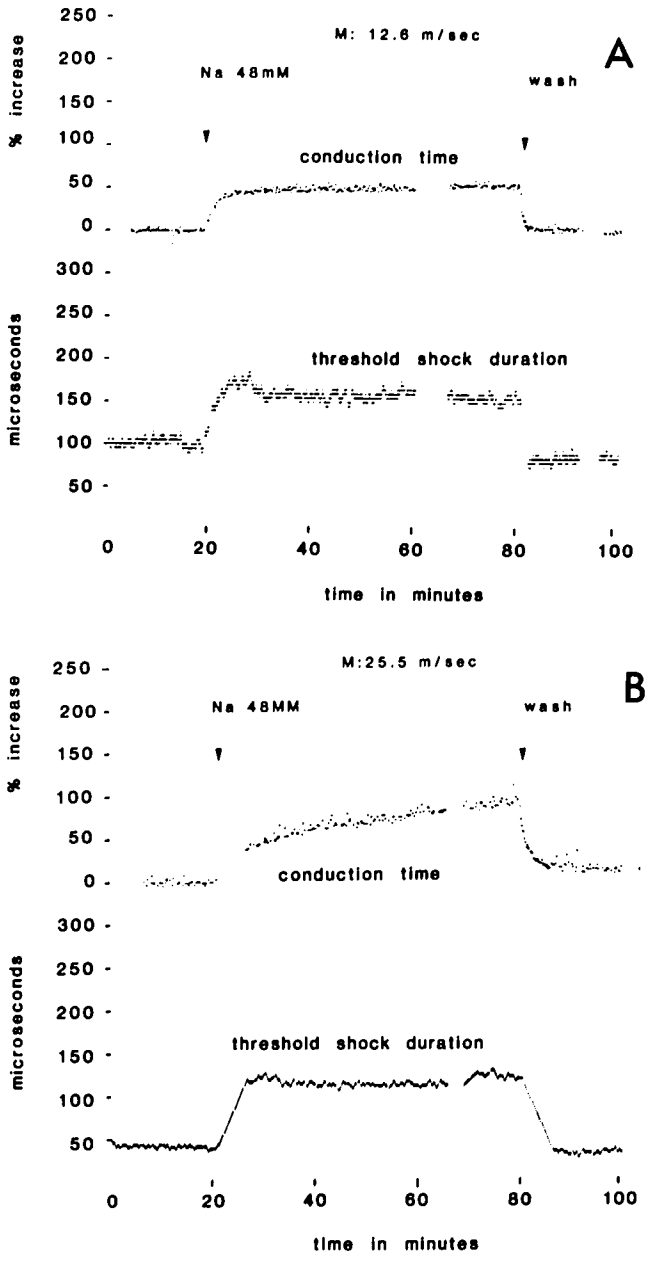

FIG. 2. Records of computer-controlled threshold "hunt" on two myelinated units. The conduction velocity of the units was measured before the beginning and after the end of the hunt, from the difference in conduction times of stimuli 1.2 times threshold strength, applied successively at the distal and proximal cathodes. Conduction time typically increased less than 5% between the start and end of the observations. During the hunt, stimulation took place throughout from the cathode farthest from the ganglion. The hunt program decreased or increased the duration of successive stimulating shocks by a fixed amount, according to whether the preceding shock excited or failed to excite the soma observed in the ganglion. The duration of successive shocks is indicated by the dots in the lower trace of each record; the upper trace is composed of dots that record the conduction time whenever a stimulus was successful in exciting a ganglionic response, expressed as per cent increase over the control conduction time. At the first arrow the nerve compartment perfusate was changed from control to hyponatric isotonic solution. The second arrow indicates the time when washing with control solution was begun. The gaps in the records are interruptions of the hunt made for the purpose of measuring the conduction velocity between the two cathodes, for comparison with the value determined immediately preceding the onset of the hunt; these values consistently agreed with the adjoining hunt values. *A.* Myelinated unit, control conduction velocity 12.6 $m \cdot s^{-1}$. *B.* Myelinated unit, control conduction velocity 25.5 $m \cdot s^{-1}$.

but approximately steady. Washing led to a rapid return toward control values. Figure 2*B* shows the corresponding record for a myelinated axon in which the control conduction velocity was 25.5 $m \cdot s^{-1}$. In this case, too, the threshold remained stable at the elevated value, notwithstanding a relatively rapid increase of conduction time during the second phase of the hyponatric effect.

A total of 11 myelinated units were tested with 48 mM sodium. The fractional decrease of conduction velocity at 20 min of exposure in the 11 units is given in figure 3. It averaged 47 ± 15% (±SD, n = 11), or, excluding the most outlying value, 51 ± 9% (n = 10); the control conduction velocity of the axons ranged from 7.8 to 34.7 $m \cdot s^{-1}$. The correlation coefficient between fractional conduction velocity decrease and control conduction velocity was 0.36 (n = 11), which was not significant ($P > 0.1$); exclusion of the outlying value decreased the correlation coefficient to 0.18. Of 10 other myelinated axons, tested in 24 mM sodium for 20 min, four remained excitable and underwent a fractional conduction velocity decrease of 83 ± 19%, which was significantly more than the decrease in 48 mM sodium ($P < 0.002$).

Tests on 12 unmyelinated axons with 48 mM sodium produced changes qualitatively similar to those on myelinated units, but the increase in threshold was less marked and the fractional decrease in conduction velocity, 35 ± 16%, significantly smaller ($P < 0.01$, df = 21).

The incidence of inexcitability in individual units during exposure to low-sodium concentrations ranging from 64 to 16 mM is summarized in figure 4. Inexcitability, if it occurred, was always reversible. In 64 and 48 mM sodium, none of the observed units became inexcitable. In 32, 24, and 20 mM sodium the incidence of inexcitability was somewhat higher among myelinated than among unmyelinated units; however, the differences did not reach statistical significance by chi-square test ($P > 0.05$). In 16 mM sodium, all units became reversibly inexcitable; the time to onset of inexcitability ranged from 1 to 17 min for myelinated axons (mean = 6 ± 5 min, n = 9) and from 1 to 13 min for unmyelinated axons (mean = 6 ± 4 min, n = 9) and did not correlate with control conduction velocity.

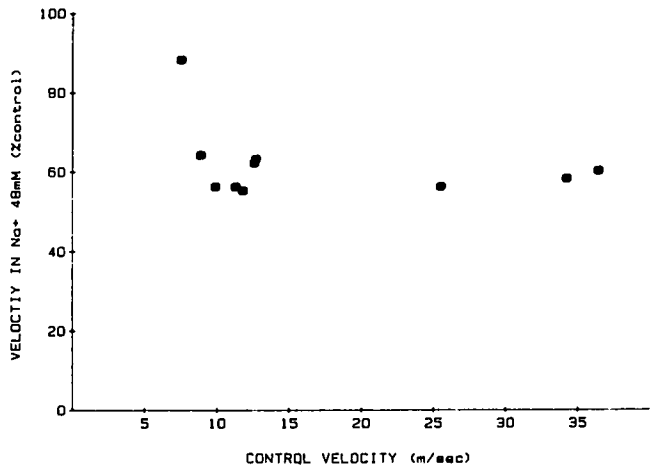

FIG. 3. Change in conduction velocity in 11 myelinated units after 20 min in a solution containing 48 mM sodium, expressed as per cent decrease from control conduction velocity and plotted as a function of the control conduction velocity.

Discussion

In the experiments where the units remained excitable in low extracellular sodium (figs. 2 and 3), the initial, nonlinear phase of the slowing of conduction was probably due to the decrease in sodium current across excited membrane in the nerve compartment.[12] Conduction was slowed because the decreased current decreased the rate of depolarization of the axonal membrane. The extent of the slowing in any given hyponatric environment depended importantly on the margin of safety of conduction: the greater the margin, the less the fractional decrease in sodium current and the less the fractional decrease in conduction velocity or fractional increase in conduction time. The percentage change in conduction time in 48 mM (fig. 3), in which none of the fibers became inexcitable, provided a basis for comparing the margin of conduction safety in axons of different conduction velocities (or different fiber sizes; for a discussion of the relation between conduction velocity and fiber size, see Brinley[13]); the data suggested that there was no significant correlation between myelinated fiber size and margin of safety of conduction.

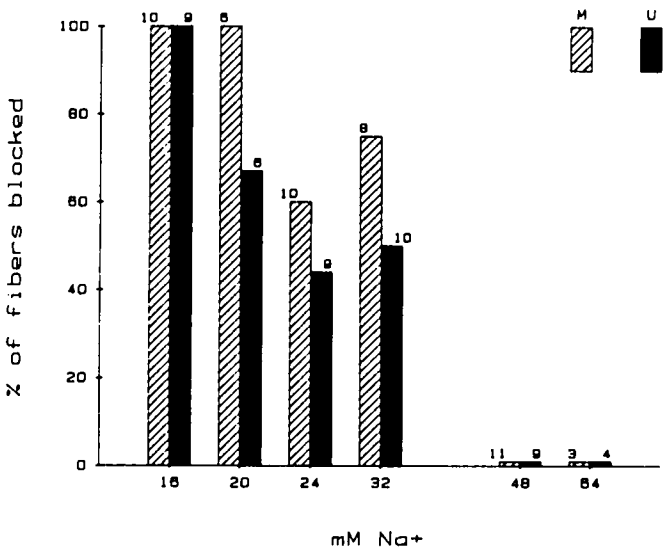

FIG. 4. Histogram showing the incidence of conduction block in myelinated (hatched bars) and unmyelinated (solid bars) units during a less than 20 min exposure to an isotonic modified Ringer's solution containing 64, 48, 32, 24, 20, or 16 mM sodium ion. The numbers above the bars indicate the numbers of units tested. Only one sodium concentration and one unit were tested per preparation.

In external sodium concentrations less than 48 mM, axons that became inexcitable did so in 1–17 min. Although this time interval again appeared to be independent of fiber size, in this case the independence may have reflected a fortuitous variation in the endoneurial diffusion distance to the axon from the surface of the nerve. This factor did not operate in the evaluation of margin of safety discussed in the previous paragraph, where the measurements were made at equilibrium.

The incidence of inexcitability in 32, 24, and 20 mM (fig. 4) sodium was approximately 50% greater among myelinated than among unmyelinated axons, *i.e.*, the ratio of extinctions in the two groups was about 3:2. The approximate minimum ambient sodium ion concentration for preserving excitability was a little greater for myelinated axons than for unmyelinated axons: 24 mM *versus* 20 mM. It is interesting to note that the above-mentioned 3:2 ratio of extinctions was approximately the reciprocal of the ratio between the average blocking concentrations of lidocaine in the two groups, determined on individual fibers, as previously reported,[6] where the mean blocking concentration for myelinated axons was 0.43 mM lidocaine, as compared with 0.63 mM for unmyelinated axons. This reciprocity is consistent with the known mechanism of action of lidocaine, which essentially decreases the sodium current by blocking the sodium channels. Both ratios tend to support the conclusion that the margin of conduction safety in myelinated axons is a little smaller than in unmyelinated axons. Teleologically it could make sense for saltatory conduction to pay a little safety of conduction in return for increased speed.

The mechanisms underlying the phases of hyponatric increase in conduction time can only be speculated upon. The curvilinear first phase (fig. 2) may depend mainly on outward diffusion of sodium from the periaxonal endoneurium, causing a first-order decrease in the transmembrane sodium concentration gradient. The steadily rising second phase represents a new observation, and its nature is quite unknown; it has not been seen with local anesthetic (unpublished observations with

lidocaine hydrochloride). It occurred without any accompanying change in threshold, which might, for example, imply that the membrane potential remained stable while the number of activatable sodium channels steadily decreased. Several other possibilities, however, including an effect on the membrane Na^+–K^+-dependent ATPase, cannot be excluded on the basis of the present data.

Some workers with compound potentials have speculated that differential block arises at the node of Ranvier because of a diffusional barrier in the nodal gap: the larger the fiber the deeper the gap and the greater the hypothesized diffusional barrier.[14] However, recently published observations on the rate of onset of block in individual axons[11] have failed to substantiate that hypothesis: no size-related difference in the time of onset of block was detected. This, together with the minor size-related variation in lidocaine blocking concentration[6] or size-related variation in margin of conduction safety evidenced in the present experiments argues against any substantial size-related differential block intrinsic to nerve fibers and suggests that the differentials observed clinically may be mainly due to other factors, such as use-dependent block[15,16] or localized differences in blood flow.

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