A Preferential Inhibition of Impulses in C-fibers of the Rabbit Vagus Nerve by Veratridine, an Activator of Sodium Channels

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In seeking a means to reverse local anesthetic block of peripheral nerve, we examined the actions of veratridine (VTD), an agent known to antagonize competitively the binding of local anesthetics to Na channels. The actions of VTD, a steroidal alkaloid “activator” of voltage-gated Na channels, were studied in the rabbit vagus nerve by two methods. In one, the effects of VTD on compound action potentials (APc) propagating through a “veratrinized” segment (11-12 mm) of nerve were measured by extracellular recording. Single volley of impulses were unaffected by VTD, but trains of impulses, triggered by repetitive stimulation, were selectively diminished. This “use-dependent” reduction was greatest for the C-fiber component of the APc, less for B-fibers, and inconsequential for A-fibers. Use-dependent inhibition was enhanced by higher stimulation frequency and by increased VTD concentration, and reversed rapidly when stimulation ceased. If the nerve sheath remained intact, the rate of VTD action was far less than in desheathed nerves, but the effects were the same. In the other experimental system, membrane potentials were measured in the veratrinized region of the nerve by a sucrose-gap method. Repetitive stimulation, particularly of C-fibers, produced a cumulative VTD-induced depolarization (VID) that was sustained over several seconds and during which the C-fiber APc was selectively reduced. We propose that this local, use-dependent VID provides the means to inhibit impulses propagating through the veratrinized region. The preferential effect of VTD on C-fibers suggests its possibilities as a relatively selective agent for block of impulse trains in nociceptive afferents. (Key words: Nerve, C-fibers: differential block; sodium channel. Anesthesiology: local. Pharmacology: veratridine.)

Among the desirable attributes of an ideal local anesthetic is specificity for certain neural modalities—such as a selective abolition of pain—with little compromise of other sensations or of motor capacity. The ability to reverse local anesthesia rapidly also would be desirable in many clinical situations.

In an attempt to find an effective reversal agent, we began to examine the ability of a classic “activator” of the voltage-gated Na channel, veratridine (VTD),1 to relieve the impulse inhibition due to the local anesthetic bupivacaine. VTD binds to and selectively stabilizes an open conformation of the Na channel,2,3 leading to a persistent increase in Na+ permeability4 and a concomitant membrane depolarization.5 VTD binding and the associated depolarization are antagonized competitively by local anesthetics5,6 and in principle, local anesthetic binding should be antagonized by VTD.

We observed, however, that the addition of VTD to a nerve during recovery from bupivacaine block potentiated rather than antagonized the residual block. Furthermore, VTD alone resulted in impulse inhibition that was highly preferential for C-fibers, in a “use-dependent” manner. In seeking to control duration with a drug, we serendipitously revealed its selectivity.

This paper reports the remarkably selective effects of VTD on the compound action potential (APc) of a mammalian peripheral nerve: the results demonstrate the dependence of inhibition on impulse frequency over a range of drug concentrations in the APc elevations of A-, B- and C-fibers. It also reports the action of VTD on impulses in a nerve with an intact sheath: since the sheath is somewhat permeable to the drug, the drug is shown to be a potentially useful local anesthetic. Also demonstrated, by the sucrose-gap method of examination of the compound (average) resting potential of the nerve, is that local depolarization of the nerve in the region exposed to VTD provides the mechanism for this selective inhibition.

Materials and Methods

Animals used in this study were maintained and treated in accordance with the guidelines of the Committee on Animals of the Harvard Medical School.

Adult male New Zealand white rabbits (3 ± 0.5 kg) were pretreated intramuscularly with a mixture of ketamine (200 mg), xylazine (20 mg), and atropine (0.4 mg) for sedation. Ten minutes after this treatment, the animals

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were anesthetized with diethyl ether. After cessation of respiration, a longitudinal skin incision was made, and the cervical portion of both vagus nerves was dissected. The vagus nerves were temporarly stored at room temperature (22°C) in a beaker containing modified HEPES Liley (HL) solution stirred constantly by aeration with a mixture of 95% oxygen and 5% carbon dioxide.

The modified HL solution used for bathing the nerve and subsequent drug perfusion consisted of (in mM): NaCl, 118.0; KCl, 5.0; CaCl₂ · 2H₂O, 2.0; MgCl₂ · 6H₂O, 1.0; α-D(+) -glucose, 11.0; NaHCO₃, 25.0; and N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) buffer, 5.0 mM. The pH was adjusted to 7.4 ± 0.05 by addition of 1 N NaOH. The perfusion solution was bubbled with a gas mixture of 95% oxygen and 5% carbon dioxide at a vigorous rate just before flowing onto the nerve. The pH was stabilized by the HEPES buffer and maintained at 7.4.

VTD free base (no. V-5754, lot 58F-7828, Sigma, St. Louis, MO) was stored in a desiccator below 0°C and was used to prepare a 2 mM stock solution in dimethyl sulfoxide [DMSO], Fisher Scientific, lot 861316), which also was stored at below 0°C. VTD, a steroidal alkaloid (fig. 1A) that is produced by certain lilies, has a solubility limited to approximately 10⁻¹⁰ M in aqueous salt solutions. The stock VTD solution was diluted to final concentrations of 0.2–10 μM by the addition of HL. The maximum equivalent content of DMSO is 0.5%, and in three control experiments we detected no change in electrophysiologic behavior with 1% DMSO.

EXTRACELLULAR RECORDING

The nerves were cleaned and desheathed by microdissection before mounting in an airtight chamber (fig. 1B), Petroleum jelly (White Petroleumum, Denison Laboratories).

FIG. 1. (A) The structure of veratridine. (B) Cross-sectional view of the extracellular recording chamber, showing the lateral stimulating and recording chambers and the central, grounded "test" pool through which veratridine was perfused. The petroleum jelly seals are shown by cross-hatching. (C) Cross-section view of the sucrose-gap chamber, showing the separate stimulus pools, the "test" pool for drug perfusion, and the "distal" pool containing the cut end of the nerve bathed in HEPES–Liley solution. A central gap of 3 mm provided a conduit for the continuous perfusion of an intermediate segment of the nerve by isotonic sucrose.
seals separated a central, 11-mm length of the nerve from the lateral pools, used for stimulating and recording with electrodes made of platinum wire. The central section of the chamber (volume 0.25 ml) was perfused with HL solution at 0.5 ml · min⁻¹. The lateral pools containing the moistened ends of the nerves adsorbed to the electrodes were filled with paraffin oil. The A_Pc were propagated over 21 mm, from the stimulating to the recording electrodes, with the proximal recording electrode located 4.5 mm from the edge of the central, drug exposure chamber. Thus, measured A_Pc were conducted in nerve exposed to drug for half of the conduction pathway, and had been conducting through drug-free medium over a distance containing at least two or three nodes of Ranvier (in the fastest-conducting fibers), before reaching the recording electrodes.

The stimulus intensities and durations, supplied by Grass S-88 stimulators (Grass Instrument, Braintree, MA), were adjusted to produce the appropriate maximum A_Pc as displayed on storage oscilloscopes (3–12 V for 0.05 ms, for A-fibers; 6–14 V for 0.1 ms, for B-fibers; and 8–24 V for 1.0 ms, for C-fibers) (model 5113, Tektronix, Beaverton, OR). The signal from the nerve was amplified 50 times (model AK 475 operational amplifier, Metametrics, Carlisle, MA) and passed to the oscilloscope preamplifiers (model 5A 22N, Tektronix). The preparation was not considered acceptable unless the A_Pc signals from the nerve exceeded 1.5 mV for A-fibers, 0.5 mV for B-fibers, and 0.3 mV for C-fibers.

Each nerve was exposed to only one VTD concentration, and each concentration was tested in four or five separate nerves, except in the sheathed nerves, for which only two nerves were used. The temperature was 20–22° C.

CONTROL RECORDING

In a series of control experiments, nerves were continuously perfused with only HL solution for a total period of 60 min (four nerves) or 175 min (one nerve). After 60 min in the control solution, a time corresponding to a 35-min exposure to VTD (see fig. 3 top), the different A_Pc components had the following values relative to the amplitudes at 25 min ("zero" time in fig. 9): A-fibers, 118 ± 13%, n = 4; B-fibers, 126 ± 19%, n = 4; C-fibers, 96.5 ± 7.9%, n = 4. In the one control nerve continuously exposed to HL for 175 min, the A_Pc amplitudes at the end, corresponding to 75 min of HL wash after 75 min exposure to VTD (see fig. 3), were the following percentages of the 25-min values: A-fibers, 147%; B-fibers, 148%; and C-fibers, 153%. These single measurements are slightly higher than the average values recorded at the end of the wash-out after VTD exposure (fig. 3 top).

SUCROSE-GAP RECORDING

Nerves were desheathed and mounted in an acrylic sucrose-gap chamber (fig. 1C). We used a method after that described by Stampfli9 (see also ref. 5). One end of the nerve was stimulated by bipolar extracellular electrodes (silver/silver chloride) in small pools (approximately 50 μl each) containing control buffer solution and isolated from the recording pools by petroleum jelly. An 8-mm length of intact, desheathed nerve was exposed to drugs in the test pool (approximately 300 μl) containing one of the silver/silver chloride recording electrodes; the other electrode rested in a HL solution that bathed the other, "intracellular" cut end of the nerve. These two recording zones were separated by a sucrose gap consisting of a hollow cylinder of 3 mm diameter that intersected the nerve to perfuse it with the insulating, nonionic sucrose (isotonic at 0.25 M). Because of the removal of extracellular ions in the sucrose gap by continuous perfusion of the non-electrolyte (2–3 ml · min⁻¹), the A_Pc cannot propagate through this region. The potential between the two electrodes is proportional to the true transmembrane potentials, averaged for the different fibers. This measurement includes a direct current (DC) offset, which is representative of the average resting potential (the compound resting potential [CRP]), plus the true monophasic action potentials from the different fiber types. In contrast to the extracellular chamber described above, which measured A_Pc in a drug-free segment after they had propagated through a VTD solution, the sucrose gap measured the behavior of the nerve membrane directly exposed to drug, the "veratrinized" nerve.

A_Pc and the CRP were recorded with the same amplifier used for extracellular recording (see above), but passed both to the DC-coupled input of the storage oscilloscope (for A_Pc) and to a strip-chart recorder (for CRP) (model 8376-20, Cole Parmer). A_Pc were elicited by supramaximal pulses with the same stimulus parameters for exciting A-, B- and C-fibers as used for extracellular recording, described above.

STATISTICAL ANALYSIS

The results reported here are means ± SEM; the numbers of independent observations are also included. A two-tailed Student’s t test was used to evaluate the significance of changes produced by the drugs.

Results

EXTRACELLULAR RECORDING

Single Stimuli

The standard protocol used for testing the action of VTD was to record the A_Pc elevations in control HL
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FIG. 2. Compound action potentials stimulated by single shocks for A- and B-fibers (A–C) and for C-fibers (D–F). The control records (A, D) were taken 5–7 min before exposure to VTD (2 μM). VTD records (B, E) were taken after 15–20 min exposure to drug, and wash records (C, F) 20–30 min after switching to a drug-free perfusate. Calibration scale: vertical, 0.8 mV for all fiber types; horizontal, 4 ms (A–C) and 20 ms (D–F). The broken lines mark the zero potential baselines.

solution for 30–60 min before adding VTD; for 60–75 min during VTD application; and for 60–75 min after replacement with VTD-free HL. Single stimuli and trains of ten stimuli, at intensities and durations sufficient to excite either almost all A-fibers alone, or all A- and B-fibers, or all A-, B- and C-fibers, were applied intermitently during these three periods.

Recordings of the separate elevations corresponding to these different fiber types in a desheathed nerve are shown in figures 2A–C for A- and B-fibers and in figures 2D–F for C-fibers in a nerve exposed to 2 μM VTD, the highest concentration we used on desheathed nerves. During the control period, the period of VTD exposure, and the wash-out period, the amplitude of the singly stimulated APc for A- and C-fibers increased continually. Some slowing and separation of the B-fiber elevation is evident in figure 2B, but this was an exceptional case, contrasting the average behavior, which is plotted in figure 3. The trend of increasing APc is shown in the top panels of figure 3, which graphs the APc elevations averaged from multiple nerves exposed to 0.5 μM VTD. Average amplitudes of single APc elevations in four nerves during periods of control, VTD exposure, and wash are (relative to the amplitudes at the beginning of VTD exposure [see fig. 4]): 92 ± 1, 85 ± 4, and 97 ± 1% (control); 117 ± 7, 105 ± 23, and 111 ± 5 (VTD); and 123 ± 14, 108 ± 31, and 133 ± 15% (wash) for A-, B-, and C-fibers, respectively.

Control experiments (in which no VTD was added) yielded action potential elevations, at times equivalent to those of VTD exposure for 35 min, of 118, 126, and 97%
for A-, B-, and C-fibers, respectively (see Materials and Methods: Control Recording). Comparison of these controls to the values for AP⁰ after 60 min in VTD shows no significant difference for A- and B-fibers ($P > 0.05$) but a significant enhancement of the C-fiber component ($P < 0.005$) from exposure to VTD. Altogether, the continuity of the AP⁰ increase in all three exposure periods and the comparable values with control measurements show that VTD at 0.5 μM has only a small effect on impulses conducted in response to a single stimulus.

Repetitive Stimulation

During a train of action potentials resulting from repetitive stimulation, the different AP⁰ elevations diminished in a characteristic VTD- and frequency-dependent manner. Examples of this behavior are shown in figure 4. At 2 μM VTD, the response of A-fibers during 100-Hz stimulation was almost identical to that in drug-free solutions before and after VTD exposure (fig. 4A–C). The elevations declined slightly (by about 15%) at the second stimulation but remained constant during the rest of the train. This effect was due to the normal refractory behavior of the A-fibers.

The B-fibers were more sensitive to VTD, and at 50-Hz stimulation frequency there was a decline (by 70%) in their AP⁰, which exceeded the decline of control AP⁰s by a factor of 3 (fig. 4D–F). However, the B-fiber component of the AP⁰ was often difficult to resolve because of its nearness to the A-fiber peak and its usually smaller amplitude. Still, as illustrated in figure 4E, we often could discriminate two separate peaks of the B-fiber component during repetitive stimulation. The selective slowing of a subclass of B-fibers (or of some other more slowly conducting myelinated fibers in the vagus nerve) that causes this peak separation accounted for a large part of the reduction in the peak height of the AP⁰. Here, the compound amplitude declined clearly as a result of conduction dispersion as well as of some extinction of impulses. Upon removal of VTD, the differential slowing was reversed, and in the single volleys the superposition was restored, sometimes leading to a B-fiber amplitude greater than that of the A-fibers. However, the use-dependent effects of VTD on B-fibers were not abolished completely by 75 min of washing with drug-free HL (fig. 4F).

Repetitive stimulation of C-fibers at much lower frequencies produced a marked fall in the corresponding AP⁰ C-fiber elevation (figs. 4G–I). During a train of AP⁰ at 10 Hz in 0.2 μM VTD, the C-fiber elevation decreased incrementally with each stimulus and reached a steady-state elevation of about 40% relative to the amplitude of the first elevation in the train. The same stimulus pattern produced a decline of only 12% before VTD, and after about 1 h of VTD wash-out, this inhibition recovered to 22%, approximately one third of the decline during VTD exposure. The peak separation evident in the B-fiber elevation was never seen in C-fibers.

The average use-dependent decline of impulses from the different fiber types are graphed in figure 3 (bottom) and are listed in table 1. During the period of VTD ex-
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<th>Table 1. Use-dependent Reduction of APc by Veratridine</th>
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<td>Fiber Type</td>
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Values are percent inhibition of the tenth APc in a train compared to the first. Means ± SEM from four separate experiments. [VTD] = 0.5 μM for all fiber types.

* Measured at 100-Hz stimulation frequency 5 min before VTD (control), after 75 min in VTD (VTD), and after 75 min of wash in VTD-free (HL).
† Measured at 50-Hz stimulation frequency 10 min before VTD (control), after 70 min in VTD (VTD), and after 70 min in VTD-free wash (HL).
‡ Measured at 10-Hz stimulation frequency 12 min before VTD (control), after 60 min in VTD (VTD), and after 60 min in VTD-free wash (HL).
§ P < 0.001, VTD versus control values.

Exposure, corresponding to the experimental times indicated on the horizontal axes of the upper panels, trains of ten stimuli were applied, and the ratio of the amplitude of the last APc to the first APc was measured (e.g., see fig. 4). Two cycles of these stimuli were applied during the 75 min of VTD treatment, the first from 0.1 to 10 Hz and the second from 0.1 to 100 Hz for A- and B-fibers but only to 10 Hz for C-fibers, ranges within their normal physiologic discharge frequencies. The selective depression of this use-dependent effect on C-fibers is evident in the comparison of the control and VTD entries of table 1.

Washing the nerves with VTD-free HL did not reverse the use-dependent depression of A- or B-fibers, but effected a partial reversal in the case of the C-fibers (table 1). In control nerves continuously exposed to HL for up to 60 min, the intrinsic use-dependent decline of APc increased by no more than 10% of the control values. Use-dependent decline of APc in VTD-free nerves recovered within 1 or 2 s of the termination of rapid stimulation in all fiber types (data not shown), unlike the VTD-induced use-dependent effects, which can take tens of seconds to recover. The endogenous use-dependent decline of APc seems to involve mechanisms different from those that mediate the actions of VTD.

Another view of this use-dependent effect is provided in figure 5, where the C-fiber responses to sequential stimuli in a train are superimposed in one record. The APc response to the second stimulus in the train not only was much smaller in amplitude than the first, but also was conducted more slowly (fig. 5B). The peak of the second wave arrived at the recording electrode with about 20% longer latency than that of the first wave. Subsequent stimuli produced a more gradual diminution in amplitude and slowing of conduction in the C-fibers. In the absence of VTD, neither the diminution in amplitude nor the slowing of conduction occurred (fig. 5A). When stimulation ceased, the APc recovered the form it had taken before the train, although this recovery sometimes took as long as 1–2 min, depending on the VTD concentration (data not shown).

We measured the use-dependent reduction in the separate elevations of the APc at three VTD concentrations and at several stimulation frequencies. The frequencies, chosen to fall in the range of normal responses to physiologic stimuli among the three fiber types, extended to 100 Hz for A- and B-fibers but only to 10 Hz for C-fibers. Averaged relative amplitudes at the end of a train are graphed in figure 6. Each fiber class is characterized by a surface topography. Any vertical plane perpendicular to the frequency axis (x axis) intersects the surface along a dose–response curve that characterizes that particular fiber type at that particular frequency. If the vertical plane is instead drawn perpendicular to the drug axis (y axis), then the surface intersect shows the frequency dependence of the inhibition for that VTD concentration.

Examination of the parallel y-axis intersects in figure 6A shows that impulses in A-fibers are modified little by VTD up to 2 μM. B-fibers show more sensitivity to this drug (fig. 6B), although we are less confident of these data since the B-fiber elevations were often small and superimposed on the "tail" of the A-fibers and bifurcated during the train (see above). The C-fiber elevations are diminished most by VTD, and at relatively low frequencies (fig. 6C).

**Recordings from Sheathed Nerves**

We tested the ability of VTD to penetrate the tissues ensheathing the vagus nerve. Nerves with intact sheaths were arranged in the same extracellular recording chamber as had been used for the desheathed nerve studies.

**Fig. 5.** Superimposed traces showing use-dependent inhibition and slowing of C-fiber APc by VTD (2 μM). (A) Control records 10 min before VTD at 2-Hz stimulation frequency show almost complete overlap of traces 1–10. (B) During VTD exposure (15–20 min), the second through tenth APcs in the train show a slowed conduction and reduced amplitude (5-Hz stimulation frequency). (C) After 20 min in VTD-free HL (wash) the use-dependent effects measured at 5 Hz have largely reversed.
just described. Because of the electrical isolating qualities of the sheath, stimulus requirements are greater and the recorded APc(s) are smaller, but have the same latency from stimulation as in the desheathed nerves. These APc also were inhibited selectively by VTD. However, the onset of the effect was much slower and the VTD dose requirement much higher: 2 \( \mu \text{M} \) VTD took approximately 30 min to affect the C-fibers detectably, and 10 \( \mu \text{M} \) took approximately 15 min (compare to the time-course shown in fig. 3). This is expected in a lipophilic base for which more than 90\%, on average, of the drug molecules are ionized.

Nevertheless, the same phenomenon that had been observed in desheathed nerves occurred: the C-fiber elevation was progressively inhibited in a use-dependent fashion. The use-dependent reduction at 10 Hz after a 60-min exposure of an desheathed nerve to 10 \( \mu \text{M} \) VTD was only approximately 30\% of that achieved at steady state on a desheathed nerve by 0.2 \( \mu \text{M} \) VTD. Therefore, if the sheath does not modify the drug’s action but only slows its access, the rate of penetration into the nerve is reduced at least 50-fold by the sheath.

The action of VTD increased throughout the exposure period (75 min) and persisted or even grew during the subsequent wash-out. In both nerves tested, a steady-state effect, reached during the wash-out period, remained constant for up to 3 h in drug-free solution.

**Sucrose-Gap Recordings in the Veratrinized Region**

Previous studies have reported that VTD has a depolarizing action on the membrane directly exposed to the drug. In order to observe this effect in the vagus preparation, we mounted desheathed nerves in a sucrose-gap chamber (see Materials and Methods). This permitted the measurement of average monophasic action potentials and resting potentials from the 8-mm length of nerve bathed by the drug.

Control APc measurements before VTD addition declined continuously but slowly with time, because in the sucrose-gap chamber the electrical activity is less stable than in the extracellular chamber. However, the CRP was steady. After the addition of VTD (0.5 \( \mu \text{M} \)), the A- and B-fiber elevations remained unchanged. The C-fiber elevation also was unaffected for a single stimulus, but a slowly rising, VTD-induced depolarization (VID) developed within about 100 ms of stimulation (fig. 7C) and decayed only slowly: the decay half-time was 3–4 s after 1 hr in 2.0 \( \mu \text{M} \) VTD (fig. 7D). At high enough stimulation frequencies, the VID and the primary C-fiber response overlapped. Because of their very slow decay, the VIDs accumulated to yield a larger sustained depolarization (fig. 8B), and the primary C-fiber elevations that were superimposed on this integrated depolarization were much smaller than were the elevations induced by single stimuli (fig. 8C and D). The VID occurred only when the stimulus

**FIG. 6.** Three-dimensional graphs of use-dependent VTD inhibition of compound action potentials of A-fibers (A), B-fibers (B), and C-fibers (C). The ratio of amplitudes of the tenth to the first APc in a train of impulses, stimulated at 0.1–100 Hz for A- and B-fibers and at 0.1–10 Hz for C-fibers, is plotted for VTD concentrations of 0–2.0 \( \mu \text{M} \). The open circle is the amplitude of a single APc in zero VTD. Broken lines fill the surface where there are no data points. Values are averages of four to five separate experiments. See text for details.

**FIG. 7.** Sucrose-gap recordings of compound action potentials in the veratrinized region of nerve. Fast oscilloscope traces (A, C) show the APc and the beginning of the veratrine-induced depolarization (VID: arrow, C). Slow strip-chart records show the time course of the VID resulting from a single stimulus (D) and its absence without VTD (B). VTD = 2.0 \( \mu \text{M} \). (Fast action potentials are not faithfully recorded in the strip-chart measurements, but the VID is followed precisely.) The broken line marks the baseline for zero change in membrane potential.
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Fig. 8. Sucrose-gap recordings of VTD effects during repetitive stimulation. Strip-chart recordings of responses to 0.5 Hz stimulation in control (A) and VTD-containing (0.2 μM) perfusate (B) show the cumulative VID during repetitive stimulation. The APs during this VID are shown for the first stimulus (C) and the tenth stimulus (D) in the train. The deflection arising from C-fiber activity, shown by the double-ended arrows, is reduced by 40% between the beginning and end of the train, whereas that from A-fibers is unchanged.

conditions were sufficient to excite C-fibers, and the elevations of A- and B-fibers superimposed on the VID were approximately the same amplitude as those from stimulation of A- and B-fibers alone. Therefore, the VID observed at these frequencies and VTD concentrations depended on activation of impulses in C-fibers, and correspondingly, affected impulses in those fibers only.

Discussion

Our results demonstrate the preferential depression of trains of C-fiber APc by VTD. In the following discussion, we consider the mechanisms that produce this selective effect and compare this phenomenon to the actions of traditional local anesthetics on APc.

The preferential slowing and amplitude reduction of propagated C-fiber APs correlate with the presence of a VID in the veratrimized segment of the nerve. Impulse propagation is affected as a function of VTD concentration and stimulation frequency in ways that are qualitatively similar to the integration of the VID. On this basis, we hypothesize that the depolarization of the veratrinized axons is the source of the changes in impulse propagation. How VTD produces this depolarization and why it occurs preferentially in C-fibers are discussed below.

Activators of Na channels, such as VTD, produce a persistent increase in Na permeability and an accompanying inward Na+ current that leads to a steady depolarization of excitable membranes. In nerve, skeletal muscle, and cardiac muscle, this action is due to a preferential binding of the drugs to an open conformation of the channel. Use dependence occurs because electrical stimulation leads to the activation of the normal open state of some fraction of a nerve's complement of Na channels and thereby provides a substrate with a higher affinity for the activator. An increased quantity of activator binds during stimulation because of an increased rate of binding, and the activator-bound open channels, being less prone to close than drug-free channels, sustain a depolarization that far outlasts the normal action potential.

At lower doses of VTD, the outward membrane currents generated by K+ channels and electrogenic pumps can repolarize or even hyperpolarize the veratrinized membrane after a single impulse. This repolarization leads to a slow reversal of VTD binding (some inactivation of VTD-bound channels also occurs in the repolarized membrane), and the prestimulus conditions are restored. When stimulation occurs at intervals too brief to allow full recovery, the depolarizations accumulate additively, as shown in figure 8B. Higher stimulation rates and the concomitant shorter interpulse intervals result in a larger summed depolarization during the repetitive stimulation, as previously reported in frog myelinated nerve at higher frequencies. When stimulation ends, the restorative outward currents dominate and the membrane hyperpolarizes.

In our studies, the use-dependent effects of VTD were relatively selective for C-fibers. Propagated APs were slowed and were reduced in peak amplitude. Probably several factors contributed to the conduction slowing in general. After the first impulse passes, the nerve is slightly depolarized (as in the VID) and some of the VTD-free Na+ channels become inactivated, while some of the voltage-gated K+ channels remain activated. Thus, the ability to generate inward Na+ current is reduced, and the membrane resistance is decreased, allowing the local circuit current that results from the action potential and drives impulse propagation to leak out over a shorter length of nerve. Both of these factors slow impulse conduction. If the depolarization is large enough and the veratrinized length long enough, then decremental conduction of impulses in the drugged axon will result in propagation failure. From the results in the current study, however, we cannot determine how much of the reduction in height of the APc is due to differential conduction slowing and how much due to impulse abolition.

The preferential reduction of the C-fiber APc also may result from several factors. Each one, however, relates to a more effective depolarization (VID) of C-fibers in the veratrinized region than the depolarization of A- and B-fibers. First, the distribution of Na+ channels in these nonmyelinated fibers probably is relatively uniform, unlike the clustering of channels in myelinated axons. In the myelinated exons, the large myelinated internodal region of the axon containing only K+ channels acts to
maintain and restore the resting potential. In the C-fibers, this relatively isolated “buffer” of membrane potential is absent, and a more uniform and larger depolarization may occur.

A second anatomic factor is the volume-to-surface ratio among different fibers and the change in intracellular [Na⁺]. Large myelinated axons may have axon diameters of 10–15 μm, whereas C-fibers in peripheral nerve are only approximately 0.75 μm in diameter, making the relative volume-to-surface ratios 20-fold smaller in the C-fibers. If the same Na⁺ flux accompanied impulses in both axons, the net increase per length in intracellular [Na⁺] in C-fibers would exceed by 20-fold that in the large A-fibers. Of course, the density of Na⁺ channels at the nodes of Ranvier of myelinated fibers (10⁴ μm⁻²)¹⁸ is much greater than their average density estimated for C-fibers (10³ μm⁻²),¹⁹ but only approximately 1% of the myelinated axon’s length is composed of the nodal membrane,²⁰ so the overall number of channels per length of axon is about the same in the two fiber types. Therefore, C-fibers experience a greater increase in axoplasmic [Na⁺] per impulse, in both drug-free and veratrinized conditions, than do myelinated fibers.

Since the outward current in C-fibers is carried largely by “delayed-rectifier” type K⁺ channels,²¹ which are blocked by intracellular Na⁺ ions,²² the greater accumulation of Na⁺ in C-axons preferentially inhibits their outward currents and promotes and prolongs the VTD. Delayed-rectifier K⁺ channels are almost absent in nodes of mammalian large myelinated axons,²³ so the effect on outward currents of the little Na⁺ that does accumulate inside is much smaller in large axons than in C-fibers.

A third factor in the preferential reduction of the C-fiber APₐ concerns the decremental conduction mentioned above. It may be that the exposure length used for these experiments, 11 mm, is long enough to allow impulses in the C-fibers, with their shorter length constant, to decrease far more then those in the myelinated A- and B-fibers. If longer lengths of these larger axons are exposed to VTD, their impulses also may show significant slowing and reduction. We think this factor is only a minor differential determinant; however, the depolarization in the sucrose gap, which is independent of the length of nerve exposed to drug, was still a C-fiber selective phenomenon—not occurring when only A- and B-fibers were stimulated—and the inhibition of C-fiber APs correlated with this depolarization.

Because these studies were conducted at room temperature and because the VID was measured by the sucrose-gap method, which hyperpolarizes the drug-exposed region of nerve,²⁴,²⁵ the effects of temperature and of membrane potential on the actions of VTD should be addressed. Cooling reduces the action of VTD. Fewer channels are bound by VTD when a cooled frog nerve rather than a warm one is depolarized.²⁶ The rate of decay of the VTD-induced permeability change, however, is slowed by nerve cooling. The net depolarization induced in single nodes of Ranvier of frog myelinated axons by VTD is highly temperature-dependent: at 14°C the average change in membrane potential is 0, but grows to +50 mV at 20°C and to approximately +40 mV at 22–24°C.²⁶ Interestingly, this is the opposite of the effect of temperature on local anesthetic action, in which cooling potentiates the impulse-blocking potency.²⁷

Membrane hyperpolarization can reduce the VTD-induced Na⁺ permeability and yet slow the rate of decay of that process after an activating depolarization. At −80 mV, VTD-induced Na⁺ permeability in frog nerve (at 13°C) is approximately twice its value at −120 mV, and it decays more slowly: the respective time constants are 2.2s and 1.7s.²⁵ Thus, the artificial experimental conditions of lower temperature and local hyperpolarization both contribute to suppress the actions of VTD, which is likely to be more potent, especially in its use-dependent actions, under the normal in vivo conditions.

The true differential susceptibility of impulses to local anesthesia remains a controversial topic.²⁸ Traditional local anesthetics have a differential potency for inhibiting action potentials propagating in different fibers of the vagus nerve. Amplitudes of APₐ from A-fibers are reduced to a greater extent than are those from B-fibers and especially those from C-fibers, by local anesthetics (e.g., lidocaine)²⁹, by the nonpeptide tetrodotoxin,²⁵ which acts at a totally separate site; and by a reduction in external [Na⁺].³⁰ We have observed this same order of susceptibility when examining, in the sucrose-gap system described here, APₐ blocked by bupivacaine.§

Fink and Cairns have examined the ablation by local anesthetics of propagated impulses in single fibers of the rabbit vagus nerve. They found that impulse failure occurred in A- and C-fibers at about the same lidocaine concentration when single stimuli were applied.³¹ However, during repetitive stimulation (e.g., at 30 Hz), impulses in the myelinated fibers were more resistant to extinction by lidocaine (0.6 mM) than were those in the non-myelinated fibers, although at the lower frequency of 15 Hz both types of fibers conducted impulses without fail.³² From these results it appears that a use-dependent block by local anesthetic may be selective for C-fibers.

Stimulation frequency is an important parameter in determining the use-dependent responses in axons ex-

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§Schneider M, Datta S, Strichartz GR: Unpublished observation.
posed to local anesthetics or to VTD. During physiologic responses, sensory afferents convey intensity information in a frequency-modulated impulse code, and any selective ablation of sensations depends on both the changes in conducted impulse frequency and on the nature of the pre- and postsynaptic integration processes in the spinal cord. C-fiber nociceptors in vivo rarely fire at frequencies above 20 Hz in response to noxious thermal stimulation, and if driven at 10 Hz for 60 s, approximately 70% of these fibers fail to conduct any impulses during the train. However, C-fibers serving other sensory modalities (e.g., cold), continue to conduct during the same stimulation protocols. Therefore, any conclusions about the drug susceptibility of different sensations, at low or high stimulation frequency, must be based on studies of the appropriate functional fiber class and not just of fibers grouped by conduction velocity, and must include the dynamic responses of the drug-free fibers as baseline behavior.

The practicality of VTD for clinical anesthesia has yet to be demonstrated. Although we have shown that VTD can penetrate the nerve sheath, which is a major permeability barrier, as it is for local anesthetics, the use of VTD for local anesthesia will be limited by both local and systemic toxicity. Years of investigation on the cardiovascular effects of VTD provide a basis for estimating at least this aspect of systemic toxicity. There is, however, a number of naturally occurring Veratrum alkaloids, of which VTD is only one, with varying effects on cardiac muscle and vascular tissues, and examination of their effects on nerve seems worthwhile. If the preferential depression of the C-fiber APc does reflect a selective abolition of impulses that occurs at natural frequencies of nociceptive signalling, and if toxic side effects are minimal, then Veratrum alkaloids may provide a useful tool with which to accomplish peripheral analgesia.

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References

22. Bezanilla F and Armstrong CM: Negative conductance caused by the entry of sodium and cesium ions into the K channels of squid axons. J Gen Physiol 60:588-608, 1972