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Neurophysiologic Actions and Neurological Consequences of Veratridine on the Rat Sciatic Nerve

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Background: **The quest for a drug that would provide analgesia with minimal motor deficiency, through the selective inhibition of impulses in small-diameter fibers, was brightened by a previous report of veratridine's C-fiber–selective actions on the isolated rabbit vagus nerve. The goal of the present research was to demonstrate the same actions on rat sciatic nerve *in vitro* and to observe the functionally differential blockade in the rat *in vivo*.**

Methods: **Sciatic nerves were removed from rats, mounted in a recording chamber, wherein a 1-cm length of the ensheathed nerve was superfused with the plant alkaloid veratridine (2 μM) in bicarbonate-buffered Liley's solution, and the compound action potential (CAP) was stimulated supramaximally to give A- and C-fiber elevations. Onset, steady-state, and recovery from veratridine effects were assayed for a range of stimulus frequencies. Open-field behavior and quantitative neurological assessments of proprioception, motor function, and nociception were tested in 15 trained rats after injection near the sciatic nerve of 0.1 ml veratridine at 0.5, 0.7, and 1.0 mM each plus epinephrine (1:200,000).**

Results: **Veratridine inhibited the C-fiber component of the CAP in a frequency-dependent manner. At 0.1 Hz the CAP was 65% of the control amplitude, 50% at 0.5 Hz, and 40% at 5 Hz. A-fiber elevations were unattenuated at stimulus frequencies as high as 50 Hz. Steady-state inhibition was reached 5 min after drug administration, and recovery from the effects was 30% complete by 15 min of drug washout. Proprioception, measured as a "hopping" or "placing" reaction, was inhibited dose dependently by maximum degree and for durations of, respectively, 0.5 mM, 61%, 180 min; 0.7 mM, 100%, 360 min; and 1 mM, 100%, 420 min. Extensor postural thrust, as a measure of motor function, was inhibited by and for 0.5 mM, 77%, 240 min; 0.7 mM, 99%, 390 min; and 1 mM, 100%, 420 min. Analgesia, as a prolonged withdrawal latency to a noxious thermal stimulus, had the following profile: 0.5 mM, 10%, 30 min; 0.7 mM, 52%, 150 min; and 1 mM, 66%, 150 min.**

Conclusions: **Despite the fact that veratridine gave a C-fiber preferential blockade in the isolated sciatic nerve, heightened analgesia over motor block was not achieved *in vivo*. Indeed, just the opposite occurred. If preferential C-fiber blockade also occurs *in vivo*, then its traditionally expected correlation with analgesia must be re-examined. (Key words: Alkaloid: veratridine. Analgesia. Local anesthesia. Motor blockade. Nerve: sciatic.**

DIFFERENTIAL control of neurological functions is needed during regional anesthesia. For example, pain relief without motor impairment would be highly desirable in ambulatory surgery, and the local treatment of muscle spasm or tremor would be better accomplished if sensation were preserved. Differential block is achieved in part by the choice of certain local anesthetics (LAs) for selected purposes. Clinically, lower doses of bupivacaine produce reliable analgesia with minimal motor impairment,[1,2] and etidocaine is known as a preferential blocker of motor function.[3] However, a fully selective block of different neural functions has not yet been achieved with any of the clinically used LAs. The development of selective functional blockade can be accomplished through pharmacologic agents based on a better understanding of the activities in peripheral nerves that underlie the different functions and their physiologic relations along the neuraxis. Although many attempts have been made to explain the observed differential block of functions by a differential pharmacologic susceptibility of fibers to LAs, electrophysiologic studies show that such a discrimination based on fiber size alone does not occur at this level of sensory

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processing.[4] Indeed, recent studies from our laboratory have shown that superfusion of rat sciatic nerve *in vivo* by lidocaine solutions of a controlled concentration show that nociceptive C-fiber afferents are significantly more resistant to block than are mechanoreceptive Aβ-fibers.[5]

Recently, when the use-dependent inhibitory effect of veratridine on the compound action potential (CAP) of the rabbit vagus nerve was reported, the inhibition was greatest for the C-fiber component.[6] It is generally accepted that afferent C-fibers are involved in nociception and efferent C-fibers represent sympathetic postganglionic fibers. This observation of preferential inhibition *in vitro* would imply that veratridine applied to the peripheral nerve *in vivo* should preferentially block nociception and the sympathetic functions subserved by this nerve.

To evaluate the influence of veratridine on the different functions of the sciatic nerve under free behavior conditions, we conducted this experimental study in rats.

Methods

Animal treatment for all studies reported here was approved by the Harvard Medical Area Committee on Animals.

Electrophysiologic Studies In Vitro

Nine Sprague-Dawley rats (weighing 250–300 g) were killed by a barbiturate overdose (100 mg/kg pentobarbital given intraperitoneally), and both sciatic nerves were removed promptly within 10 min. The ensheathed nerves were perfused in a modified HEPES Liley (HL) solution, buffered with both CO_2 and HCO_3 and the synthetic buffer HEPES (described subsequently). Nerves were maintained in their perineurial enclosures and mounted in an extracellular chamber. The test pool of the nerve chamber contained about 0.6 ml total volume; the perfusate passing through this pool was pumped back to the perfusion reservoir, which contained 10 ml, bubbled continuously with 95% oxygen and 5% carbon dioxide, and again superfused around the nerve. These studies were conducted at room temperature (22–24°C).

The modified HL solution contained 118 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 1 mM $MgCl_26H_2O$, 10 mM glucose, 5 mM HEPES, and 5 mM $CaCl_2 \cdot 2H_2O$, which was added only after the initial, vigorous bubbling of the solution with carbon dioxide to prevent the precipitation of $CaCO_3$. The final *p*H of this solution, measured in the perfusion chamber, was 7.3–7.4. Addition of veratridine to the HL solution contained ethanol to a final concentration of 0.04%, an ethyl alcohol concentration that had no effect on the action potential.

A recording chamber identical to the one described by Schneider *et al.*[1] was used for recording the CAP. The length of the chamber's portion wherein the nerve was exposed to drug solution was 11 mm, and the total conduction distance from recording to stimulation electrode was 21 mm. The two ends of the nerve were stimulated by or recorded from using platinum-blacked/platinum electrodes in separate chambers from which HL was removed and replaced with mineral oil (Dow Corning, Midland, MI). The nerves were stimulated by 0.1–0.5-ms-long cathodal pulses (model 44 Stimulator; Grass Instrument Co., Braintree, MA) and the propagated CAP was amplified (50x, model 47 amplifier; Metametrics, Cambridge, MA), recorded on a storage oscilloscope (model 5113; Teletronix Corp., Bend, OR), photographed, and analyzed manually. The preparation was not considered acceptable unless the CAP signals from the nerve exceeded 1.5 mV for A-fibers and 0.3 mV for C-fibers.

Each of five separate nerves was exposed to 2 μM veratridine. Single and repetitive stimuli were each applied at 5-min intervals for 10–15 min before drug was added to establish baseline control values and every 5 min after veratridine was added until a steady-state response occurred (15–20 min), followed by a washout period of 15–30 min, sufficient to achieve at least partial recovery of veratridine effects. The temperature of the bathing solution was 20–22°C. At the A-fiber stimulus level, the nerves were repeatedly stimulated by ten pulses at 50 Hz and at C-fiber levels by ten pulses at 5 Hz. With this protocol the apparent use-dependent inhibition was quantified as the amplitude of the tenth CAP divided by the amplitude of the first CAP in the train.

Behavioral Testing

Thirty-six experiments were performed in 15 handled, male Sprague-Dawley rats weighing 250–300 g (Charles River Animals, Wilmington, MA). The rats arrived at an age of 40 days and were handled daily for the next 15 days. Except for the few hours when experiments were done (10.00 AM–1:00 PM for handling and 10.00 AM–6:00 PM for pharmacologic experiments),

they were kept in separate cages in rooms with rats of other investigators in the animal housing facilities of Brigham and Women's Hospital, with controlled humidity, room temperature, and 12-h light–dark cycles.

The handling was performed to familiarize animals with the experimenter, the environment in which the studies were done, and the manipulations used in the experiment. This familiarization minimizes the stress-response during the experimental period.[7]

The experiment consisted of monitoring the neurological status of the animal before and after administration of veratridine or vehicle close to the sciatic nerve. We evaluated general behavioral reactions and examined specific neural functions mediated by the sciatic nerve of both hindlimbs: proprioception, motor function, response to noxious stimulation, and skin temperature.

Preparation of Veratridine Solution. A stock solution of veratridine (Sigma Chemical Co., St. Louis, MO) was prepared by dissolving 3.4 mg veratridine in 5 ml 4% solution of dimethylsulfoxide in 0.9% NaCl. All working solutions were prepared on the day of the experiment, no more than 30 min before injection. The final concentration of each solution was delivered in an injected volume of 0.1 ml. Epinephrine (Abbott Laboratories, N. Chicago, IL; ampule of 1 mg/ml) was added to a final dilution of 1:200,000, and the *p*H was adjusted just before injection to 6.5–7.

Administration of Drug. Veratridine or vehicle was injected without any sedation or general anesthesia. The rat was placed in lateral recumbency; the greater trochanter and ischial tuberosity were localized by palpation and the injection needle was advanced until the ischium was encountered. Then 0.1 ml of drug or control solution was injected.

Three series of experiments were performed with three doses of veratridine: 0.5 mM, 0.7 mM, and 1 mM. Two series of control experiments were done; first with the injection of only vehicle — 4% solution of dimethylsulfoxide in saline; and second with the injection of 4% dimethylsulfoxide in saline with 1:200,000 epinephrine. Each series contained six experiments conducted on six animals. Each hindlimb was injected once only.

Experimental Design. The individual behavioral reaction to administration of veratridine or vehicle was assessed by comparison of behavior in an open field before and after injection. Every animal was observed for latency of grooming, number of rearings, presence of postural freezing, and number of fecal boli during a 3-min period. These observations were performed before injection and at the time of maximal neurological change (see Results). At the peak of the pharmacologic effect, the relation with an intruder rat was also observed.

Neurological status was assessed, and general behavior, gait, posture, and locomotor activity were monitored during the observation period under free behavior conditions. Special attention was paid to gait, posture, and locomotion of the hindlegs. The noninjected leg served as a control for qualitative evaluation of neurological functions. The time of appearance of any noticeable asymmetry indicated the onset of pharmacologic effect and served as a sign for the beginning of testing of sciatic neural functions of both hindlegs.

The order of quantitative testing procedures was measurement of skin temperature and evaluation of the postural reactions, of the "extensor postural thrust," and of withdrawal response measurement to noxious stimulation of the foot. In pilot experiments, optimal time intervals for this testing were established: 15 and 10 min before injection; at 1, 5, 10, 20, 30, 45, and 60 min; and then every 30 min until complete recovery.

Assessment of Functions. Skin temperature (SkT) was measured using a model 871 Omega digimeter thermometer (±0.1°C; Omega Instruments, Stamford, CT). The thermocouple was placed on the surface of the hairy skin at two loci, both of which are innervated by the sciatic nerve of the respective hindleg: on the base of the fifth "toe" and at the "heel."[8] The maximum possible difference (core temperature − room temperature) was used to normalize the postinjection changes; that is, the measured difference of the experimental SkT − control SkT at "toe" and "heel" was divided by the maximum possible temperature change, because under physiologic conditions SkT cannot be lower than the temperature of the environment or higher than the core temperature. The latter was taken as 38.5°C.[9]

The evaluation of proprioception was based on quantification of two postural reactions, "hopping" and "tactile placing."[10] When an animal supports its weight on one extremity only, sideward movement of the body induced "hopping" of the weight-supporting limb laterally in the direction of the movement. "Tactile placing" is the ability to reposition the knuckled toes such that the plantar surface of the foot rests flat on the support surface. The functional status was graded as 3 (normal), 2 (slightly impaired), 1 (severely impaired), or 0 (absent). For more detailed description of these tests, see Thalhammer *et al.*[7]

Motor function was evaluated by the "extensor pos-

tural thrust," measured in grams on an electronic digital pan balance (Ohaus LoPro; Fisher Scientific, Florham Park, NJ). Normal motor function was established by measuring the applied force necessary to overcome the extensor thrust, by bringing the heel down to touch the platform. When a rat is held upright with the body weight resting on this hind limb, it extends the tibiotarsal joint (extensor postural thrust) to keep its posture. The reduction in this force, representing the loss of extensor muscle tone, was considered a quantitative test for block of motor function.[7]

Nociception was evaluated by the latency of the withdrawal response to application of a hot (51 ± 0.5°C) metal probe (3 mm diameter) to the dorsal surface of the lateral or medial margin of the metatarsus. Prolongation of latency to 10 s (cut-off time) was considered complete nociceptive block.[7]

Durations of motor and nociceptive deficits were compared by careful observation of the onset of impairment and recovery of full function. Specifically, when rats showed changes in leg posture and movement that altered their gait during free behavior, the time was noted and the extensor postural thrust immediately tested. When these locomotor deficits disappeared, the time was also noted and the difference in onset and recovery, measured to within 1–2 min, was assigned as the duration of functional motor block. (The corresponding reduction in extensor postural thrust was ~50% maximal possible effect [MPE].)

For impairment of nociception, rats were scored as being impaired when the latency for withdrawal to the thermal stimulation was at least two times that of the baseline, predrug values. Because baseline latency varied by as much as 20% (see table 1 of Thalhammer *et al.*[7]), a doubling in latency can be reliably detected as a change in nociception. In this article, we used this identical procedure to compare the relative duration selectivity for impairment of motor and nociceptive function by veratridine with a traditional local anesthetic, bupivacaine.[9]

Treatment of Behavioral Data and Statistics. Changes of each function were evaluated as percentages of MPE in each experiment at a specified time after injection. Maximal possible effect was used for a general comparison of functions measured with different numeric scales. Percentage of MPE was calculated for every functional change, considering 100% of MPE as a complete block of function, and by comparing the magnitude of evoked response with the magnitude of the predrug control measurement on the same leg. We believe emphatically that such separate, normalized functional parameters cannot be compared for significant differences; that is, 50% MPE for nociceptive impairment is not validly comparable to the 50% MPE for motor impairment.

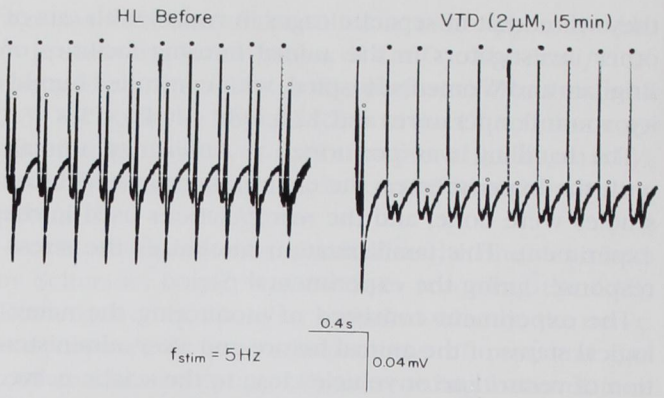

Fig. 1. A train of compound action potentials showing the peaks of elevations from A-fibers (●) and C-fibers (○). The stimulus was supramaximal for both fiber types and was applied at a frequency of 5 Hz. (The stimulus artifact overlaps the base of the A-fiber compound action potential and therefore cannot be detected.) Note the relative constancy of both types of elevations during the train in control, Hepes-Liley (HL) solution, contrasted with the preferential, large decrement in the C-fiber elevation after the first response of the train in the veratridine-incubated nerve.

A one-way analysis of variance (using SAS software; SAS Institute, Cary, NC) was performed to analyze the dose-dependent effect of veratridine on each function. The following effects between different doses of veratridine were compared based on the time to full recovery of each function. The duration of 50% and greater than 50% MPE of motor impairment, with the duration of 20% and greater than 20% MPE of nociceptive changes was compared. A Tukey's studentized multiple-range test and Duncan's multiple-range test were used as *post hoc* tests. Probability values are reported in the text. Probability values less than 0.05 were considered significant.

Results

Electrophysiologic Data

The elevations of the CAP from A-fibers and C-fibers could be separated by their conduction velocities, which were 3–5 m/s and 0.3 m/s, respectively (at room temperature), and by their different waveforms as detected in the records, with C-fibers having a more pronounced biphasic character than A-fibers (see figs. 1 and 2). Repetitive stimulation of sciatic nerves *in vitro*

at intensities that can excite both A-fibers and C-fibers led to a use-dependent reduction of the C-fiber elevation of the CAP after nerve equilibration (15 min) in 2-μm veratridine (fig. 1). Reduction of the C-fiber amplitude was accompanied by a slowing of the group conduction velocity (fig. 2 and fig. 4B). The area under the impulse was also reduced, indicating that the amplitude of single impulses and the number of activated individual fibers was decreased, in addition to the conduction being slowed. Little depression by veratridine of the A-fiber elevation of the CAP occurred (fig. 4A), and this required much higher stimulation frequencies at 50–100 Hz (data not shown).

The depression of the C-fiber component by 2 μM veratridine depended closely on the frequency of stimulation. Single volleys showed little depression (fig. 2), but with a train of ten stimuli, CAP depression reached 50% at 0.5 Hz and 75–80% at 5 Hz (fig. 3). Full return to control amplitudes occurred when repetitive stimulation was stopped.

The selective use-dependent depression of C-fiber elevations reached its full effect after a 15-min exposure to 2 μM veratridine, and began to recover after 5–10 min in veratridine-free solution (fig. 2 and fig. 4A). When two nerves were exposed for 30 min to vehicle alone (0.04% ethanol in HL), no tonic or use-dependent change in the CAP occurred.

Slowing of conduction accompanied veratridine's use-dependent decrease of the CAP, particularly at higher stimulus frequencies (figs. 2 and 3). Interestingly, although the C-fiber CAP amplitude recovered partially during the first 15 min of washout (fig. 4A), no reversal

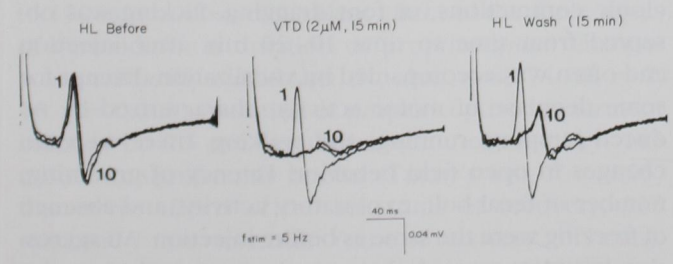

Fig. 2. Superimposed traces of compound action potentials from C-fibers stimulated supramaximally by ten stimuli at 5 Hz. The first and tenth CAP traces in the train are labeled. (*Left*) In HEPES-Liley (HL) solution. (*Middle*) After a 15-min exposure to 2 μM veratridine. (*Right*) After a 15-min wash in drug-free HL solution. The bottom portion of the truncated A-fiber elevation is on the left of each trace, and although its peak is off scale at this gain (to measure the C-fibers), the parts of the trace that are visible closely superimpose at 5 Hz stimulation.

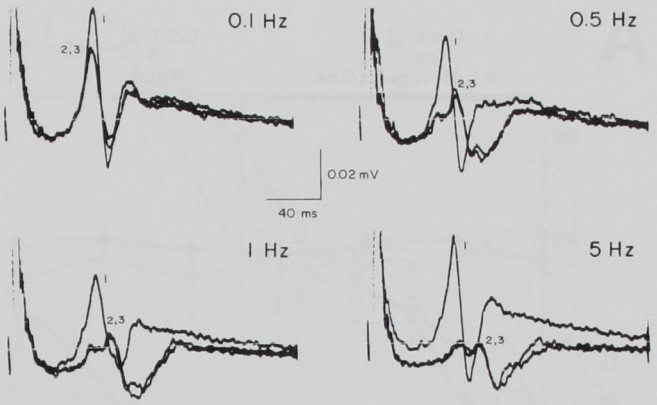

Fig. 3. Frequency dependence of C-fiber elevations. Nerves had been exposed to 2 μM veratridine for 10–15 min and then stimulated supramaximally at the frequencies noted on each panel (0.1–5 Hz). The number next to each trace identifies the particular action potential in a set of three. The steady state of use-dependent reductions was reached after three stimuli.

of the conduction slowing occurred (fig. 4B). Conduction velocity of the A-fiber CAP was insignificantly changed by 2 μM veratridine (data not shown).

Neurobehavior

Every injection of veratridine caused vocalization by the rats as soon as the skin was penetrated by the hypodermic needle, a reaction that never occurred with lidocaine.[7] This initial vocalization was followed by intermittent periods of squealing for 5–10 min. Besides vocalization, sometimes the rats also licked the injection site. The rapid development of directed licking and the absence of generalized behavioral abnormalities excluded the possibility that this behavior results from systemic veratridine.

Posture and gait were affected before any other visible functional changes, and their impairment (particularly the posture of toes) lasted longer. Changes in resting posture and in gait were observed within the first minute after veratridine injection. At 1 min the toes became first fully extended, ventroflexed, and curled; the stifle was extended and the hock dropped. The magnitude of these changes appeared to increase with increasing dose and to progress in time.

After injection of 0.5 mM veratridine in all animals, muscular twitches were elicited by locomotion involving the proximal limb and thigh muscles, without any involvement of the distal limb muscles innervated by the sciatic nerve, for up to 25 min. Injection of 0.7 mM veratridine induced intermittent spontaneous twitches and some tonic contrac-

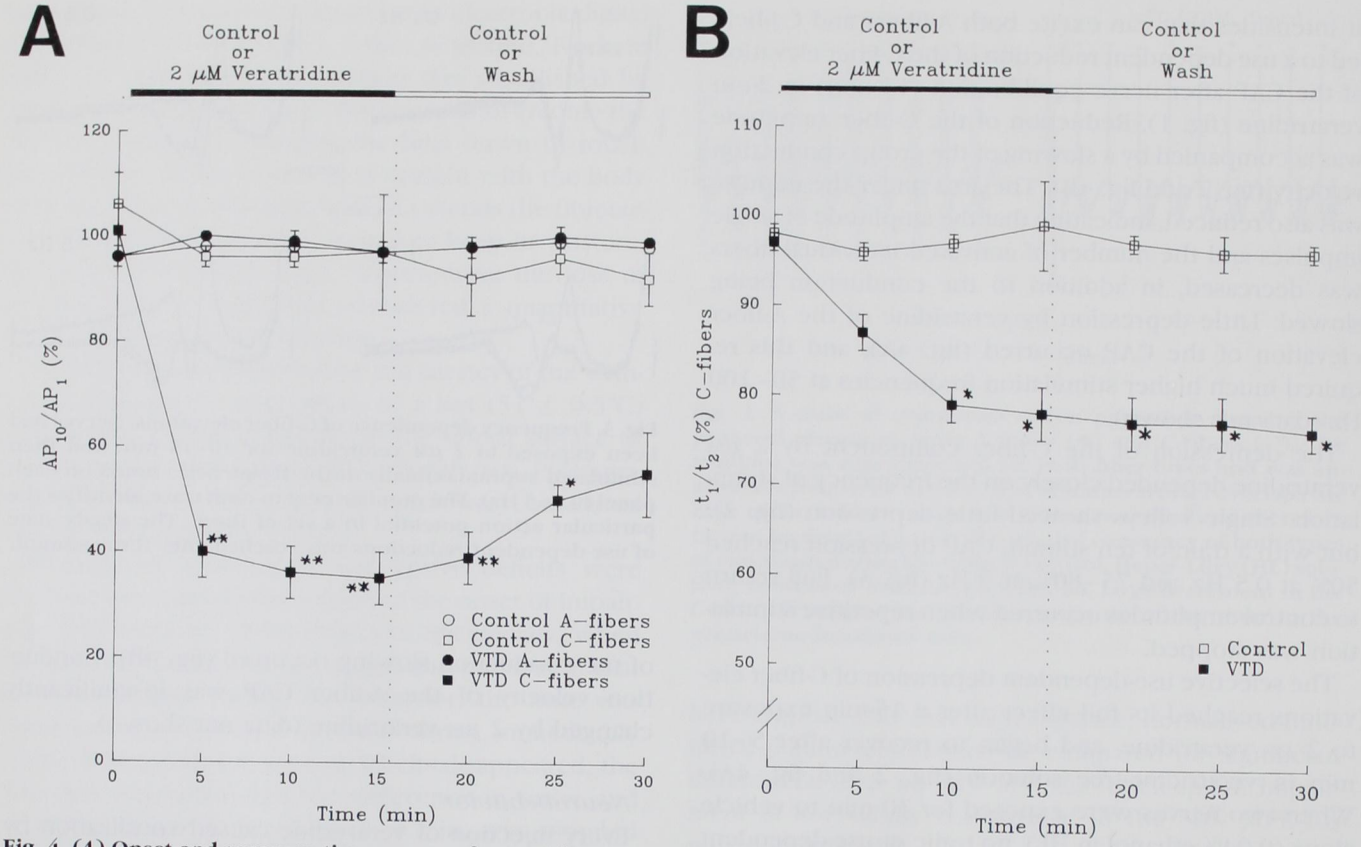

Fig. 4. (*A*) Onset and recovery time course of veratridine actions on isolated rat sciatic nerve. Compound action potentials in C-fibers (stimulated at 5 Hz) and A-fibers (stimulated at 50 Hz) were counted by the ratio of peak amplitudes of the tenth to the first action potential in a train (see figures 1 and 2, for example). For veratridine-exposed nerves, n = 5; for control nerves, n = 4. *$P < 0.05$, **$P < 0.01$, compared with control. (*B*) Ratio of the time to peak, after the stimulus artifact of the first to the second C-fiber elevations from a pair of stimuli separated by 0.2 s. Data were taken from traces such as those in figure 2. Veratridine-exposed nerves, n = 5; control nerves, n = 4. *$P < 0.05$.

tion of the thigh muscles, which was combined with curling of the toes. Injection of 1.0 mM veratridine produced clonic contractions of thigh muscles immediately after injection, simultaneously with flaccid paralysis of the tarsus. The magnitude of visible local muscle twitches was greatest at the site of injection.

Changes in gait were dose dependent: The entire plantum of the injected limb touched the supporting surface with more weight bearing on the lateral margin (0.5 mM veratridine) or with weight bearing on the knuckled paw (0.7 mM veratridine). With 1 mM veratridine, the hip became more flexed, preventing the leg from dragging on the supporting surface.

Observation under conditions of free behavior showed that throughout the testing period the animals exhibited normal exploratory activity and were responsive to the environment. The rats appeared to pay no specific attention to any functional impairment or muscle twitching. No correlation could be found among rare licking of the injection site and muscle twitching, clonic contractions, or foot dragging. Licking was observed from time to time 10–20 min after injection and often was accompanied by vocalization. Except for some decrease of motor activity, characterized by reduced jumping, running, and walking, there were no changes in open field behavior. Latency of grooming, number of fecal boli, exploratory activity, and absence of freezing were the same as before injection. An aggressive reaction toward the experimenter and to an intruder rat also was absent.

In control studies, no neurological changes could be detected in rats injected with saline (n = 6) or with saline plus dimethylsulfoxide with or without epinephrine (n = 6). The rats did not vocalize on injection or lick the injected area, and no local fasciculations were observed.

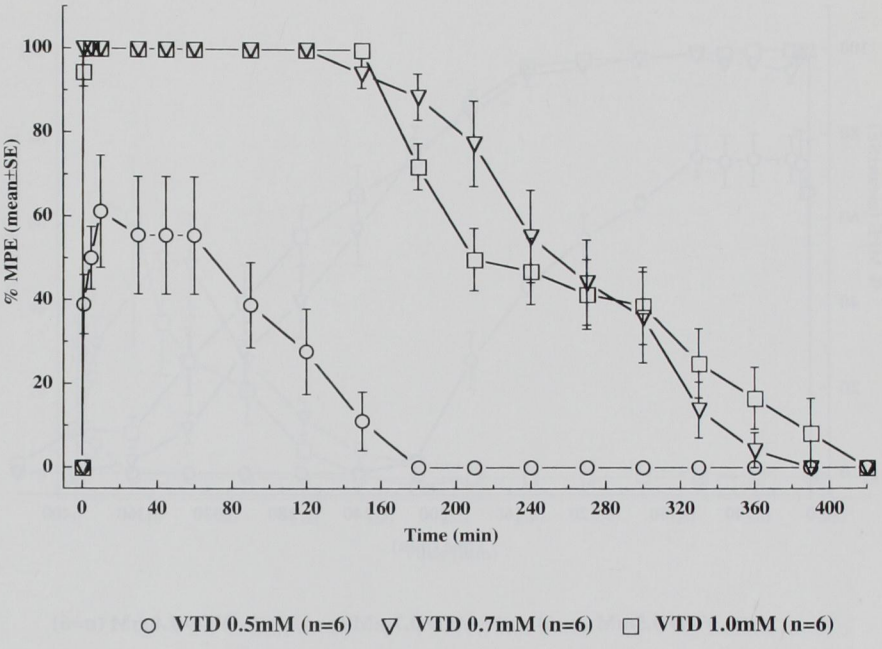

Fig. 5. The time course of proprioceptive deficits caused by veratridine: (○) 0.5 mM (n = 6); (▽) 0.7 mM (n – 6); and (□) 1 mM (n = 6). All doses give blocks at 5 min that persist for 145 min after 0.5 mM veratridine, for 335 min after 0.7 mM veratridine, and for 345 min after 1 mM veratridine, differing significantly (P = 0.05) only between 0.5 mM and other doses. Time recovery data for proprioceptive function after 0.7 mM and 1 mM were not significantly different from each other.

Proprioception. Unilateral changes in resting posture and gait were accompanied by changes in postural reactions of the injected leg as early as 1 min after injection of veratridine in all animals. The "tactile placing" and "hopping" responses were partially impaired with 0.5 mM and absent with 0.7 mM and 1 mM at the first minute after injection (fig. 5). For 0.5 mM veratridine, maximal changes of percentage MPE reached 61.1% ± 13.4% (means ± SE) within 10 min. Complete block of proprioception after 0.7 mM lasted for 150 min. Similarly, administration of 1 mM veratridine caused complete absence of postural reactions from 5 to 150 min. A dose-dependent effect of the three doses of veratridine on duration of proprioceptive changes was statistically significant (F value = 83.74; df = 2, 15; P = 0.001). Duration of proprioceptive impairment induced by 0.5 mM veratridine (time of recovery, 145 min) was significantly briefer (Tukey's test, P = 0.05) than were effects of 0.7 mM (time of recovery, 335 min) and 1 mM veratridine (time of recovery, 345 min). Duration of proprioceptive impairment after 0.7 mM and 1 mM veratridine were not significantly different from each other. Apparently the postural reactions were fully inhibited by the intermediate dose of veratridine (0.7 mM) and, interestingly, duration of effect did not increase with dose.

Motor Function. As soon as proprioceptive deficits were apparent, locomotor activity also decreased during free behavior, and a diminution of extensor postural thrust could be detected in all veratridine-injected animals (fig. 6). As with the impairment of proprioception, the first changes of postural thrust were observed within 1 min after injection with all three doses of veratridine; the maximal effects from 0.7 and 1 mM were reached at 1 min, whereas the maximal effect from 0.5 mM was reached at 5 - 10 min.

The amplitude and duration of motor impairment were dose dependent. The greatest inhibition of postural thrust after injection of 0.5 mM veratridine, 77.1% ± 5.1%, was reached at 10 min; 99% ± 0.8% inhibition was reached at 1 min after 0.7 mM, and 99.9% ± 0.1% inhibition was reached at 1 min after 1 mM. One-way analysis of variance was performed to compare times of recovery of the motor effects induced by three doses of veratridine. Motor function was restored after 210 min from 0.5 mM, after 335 min from 0.7 mM, and after 350 min from 1 mM veratridine (F value = 36.55; df = 2,15; P = 0.0001). A Tukey's studentized multiple-range test (P = 0.05) showed that time of recovery after 0.5 mM veratridine was significantly less than after 0.7 and 1 mM veratridine. Times of recovery of motor function after 0.7 mM veratridine and after 1 mM veratridine were not significantly different from each other. Impairment of motor function to 50% and more of MPE lasted 138 ± 14 min after 0.5 mM veratridine; 255 ± 13 min after 0.7 mM veratridine; and 275 ± 18 min after 1 mM veratridine.

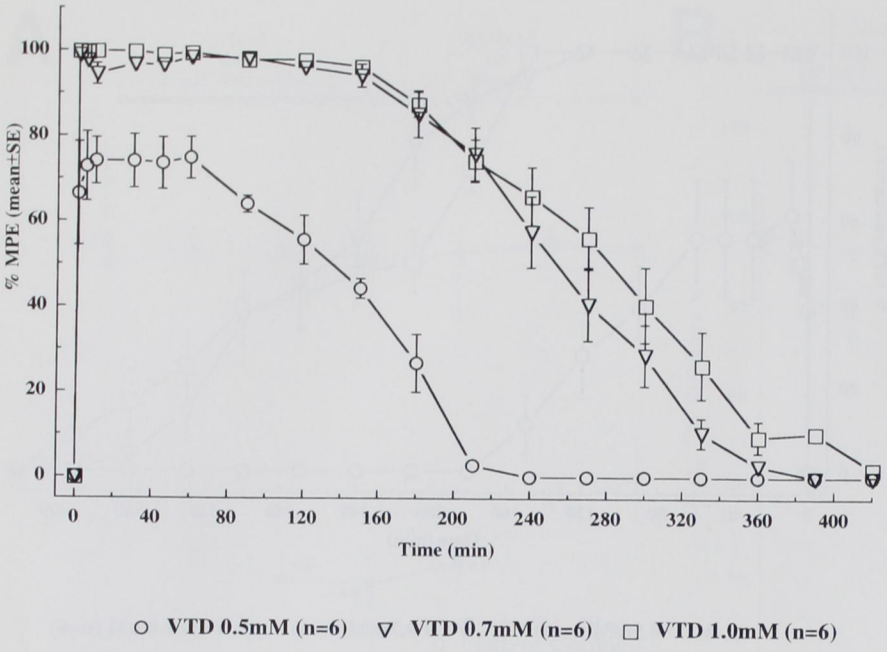

Fig. 6. The time course of motor deficits after veratridine injection. Weakness in extensor postural thrust occurred as early as the first test (5 min) and persisted for 210 min after 0.5 mM veratridine (○), for 335 min after 0.7 mM veratridine (▽), and for 350 min after 1 mM veratridine (□). Motor block was significantly (P = 0.05) briefer after 0.5 mM than after 0.7 mM and 1 mM. The difference between duration of motor block after 0.7 mM veratridine and 1 mM veratridine was not significant.

Statistical analysis of this parameter confirmed the dose-dependent effect of veratridine on motor function (F value = 24.23; df = 2.15; P = 0.0001), but, again, only the effect of 0.5 mM veratridine was significantly different (Duncan's test) from effects of 0.7 mM and 1 mM veratridine. As with proprioception, the inhibition of motor function was saturated at the higher doses of veratridine.

Nociception. The incremental increase of the withdrawal latencies of responses to noxious heat stimulation was dose dependent, but these changes were not as pronounced or as persistent as proprioceptive and motor inhibition, although they could be detected in all animals. The lowest veratridine dose (0.5 mM) resulted in a minor inhibition of nocifensive reactions (fig. 7), reaching 10.2% ± 4% at 1 min and fully recovering after 10 min. Animals receiving 0.7 mM veratridine showed some increase of the latency to the heat stimulation of the lateral tarsus, considered as mild analgesia. The maximal analgesic effect of 0.7 mM veratridine, 52.1% ± 13.4%, was reached at 45 min. The average of maximal nociceptive changes after 1 mM veratridine reached 66.3% ± 8.6% at 10 min.

Veratridine produced dose-dependent effects on nociceptive function (f = 9.56; df = 2,15; P = 0.0021). However, the duration of nociceptive inhibition did not increase monotonically with veratridine dose. Although 0.5 mM veratridine gave the shortest duration of nociceptive inhibition (10 min), 0.7 mM gave the greatest duration (195 min) and 1 mM veratridine yielded gave an intermediate duration (93 min).

In every animal, antinociception after 0.5 mM veratridine never exceeded 20% of MPE. Nociceptive impairment of 20% and more of MPE lasted 58 ± 20 min after 0.7 mM veratridine and 60 ± 10 min after 1 mM veratridine. Statistical analysis of this parameter established the dose-dependent effect of veratridine on nociceptive function (F value = 5.80; df = 2.15; P = 0.0136). The effect of 0.5 mM veratridine was significantly different (Duncan's test) from effects induced by 0.7 mM and 1 mM veratridine.

Only one rat showed 100% MPE of analgesia (fig. 8); that is, there was no response to heat or even to pinch for 20 min at the highest concentration of veratridine (1 mM). But even in that rat, impairment of the nocifensive response was much briefer than the complete blocks of other functions: proprioception at 180 min and motor function at 120 min.

There was a simultaneous increase of the withdrawal latency to heat stimulation at the medial margin of the injected limb's metatarsus, which is innervated by the saphenous nerve, of the noninjected hindlimb (fig. 8), and even of the forelimbs. However, any difference between inhibition of the nocifensive reflex in contralateral hindlimbs by 0.7 and 1 mM veratridine did not reach statistical significance. Injection of 0.5 mM veratridine did not cause any

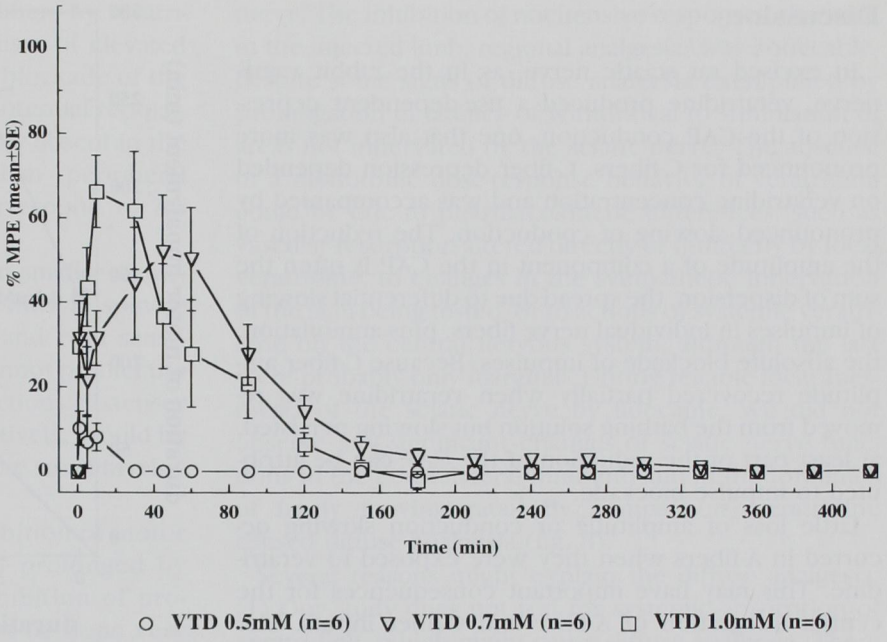

Fig. 7. Time course of antinociceptive actions of veratridine. The withdrawal response latency to movement of the hindpaw away from a 51°C probe was normalized by a cut-off latency of 10 s and a baseline latency of 2–3 s. Only duration of nociceptive block after 0.5 mM veratridine (○) for 10 min and after 0.7 mM (▽) for 195 min differed significantly. There is no significant difference of nociceptive effects among the doses of 0.7 mM and 1 mM (□) for 93 min.

impairment of the response to heat in the noninjected foot.

Skin Temperature

The SkT of the hairy skin of the injected hindlimb increased after 0.7 and 1.0 mM veratridine administration. The maximal changes (percentage MPE; see Methods for the scale) measured at heel (H) and toe (T) after the injections were 0.7 mM veratridine, +17 ± 4.2 at 30 min (H) and +9.3 ± 1.5 at 45 min (T); 1 mM veratridine, +13.2 ± 1.2 at 60 min (H) and 30.2 ± 7.7 at 60 min (T). The elevations of SkT disappeared by 300 min after injection of 0.7 mM veratridine but were still present 360 min after 1 mM veratridine.

The largest of these changes in percentage MPE, 30% ± 7.7%, corresponds to a temperature increase of 4.7 ± 1.1°C.

Reversibility. All neurobehavioral changes induced by veratridine in the doses studied were completely reversible.

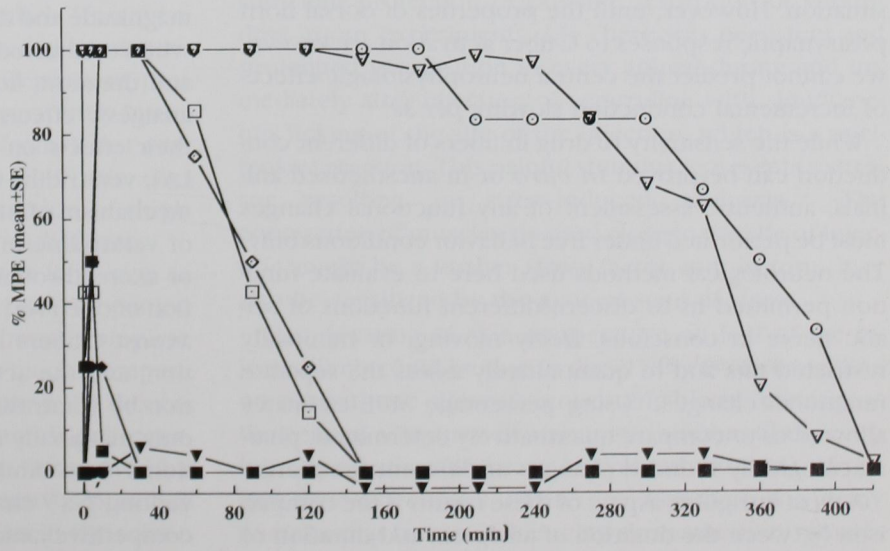

Fig. 8. Time course of functional impairment in ipsilateral and contralateral limbs induced by 1 mM veratridine in the one rat that achieved 100% analgesic effect with veratridine. Proprioceptive reactions were completely inhibited in the injected limb (○) but not in the noninjected limb (●). Complete motor block in the injected hindlimb (▽) was not accompanied by equal changes of motor function of the noninjected hindlimb (▼). Inhibition of the heat response (□) and the pinch response (△) of the injected limb was accompanied by lesser and briefer inhibition of the heat (■) and pinch (▲) responses of the noninjected limb.

Discussion

In excised rat sciatic nerve, as in the rabbit vagus nerve, veratridine produced a use-dependent depression of the CAP conduction, one that also was more pronounced for C-fibers. C-fiber depression depended on veratridine concentration and was accompanied by pronounced slowing of conduction. The reduction of the amplitude of a component in the CAP is often the sum of dispersion, the spread due to differential slowing of impulses in individual nerve fibers, plus annihilation, the absolute blockade of impulses. Because C-fiber amplitude recovered partially when veratridine was removed from the bathing solution but slowing persisted, at least part of the reduction of the CAP can be attributed to impulse blockade.

Little loss of amplitude or conduction slowing occurred in A-fibers when they were exposed to veratridine. This may have important consequences for the central integration of A-fiber and C-fiber inputs. The simultaneous activation of tactile A-fibers and nociceptive C-fibers blunts the response of dorsal horn neurons to the latter.[11] However, the temporal relation between the arrival of these two signals that are required for such primary afferent inhibition to be effective is not known. Therefore the neurological consequences of differential conduction slowing cannot be predicted. If the effective exposure length *in vivo* is like that *in vitro* (11 mm) and C-fiber conduction slows by approximately 30 ms at the end of a train *in vitro* at 20–22°C (fig. 2), where the calculated group conduction velocity is 0.3 m/s, then the delay in the arrival time of the last impulse might be 15 ms compared with the drug-free situation. However, until the properties of dorsal horn postsynaptic responses to C-fiber activation are known, we cannot predict the central neurophysiologic effects of incremental conduction slowing *per se*.

While the sensitivity to drug in fibers of different conduction can be proved *in vitro* or in anesthetized animals, authentic assessment of any functional changes must be performed under free behavior conditions only. The neurological methods used here to evaluate function permitted us to discern different functions of sciatic nerve in conscious, freely moving, or minimally restricted rats and to quantitatively assess the separate functional changes.[7] Using percentage MPE estimates allowed us to compare quantitatively determined, pharmacologically induced changes of different functions.

A most intriguing aspect of these results is the comparison between the duration of analgesia and duration of motor block induced by veratridine (figs. 6, 7, and 8). This comparison shows the preferential inhibition by veratridine of motor function over nociception in both magnitude and duration. The comparison is noteworthy when contrasted to the effects of LAs. In terms of depth and duration, lidocaine[7] and bupivacaine (fig. 9) have analgesic effects that are as strong and act as long as their effects on motor activity. As with all traditional LAs, veratridine affects sodium channels. However, the mechanism of impulse inhibition at low concentrations of veratridine involves a use-dependent depolarization of axons through the discrete binding to and stabilization of open Na^+ channels.[12–15] Among peripheral nerve axons, C-fibers are particularly susceptible to this action, and the accumulating, use-dependent depolarization by veratridine leading to impulse blockade occurs only marginally in other fiber types.[6] In contrast, traditional LAs inhibit impulses mechanistically by preventing Na^+ channels from opening; in fact, LAs are competitive antagonists of veratridine on Na^+ chan-

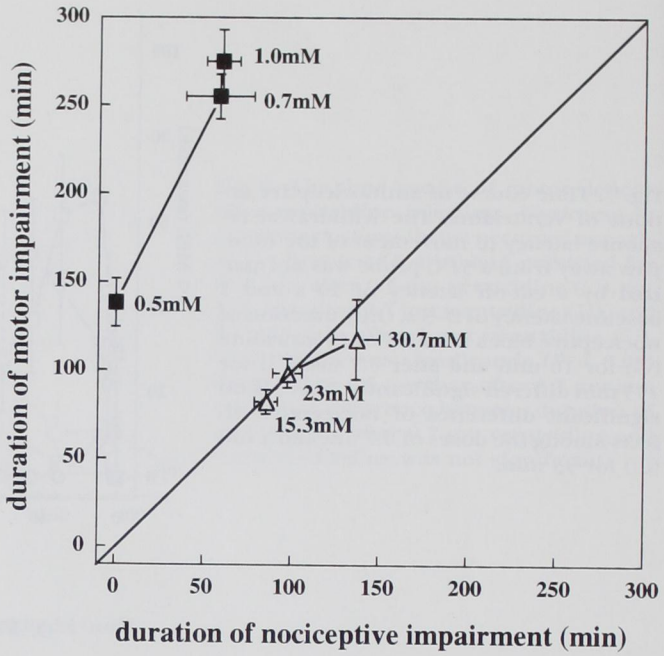

Fig. 9. Comparative duration of impaired motor function and impaired nociception in rats injected at the sciatic nerve with bupivacaine (△) or veratridine (■). Sciatic blocks were performed identically with either drug, using 0.1 ml and 1% (30.7 mM), 0.75% (23 mM), or 0.5% (15.3 mM) commercial bupivacaine (Marcaine; Astra Pharmaceuticals; Westborough, MA) solutions. The 45 degree line of equivalence shows the locus for blocks of equal duration. Location of veratridine points high in the upper quadrant indicates clearly the motor selectivity of this drug.

nels.[16-18] Selective depolarization of C-fibers by veratridine has been explained by a mechanism of elevated intracellular Na^+, leading to molecular blockade of the K^+ channels that contribute to action potential repolarization.[6] Such K^+ channels are apparently absent in the large myelinated axons of mammalian peripheral nerve,[19,20] axons whose diameters correspond to Aα motor fibers and Aβ tactile fibers.

The presence of these K^+ channels in smaller-diameter myelinated fibers, which maintain muscle spindle tone (Aγ-fibers) or conduct first pain and cold sensations (Aδ-fibers), is not known, so we cannot predict the selectivity of veratridine for these functions. Extensor postural thrust and nociception, respectively, would be susceptible to impulse disruption in these small myelinated Aγ-fibers and Aδ-fibers.

Proprioceptive impairment and inhibition of motor function were most pronounced and prolonged by veratridine. The differentiation of inhibition of proprioception from motor function must be done very carefully because these two functions are related. Both functions are normal only when both afferent (proprioceptive) and efferent (motor) activities are fully preserved. Careful observation of the phases—initiation and follow-through—of the "hopping" response is helpful for conjectural differentiation. Poor initiation of the "hopping" reaction suggests sensory (proprioceptive) deficits, whereas poor follow-through implies motor deficits (paresis).[21] Therefore there was "behavioral" evidence for the prevalent impairment of motor *versus* proprioceptive function by veratridine. In our experiments, the degree of impairment of both functions and the kinetics of their inhibition appeared to be closely parallel. However, muscle contracture and twitching induced by veratridine made it difficult to properly differentiate proprioceptive and motor changes. The apparently faster recovery of proprioceptive function from 1 mM *versus* 0.7 mM at the time interval 180–240 min (fig. 5) may be a result of the tonic contraction of thigh muscles, which is more pronounced with 1 mM.

With increasing doses of veratridine, the inhibition of motor and proprioceptive functions were accompanied by signs of nociceptive impairment. Although complete withdrawal was abolished by the motor block, comparison of the delays of incomplete withdrawal (by flexion in the hip) to noxious stimulation of lateral (*i.e.*, innervated by nerve sciatic) and medial (*i.e.*, innervated by nerve saphenous) margins of the metatarsus provides evidence for some nociceptive inhibition in the sciatic nerve. The inhibition of nocifensive responses observed in the injected limb, regional analgesia, was noticeable, despite some signs of diffuse analgesia exemplified by prolongation of latency of withdrawal to stimulation of areas not innervated by the sciatic nerve. The absence of a monotonic dose-response behavior of veratridine could be due to pharmacokinetic differences, such as vascular resorption altered directly or indirectly by local veratridine, to changes in the sympathetic innervation of the skin being tested, or to actions of systemic veratridine on the central nervous system, although the last were probably only marginal. During reliable local anesthesia of the sciatic nerve by lidocaine, we did not observe any significant changes of sciatic nerve functions in the contralateral hindlimb and in the forelimbs of freely moving rats.[7] By comparison, veratridine caused diffuse analgesia (fig. 8).

Several reasons might explain the diffuse analgesia. (1) Our study does not test for systemic absorption of veratridine, which might cause diffuse analgesia. There are two indirect arguments against the resorptive effect of veratridine, however. First, there was an immediate onset of diffuse analgesia after the injection. Second, no other signs of resorption, such as any changes of behavior, were seen. We observed only a minor decrease of voluntary locomotion under open-field conditions, which is compatible with our observations of unilateral block by LAs[7] or after transection of sciatic nerve (Thalhammer and Vladimirov, unpublished data). It is not a result of resorption but rather relates to the decrease of motor function of the injected leg. Therefore the addition of epinephrine appeared to provide sufficient protection of possible resorption of veratridine in our experiments. (2) There was persistent and prolonged vocalization in every animal during and immediately after injection of veratridine with simultaneous licking of the site of the injection, which is a nocifensive reaction. This painful stimulus represents a stressor resulting in stress-induced analgesia.[22] The contraction of muscles situated close to the site of injection might be a further stress factor and, in turn, may also be sensitized by the general state of stress.

The elevation of skin temperature on feet of the injected limb could be due to direct effects of veratridine on sympathetic efferents innervating blood vessels plus the general activation of autonomic afferent fibers causing a generalized increase in sympathetic tone of the posterior extremities. There are, however, too many contributing factors influencing sympathetic responses

for us to isolate one as the singular cause of elevated skin temperature.

The presence of clonic contractions cannot be explained by the actions of veratridine on the sciatic nerve, because they do not occur in any muscles innervated by the sciatic nerve distal to the site of injection, but in muscles close to injection site only. This suggests that such a focal response of musculature is likely to arise either from the direct action of veratridine on the muscular membrane[12,13,23] or from the action of veratridine in very high concentrations on small regional nerve branches that innervate these muscles.

Conclusions

The effect of veratridine on isolated nerve is a preferential, frequency-dependent inhibition of the C-fiber component of the sciatic nerve CAP, confirming the preferential inhibition originally reported in the rabbit vagus nerve.

In conscious, handled rats veratridine injected around the sciatic nerve results in significant motor and proprioceptive block with little nociceptive inhibition, although it is accompanied by signs of diffuse analgesia and by muscle twitching close to the site of injection. All of these effects were dose dependent.

The results of the neurobehavioral evaluation of locally administered veratridine is generally compatible with electrophysiologic findings: Veratridine leads to impairment of neural functions *in vivo* and to the selective depression of certain elevations of the CAP *in vitro*, although the expected preferential inhibition of nociception was not observed. The nature of differential functional block is too complex to be explained through electrophysiologic data from isolated nerves alone.

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