After-Potentials and Large Depolarizations of Single Nodes of Ranvier Treated with Veratridine

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ABSTRACT Potential differences between normal nodes of Ranvier (single fiber from the sciatic nerve of the frog, air-gap method) and a node exposed to 1 to 2.5 × 10^{-8} gm veratridine per ml were measured. Negative after-potentials occurred immediately after application of the alkaloid when spike configuration and resting potential were virtually unchanged. The after-potentials decreased in magnitude and their time constant increased as the resting membrane was depolarized either by outward currents or by a train of impulses. Increase of (Na), markedly increased the amplitude of the after-potential. After prolonged application of veratridine or with higher concentrations, a large slow depolarization (rate of potential change about 7 mv per second) could be triggered by a train of impulses or even a single spike. This depolarization could promptly be terminated by withdrawing Na. It is concluded that, once the nodal membrane has become permeable to Na (as during a spike), veratridine prevents the normal return of $P_N$ to its resting value.

The action of veratrine on excitable cells and tissues has been studied for a long time (see review by Krayer and Acheson, 1946, and for more recent work see Shanes, 1958). In spite of this, the nature of the action of the alkaloids is not well understood. Straub (1956) studied the effect of veratridine on the permeability of the resting membrane in bundles of myelinated nerve fibers. His most important finding was that of an increased permeability to sodium resulting in spontaneous depolarization with high concentrations of the alkaloid. However, marked effects are seen with alkaloid concentrations that cause little or no change in resting membrane potential. The question arises whether these effects can be ascribed to the same mechanism which is affected at higher drug concentrations and results in spontaneous depolarization. Also, the limitations of multifiber preparations are well known, and only two studies have been reported in which veratrine has been
applied to single nerve fibers. Tasaki and Mizuguchi (1949) measured the impedance of veratrine-treated myelinated frog nerve fibers, and Hodler et al. (1950) reported long lasting after-currents and exponential decline of action currents during repetitive stimulation of single myelinated nerve fibers in veratrine. The technique of the single superfused node of Ranvier (Stämpfli, 1956, 1959) is especially suited for experiments concerning drug actions since it combines measurement of potential changes with the possibility of rapidly applying various experimental solutions directly to the node under study. Factors such as access of the drug to its site of action and control of drug concentration at the site of action are minimized if the site of action is likely to be on the outer surface of the nodal membrane.

The present investigation makes use of this technique to study the effect of a pure alkaloid, veratridine. Membrane potential during rest and activity was observed in the presence of different concentrations of the alkaloid and with solutions of different ionic composition. It will be shown that the prolonged after-potentials following a spike and large depolarizations are both linked to long lasting changes in \( P_{Na} \), the membrane permeability to sodium ions. The after-potential appears to be due to an unusually long sustained increased \( P_{Na} \) following the spike, while the large depolarizations are the result of a further slow increase in this permeability.

**METHOD**

Single motor fibers were isolated from the sciatic nerve of *Rana pipiens* and were transferred to a chamber described in detail by Stämpfli (1959). The node under investigation was placed in a small slit across a thin polyethylene tube (inside diameter 0.6 mm) which was connected to a stopcock with minimal dead space. In this arrangement the node was continuously superfused. The neighboring nodes and the rest of the nerve trunk were situated in larger side pools containing cocaine-Ringer's solution and were separated from the tube by air gaps. Electrical contact to the pools and the tube was made through KCI-agar bridges by means of Ag-AgCl electrodes. Stimulating and lasting subthreshold currents were applied between one side pool and the tube which was held at ground potential. Membrane potentials were recorded between ground and the other side pool by a negative-capacitance electrometer amplifier in connection with a double beam oscilloscope. The finite resistance of a small fluid film around the internode exposed to air caused an attenuation of about 20 per cent. Potentials given in text and figures were not corrected for this short-circuiting.

The normal Ringer's solution contained (in mM): NaCl 111.3, KCl 2.5, CaCl₂ 2.0, and NaHCO₃ 2.5. In sodium-deficient solutions NaCl was replaced by equivalent amounts of choline chloride. High Na solutions were prepared by adding appropriate amounts of crystalline NaCl to Ringer's solution. A stock solution of veratridine in a concentration of 1 mg per ml (1.49 mM) was prepared under addition of equimolar amounts of hydrochloric acid. The desired final concentrations were prepared from this stock solution before each experiment.
RESULTS

Apart from producing after-potentials the early effects of moderate concentrations of veratridine (10^{-6} to 2.5 \times 10^{-6} gm per ml) on nodes of Ranvier were usually small. The resting potential either remained unchanged or a slight depolarization up to 6 mv was observed. Occasionally a small decrease of spike amplitude was seen. Hodler et al. (1950) reported a 10 per cent decrease on singly evoked action currents in 1.5 \times 10^{-6} gm veratrine per ml. One may conclude that veratridine can produce after-potentials with little or no change of spike configuration.

With inward currents lasting 1 to 2 seconds current-voltage curves were measured and the constant slopes of their anodal portions were determined to obtain information on the resting membrane resistance. In 8 fibers it was found to be 123 \pm 15 per cent (se) of the resistance in normal Ringer's solution. This increase is not statistically significant (P > 0.1). However, Tasaki and Mizuguchi (1949) also observed a 15 per cent increase in 5 \times 10^{-5} gm veratrine per ml.

(A) After-Potentials

1. AMPLITUDE AND DURATION

Spikes elicited soon after the application of veratridine (10^{-6} to 2.5 \times 10^{-6} gm per ml) were followed by an after-depolarization ("negative" after-potential). The after-potential had an amplitude of a few millivolts and a half-time of decline of 1 to several seconds. However, there was considerable scatter among individual preparations. It was observed that the after-potentials depended on the resting potential: their amplitude decreased and the duration increased as the resting membrane was depolarized. This is illustrated by Fig. 1 which shows the superposition of three after-potentials at high gain and slow sweep speed. In this experiment the change in membrane potential was brought about by the flow of appropriate currents; however, it will be shown later that current flow per se was not responsible for this effect. About 0.3 second after their start the after-potentials declined exponentially and the time constants given in the legend to Fig. 1 refer to this exponential portion. If the membrane was further depolarized a critical potential was reached at which the after-potentials vanished; i.e., there was no difference in the membrane potential immediately before and after a spike. The dependence of the after-potential on the resting potential was probably the main cause of the scatter mentioned.

2. MEMBRANE RESISTANCE DURING AFTER-POTENTIAL

In the manner described above the membrane resistance 4 milliseconds after the peak of an action potential was determined in 8 fibers and expressed as
percentages of the resting resistance measured at the same potential in the respective solution. The resistance during the after-potential was 88 ± 2 per cent (SE) in Ringer's and 70 ± 5 per cent in veratridine-Ringer's, the difference being significant \((P < 0.02)\).

**Figure 1.** Dependence of after-potential on resting potential in \(10^{-6}\) gm veratridine per ml. Resting potential displaced by constant current; depolarization upward. Spike potentials not seen because of slow sweep speed. Maximum value of after-potentials (from top to bottom) 4.0, 5.4, and 7.0 mv. Corresponding time constants of decline 2.6, 1.14, and 0.6 seconds. 28°C. 2-13-64 II.

**Figure 2.** Effect of \((\text{Na})_0\) on after-potential. Only lower portions of action potentials seen; depolarization upward. 1 Na = 113.8 mM NaCl, 2 Na = 227.6 mM NaCl. After-potentials in 2 Na photographed 15 seconds after change to this solution. 27°C. 2-18-64 I.

### 3. EFFECT OF NA IONS

Since it is known that Na ions are essential for the spontaneous depolarizations in veratridine (Straub, 1956), the effect of these ions on veratridine after-potentials was studied. In order to maintain the nodes fully excitable an increase in \((\text{Na})_0\) was preferred to a decrease. Solutions of increased \((\text{Na})_0\) were applied for a maximum of 15 seconds and caused an immediate increase in spike height. Hypertonicity effects are known to be small for this short application (Stämpfli, 1956; Frankenhaeuser and Moore, 1963). Fig. 2 shows that twice the normal \((\text{Na})_0\) depolarized the resting membrane by
about 4 mv in the absence of veratridine (left traces). This is what one would expect if there is a small but definite Na permeability of the resting membrane. In $10^{-4}$ gm veratridine per ml and normal Na content the membrane was depolarized by about 6 mv and there was a small but clearly visible after-potential. A change to $2 \times (\text{Na})_o$ in the presence of veratridine markedly increased the after-potential in spite of a further depolarization of the resting membrane. This suggests that veratridine not only induces after-potentials by increasing $P_N$ but also, to a smaller extent, increases the $P_{Na}$ of the resting membrane. This conclusion is in agreement with an increased hyperpolarization by Na-free solutions in the presence of the alkaloid (Straub, 1956) and does not necessarily contradict the finding of a raised membrane resistance at rest, as will be discussed later.

![Figure 3](image_url)

**Figure 3.** Summation of after-potentials. Left column, in normal Ringer's solution, right column, in $10^{-4}$ gm veratridine per ml. Spike potentials not seen because of slow sweep speed; depolarization upward. Rate of stimuli on right hand margin. Second stimulus at 7 per second in veratridine failed. 1-28-64.

### 4. Summation of After-Potentials

A spike potential elicited during an after-potential was itself followed by an after-potential which, although of smaller magnitude, was added to the remainder of the preceding one. Therefore, a marked summation of after-potentials occurred during a train of spikes as shown in Fig. 3. Because of the long duration of the veratridine-induced after-potentials summation was observed even with fairly low stimulation frequencies (4 per second and less). The bottom trace of the right hand column in Fig. 3 shows that each impulse caused a smaller increment in net after-potential as the membrane was depolarized. This is essentially the same finding as in Fig. 1, the difference being that the depolarization in Fig. 3 resulted from summation of after-potentials.

In a few cases both means of depolarization were studied in the same preparation. If allowance is made for the voltage drop across the membrane resistance during the after-potentials, depolarization by current flow yielded the same amplitudes of after-potential as a function of “resting” potential as depolarization during a train of impulses.
For a closer study of the summation phenomenon action potentials during a train were photographed on moving film and the difference between the membrane potential immediately before each spike and the original resting potential was plotted against time from the beginning of the train as in Fig. 4. This figure demonstrates the tendency of the membrane potential to reach a steady state value during a train; it also shows that this stationary potential corresponds to a larger depolarization as the repetition rate was increased. An interesting, although still unexplained, feature of the curves in Fig. 4 is the hump after an initial exponential part which is most clearly seen in the curve for 10 stimuli per second and which was repeatedly observed.

Because of the slow time base employed to obtain Fig. 3 the spikes did not show on the print. However, the photographic records underlying Fig. 4 showed that during a train the spike amplitude decreased as the membrane became more depolarized. Depolarizations caused by summation of after-potentials reduced the spike height to virtually the same extent as those caused by appropriate currents lasting several tens of milliseconds.

The present study did not allow for the establishment of a correlation between the height of an action potential and the size of its after-potential, so that for the time being only some limiting remarks can be made. There is no after-potential following a subthreshold response. During a train of impulses at a rate of 10 or 20 per second the action potentials could decrease to half their normal amplitude or even less without a change in their after-effects; i.e., these small spikes are sufficient to produce after-potentials which kept the membrane depolarized while there was an immediate repolarization upon cessation of the stimuli.
(B) *Large Depolarizations*

The effects of veratridine described thus far were observed during the first one-half to 1 hour of application. For longer periods of contact, or if higher concentrations of the alkaloid were applied, the nodal membrane tended to undergo large depolarizations. In general, these depolarizations were triggered by a train of spikes or even a single action potential. An example is given in Fig. 5. Here, after a single spike the membrane potential first fell to a value corresponding to the beginning of a large after-potential, but instead of repolarizing the membrane started to depolarize slowly. During the following sweep the perfusate was switched to a solution containing the same concentration of veratridine but in which 98 per cent of the Na was replaced by choline chloride. The immediate effect of this solution was a fast repolarization followed by a slower phase and terminating in a hyperpolarization with respect to the membrane potential prior to stimulation.

Fig. 5 also reveals a slight tendency of the membrane, once depolarized, to repolarize spontaneously as can be seen in the upper trace before the change of solutions. Spontaneous repolarizations were occasionally observed, especially in preparations whose resting potential was low after prolonged treatment with veratridine and which had to be repolarized by an inward current to restore excitability. In other words, during the flow of a constant current...
the membrane underwent a slow depolarization following an action potential, reached a plateau, and finally repolarized equally slowly, the whole cycle of events lasting up to several minutes. In the advanced state of veratridine poisoning a depolarization could be triggered by a mere change from a Na-poor solution to one containing the normal amount of Na. This is shown in Fig. 6. Upon return to the Na-poor solution a fast repolarization resulted. Thus, the most striking feature of Fig. 6 is the great difference in the time constants for de- and repolarization.

The depolarization as illustrated by Figs. 5 and 6 is a very slow process and its time course appeared to be independent of the means by which it was triggered. The maximum rate of depolarization in 15 measurements on 8 nodes was 7.1 ± 0.5 mv per second. The slow depolarization shown in Fig. 6 was not the result of a slow change of solutions, for with an equally fast change to a low Na solution the membrane was capable of a quick repolarizing response. Moreover, the difference in time course cannot simply be attributed to the direction of potential change since in a preparation giving results as in Fig. 6 switching to isotonic KCl solution led to the usual fast depolarization (Ulbricht, 1963).

The main purpose of such interspersed tests with isotonic KCl, however, was to obtain a reference potential for the veratridine depolarization since it is generally assumed that in isotonic KCl the absolute membrane potential is equal to or very close to zero. It was found that in most fibers veratridine
did not lead to a complete depolarization of the nodal membrane but in two cases it clearly changed the potential beyond the level in isotonic KCl.

Only in a few preparations could a massive depolarization be obtained at the first application of $2.5 \times 10^{-6}$ gm veratridine per ml. In these cases a change back to normal Ringer's solution usually repolarized the membrane, but it did so very slowly (maximum rate of repolarization $-1$ to $-2$ mv per second). Repolarizations at rates comparable to those for depolarizations could be initiated by a change to K-free Ringer's or by an increase of $(\text{Ca})_o$. A solution with 50 per cent normal $(\text{Na})_o$ was also found sufficient to repolarize the membrane.

![Figure 7](image)

**Figure 7.** Effect of break and make of inward current during depolarization in $2.5 \times 10^{-6}$ gm veratridine per ml. Ordinate, membrane potential (depolarization upward), 10 mv per major division. Abscissa, 1 second per major division. 26°C. 1-31-64.

A. In lower left hand corner common starting level during current flow for all traces. (1) After-potential following action potential (not visible) triggered by a brief release of current. (2) Repolarization after break of current for 1 second; during break trace was identical with first portion of (3). (3) Break lasted for remainder of sweep. Note "creeping" of membrane potential in (3) while current was switched off. B. Make of same inward current as in A. Slow repolarization after fast voltage drop.

Finally, a repolarization could be achieved by an inward current as one might expect. The interesting point, however, is that after a sudden potential change due to the voltage drop across the momentary membrane resistance the repolarization again was a slow process as demonstrated by Fig. 7 B. In Fig. 7 A the effect of a release of this current is depicted. A very short release elicited an action potential which was followed by an after-potential (trace 1). If the current was kept switched off (trace 3) the potential crept towards its steady state level which is also the starting level of Fig. 7 B. It is quite clear that the slow changes of membrane potential cannot be explained by a mere change in nodal resistance since in trace 3 of Fig. 7 A no current was applied.

**Discussion**

It was shown that immediately after a spike the veratridine-treated nodal membrane remains depolarized with respect to the resting potential and that
it is a matter of concentration and duration of treatment with the alkaloid whether during the following period of time the membrane slowly repolarizes (Fig. 1) or again depolarizes (Fig. 5). Either after-effect is sensitive to changes in \((Na)_0\): the after-potentials markedly increase in high \((Na)_0\) (Fig. 2) and the large depolarizations can promptly be terminated by withdrawal of this ion (Figs. 5 and 6). This suggests an increased ratio \(P_{Na}/P_K\) during the after-depolarization which, because it is accompanied by a decreased membrane resistance, cannot be due to a mere decrease in \(P_k\). Hence, one may conclude that the depolarization after a spike is due to an increased \(P_{Na}\) which slowly subsides in the case of the after-potential but which further increases during a large depolarization. In either case the time constants of potential change are several orders of magnitude greater than any time constant determining the normal spike configuration (Frankenhaeuser and Huxley, 1964). On the other hand, a node treated with veratridine is capable of producing spike potentials whose fast phases do not greatly differ from those observed in normal Ringer’s. Therefore, at least part of the sodium permeability must remain time- and voltage-dependent as in the untreated node. Since the falling phase of the spike is virtually unchanged this part of \(P_{Na}\) must also be affected by a fast inactivation process for it seems established from computations using voltage clamp data that the falling phase of a spike is mainly determined by this process (see Fig. 10 in Frankenhaeuser and Huxley, 1964).

In cases in which veratridine depolarizes the resting membrane it presumably does so by increasing the resting \(P_{Na}\) (Fig. 2). Calculations from the data of Frankenhaeuser and Huxley (1964) show that the steady state sodium current, \(I_{Na}\), although very small, increases with increasing depolarization for \(V < 30\) mv resulting in an \(I_{Na}-V\) curve with a negative resistance for this region. Therefore, if \(P_{Na}\) is sufficiently increased by the alkaloid a portion of this negative resistance might become measurable as an increase of the total resting membrane resistance. A formally similar phenomenon was seen at moderately increased \([K]_0\) (see curve for 10 mM K in Fig. 7 of Ulbricht, 1963).

Of other possibilities to explain the slow after-effects, two can be excluded directly by our experiments. It was mentioned that a veratridine-treated node responds promptly to quick changes of the external K concentration. Furthermore, Figs. 5 and 6 show that the membrane repolarizes fast as Na is withdrawn. From these facts one can conclude that the treated membrane remains easily accessible to the external medium. It seems, therefore, very unlikely that potassium released during activity accumulates outside the membrane (thereby depolarizing it) and that its diffusion away is reflected by the after-potential as was at one time discussed by Shanes (1952).

From impedance measurements Shanes et al. (1953) tentatively concluded
that during the veratrine after-potential in squid axons the chloride permeability, \( P_{Cl} \), is elevated. Although there is much uncertainty about \( P_{Cl} \) of the nodal membrane, the increased after-potentials observed in a solution with a high concentration of NaCl (Fig. 2) exclude an appreciable increase of \( P_{Cl} \) since it is in contrast to the concomitant shift of \( E_{Cl} \) in the opposite direction.

Care has to be taken in comparing results from nerve trunks with those from single nodes. It has repeatedly been reported that veratrine or veratridine enhances repetitive firing in frog nerve (Lorente de Nó, 1947; Shanes, 1951) which was never seen in our experiments. A possible explanation for this difference is that the sciatic nerve contains sensory fibers which, in contrast to motor fibers, tend to respond repetitively even in normal Ringer's (Erlanger and Blair 1938; Schmidt and Stämpfl, 1964). Also, in bundle experiments involving a change of solutions diffusion to the nodes is often the rate-limiting process. Thus, removal and readdition of Na in the presence of veratridine result in about equally fast potential changes in bundles (Straub, 1954) while with single nodes a very marked difference in time constants for re- and depolarization is observed (Fig. 6).

Our findings can be summarized as follows: Although there are signs of an increased resting \( P_{Na} \) (see Fig. 2), the alkaloid appears mainly to keep the membrane passable for sodium ions once a spike has been elicited. Since the spike can be unchanged while a marked after-potential is observed one may assume that in the early stage of treatment only part of the sodium "channels" are affected by the alkaloid. Thus large depolarizations and afterpotentials appear to be only quantitatively different expressions of one basic drug effect.

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