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# The Interaction between the Activator Agents Batrachotoxin and Veratridine and the Gating Processes of Neuronal Sodium Channels

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**SUMMARY**

The depolarization of frog sciatic nerves by the Na channel-activating toxins, batrachotoxin and veratridine, was studied using the sucrose-gap technique. To study the interaction between the activators and the gating processes of Na channels, we measured the depolarizations of unstimulated nerves, of nerves during repetitive stimulation, and of nerves whose Na channel inactivation process had been pharmacologically modified. Stimulation enhanced the rates of depolarization by the activators but did not effect the steady state depolarization values. Of the three inhibitors of Na channel inactivation that were tested (*Leiurus* $\alpha$-scorpion toxin, chloramine T, and $Ni^{2+}$), only *Leiurus* toxin enhanced the potencies of the activators. Neither chloramine T nor $Ni^{2+}$ had any effect on the steady state level of depolarization produced by either activator. Both chloramine T and $Ni^{2+}$, however, enhanced the rate of batrachotoxin action, although neither affected the rate of veratridine action. *Leiurus* toxin also potentiated the effects of the activators in chloramine T-treated nerves. We tested the interaction between the Na channel activators and a class of agents, local anesthetics, that stabilize a non-conducting state of the Na channel. The presence of lidocaine inhibited the depolarization produced by addition of either activator, although the addition of lidocaine subsequent to the development of batrachotoxin-induced depolarization produced repolarization very weakly and slowly. We also found that the lidocaine homologue, RAC 109I, was about 3 times as potent as its stereoisomer, RAC 109II, in its ability both to reduce the compound action potential amplitude and to inhibit the veratridine-induced depolarization.

The sodium channel-activating drugs, BTX and VTD, exert their depolarizing effects on excitable cells by increasing resting Na permeability (1, 2). This permeability is inhibited completely by tetrodotoxin and is thus believed to be mediated by voltage-dependent Na channels. Both BTX and VTD alter many properties of Na currents observed under voltage clamp conditions, but a cardinal feature of their actions is that they both inhibit Na channel inactivation (2, 3). In addition, there is a hyperpolarizing shift of the voltage dependence of activation such that a significant fraction of the Na channels is activated at potentials near the resting potential (2, 3). In a resting nerve these two effects (inhibition of inactivation and a shift of the voltage dependence of activation) lead to a steady inward Na current and the steady depolarization observed. The relative magnitude of this depolarization depends on the fraction of Na channels modified by the activators at steady state.

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Previous investigations have indicated that the interaction of activators with the Na channel can be modified by the state of the channel. For example, the more rapid onset of the effects of BTX during nerve stimulation has led to the suggestion that BTX binds more readily to open channels than to closed channels (3, 4). The ability of the activators to induce a steady state sodium permeability is enhanced in the presence of specific neurotoxins, which themselves act uniquely to inhibit Na channel inactivation (5). Activator dose-response curves show that this toxin, from *Leiurus* scorpion venom, increases the potency of the activators as well as the efficacy of the ones characterized as "partial agonists" (5, 6).

Can the pharmacological modulations of activator action be accounted for satisfactorily by the known effects on Na channel processes of the modulators alone? The potentiation by *Leiurus* toxin has been explained by two schemes. In the first, the toxin is postulated to modify an equilibrium distribution of channels between two "states," shifting channels toward the state with the higher affinity for activator molecules (5). However, these states are not directly identified with any form of the channel

**ABBREVIATIONS:** BTX, batrachotoxin; VTD, veratridine, CAP, compound action potential; CRP, compound resting potential; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; BTX-B, batrachotoxinin-A 20$\alpha$-benzoate.

inferred from electrophysiological studies. In the second scheme, the specific ability of the scorpion toxin to inhibit Na channel inactivation is invoked to model the potentiating effects on activator actions (6). By preventing inactivation the toxin is thought to increase the fraction of channels that can be opened by the activators. The first hypothesis is difficult to test explicitly, but the second can be addressed directly by experiments that examine the effects on the activators of a variety of treatments that inhibit the inactivation of Na channels.

The actions of the activators and the specific binding to channels of certain radiolabeled activator derivatives are antagonized by local anesthetics (7–9). This antagonism, although competitive in manner, involves changes in both binding and dissociation rate constants of the channel/activator complex and thus cannot be explained by simple, direct competition for a common site (9). The dose-dependent effects of anesthetics on both normal Na channels and on activator actions have not been measured in the same preparation. There is, however, evidence for a resistance of activator-modified channels to inhibition by local anesthetics (3, 10) and qualitative correlation between stereospecific anesthetic actions on both drug-activated (9) channels and voltage-activated channels in some (11) but not all (12) nerves.

Furthermore, most of the detailed data on local anesthetic actions come from electrophysiological studies on axons where the actions of BTX appear to be irreversible, even after extensive washing (Refs 1, 3, and this report), whereas the kinetic analyses of the BTX-local anesthetic interaction were conducted on vesicles from brain (8) or cultured cells (7, 9) where BTX action and binding can be reversed by washing.

For the experiments reported here, we have used the sucrose-gap technique to test the effects of inactivation inhibitors and of local anesthetics on the actions of the Na channel activators, BTX and VTD, on frog sciatic nerves. To explore the state-dependent interactions of the activators with the Na channels we have tested BTX and VTD on nerves during repetitive stimulation (to increase the probability of channels being in the open state) and on nerves in which the inactivation process has been pharmacologically inhibited. The modulation of activator action by local anesthetics has been assayed, using both activators, by applying stereoisomeric analogues of lidocaine as well as that drug itself. Our results support the hypotheses that activators bind preferentially to open channels and that local anesthetics inhibit the actions of the activators. However, our data strongly indicate that inhibition of inactivation, per se, is not sufficient to potentiate the actions of BTX and VTD; and, although the inhibition of VTD-depolarization by local anesthetics is competitive, the inhibition of the BTX-depolarization is not. The action of BTX appears to be essentially irreversible in this preparation, unlike that in cultured cells, and the differences between the kinetics of BTX and VTD actions and their inhibition by local anesthetics are representative of their relative reversibilities.

## Materials and Methods

**Electrophysiology.** Experiments were carried out on sciatic nerves from frogs of the species *Rana pipiens*. The sciatic nerves were dissected, desheathed, and split longitudinally into two bundles. The CAP and CRP were assayed using the sucrose-gap technique (13, 14). Ag/AgCl wires were used for both stimulation and recording. Stimulation by 0.05-msec supramaximal pulses was applied to a region of nerve not directly exposed to drugs. The recording electrodes were coupled through an FET input preamplifier to the input of a storage oscilloscope (Philips PM 3234), for recording the CAP, and to the input of a strip chart recorder (Honeywell Electronik 194). The strip chart record shows a DC offset which we refer to as the compound resting potential (see below). The chart recorder was used to measure changes of the CRP and also to measure the duration of the CAP when the nerve was treated with inhibitors of Na channel inactivation (see Fig. 3). Under these conditions, the repolarization phase of the CAP is sufficiently slow that the chart recorder can faithfully follow its time course and the CAP duration can be measured accurately.

The sucrose-gap method does not permit the direct measurement of sodium currents available from voltage clamp, and the recorded potential changes are somewhat smaller than those from intracellular microelectrode penetrations. Yet the method provides sufficiently good data, in terms of stability, signal-to-noise ratio, and specificity, to answer the questions we are addressing. Compound action potentials normally ranged from 50 to 60 mV and CRPs were about 35 mV, whereas the baseline noise and drift were typically <1 mV and <4 mV·hr$^{-1}$, respectively. Drug-induced changes in CAP amplitude and in the CRP level usually will not be proportional to channel occupancy, but functional, reproducible dose-response relations can be constructed, and these provide accurate measures of relative potencies (see Results and Ref. 15).

**Solutions and chemicals.** Ringer's solution contained 110 mM NaCl, 2.5 mM KCl, 2.0 mM $CaCl_2$, 5 mM HEPES buffer, and 12 mM tetraethylammonium chloride to block the delayed K current. All solutions were titrated to pH 7.2 with 1 N NaOH. The $\alpha$-toxin from the scorpion *Leiurus quinquestriatus* was purified (Ref. 16; toxin II$\alpha$) from venom obtained from Sigma Chemical Co., St. Louis, MO. Batrachotoxin was generously supplied by Dr. John Daly, Laboratory of Bio-Organic Chemistry, National Institutes of Health (Bethesda, MD); veratridine was obtained from Sigma, and chloramine T was purchased from Fisher Scientific (Pittsburgh, PA). The local anesthetics lidocaine, RAC 109I, and RAC 109II were generously provided by Dr. Bertil Takman of Astra Pharmaceuticals, Worcester, MA.

**A note on the measurement of the CRP.** The DC offset measured with the sucrose-gap technique is some averaged value of the resting potentials of the fibers in the bundle, reduced by current shunts through the gap. Thus, the measured offset would be expected to be less than the resting potential of healthy myelinated axons (~ −70 mV) that account for most of the axoplasmic cross-sectional area of the sciatic nerve. In order to estimate the absolute value of the CRP, in several preparations Ringer's solution was replaced with isotonic KCl, which should depolarize the CRP to 0 mV. In four nerves tested, the average magnitude of the measured depolarizations was 36 mV (range: 33–40 mV). We used the CRP to measure the potencies and efficacies of pharmacological treatments in increasing the resting Na permeability. There is clearly a nonlinear relationship between the number of Na channels activated and the resultant depolarization, and it may be that a maximal depolarization occurs with activation of only a small percentage of available channels. Nevertheless, quantitative comparisons of potencies can be made using standard dose-response relationships. Furthermore, conclusions of the relative efficacies of two treatments are valid except when both are able to produce the maximal response of the system (i.e., about 35 mV depolarization).

All of the averaged data reported in this paper are expressed as mean values ± standard deviation, with the number of observations noted in parentheses.

## Results

**Actions of activator drugs alone.** Before describing the modulations produced by other drugs, we first will describe the actions of the activators BTX and VTD alone. These agents are characterized by their rates of action on resting and stim-

ulated sciatic nerves, their potencies and efficacies, and the reversibility of their effects upon washing. BTX and VTD caused a persistent depolarization of the frog sciatic nerve (Fig. 1). BTX was far more potent and slightly more effective than VTD, producing a maximal effect (~35 mV depolarization) at a concentration of 5 $\mu$M, whereas VTD was maximally effective (~30 mV depolarization) at around 300 $\mu$M (see Fig. 5). At VTD concentrations approaching 100 $\mu$M, we always observed a transient overshoot of the depolarization before it settled to a steady state, as shown in Fig. 1. This was never seen with BTX. The potency and the effectiveness of VTD in producing the transient depolarization exceeded the values of these parameters for the steady state depolarization (*dashed line*, Fig. 5B). In fact, for the peak response, VTD was as effective an agonist as BTX (see Fig. 5).

After being depolarized by BTX, a nerve could not be repolarized by washing with Ringer's solution, even after 2 hr of continuous perfusion. BTX thus acts virtually irreversibly in this system. The BTX-induced depolarization was rapidly abolished upon addition of 1 $\mu$M tetrodotoxin, but when the tetrodotoxin was washed away, the nerve again depolarized.[1]

In contrast, the depolarization produced by VTD was readily reversed by washing with Ringer's solution (Fig. 1B). When tetrodotoxin was applied to a nerve depolarized by VTD in the continued presence of VTD, a rapid repolarization occurred as with BTX. In addition to depolarizing the nerve, VTD also caused a prolongation of the CAP and this effect could be reversed only after extensive washing with Ringer's solution.

These findings are consistent with reports from other systems of excitable membranes (1–6) and show that these activators interact specifically with the voltage-dependent Na channels, causing them to open and to remain open at the resting potential and at more depolarized potentials. The partially transient response observed with higher VTD concentrations, but not with BTX, suggests that some of the VTD-modified channels convert to a non-conducting state after initially opening. We were interested in the relationship between the activator-induced depolarization and the gating processes of the Na channel, namely, activation and inactivation. In subsequent experiments, we compared the rates and steady state levels of the depolarizations by the activators in nerves whose Na channels were repeatedly activated (by stimulation) or whose Na channel inactivation was pharmacologically modified (either inhibited or stabilized). To observe changes in the rates or steady state levels of depolarization, in most experiments we used concentrations of the activators which produced intermediate results, namely, 0.1 $\mu$M BTX and 10 $\mu$M VTD (Table 1; see also Fig. 5). In Fig. 1, 0.1 $\mu$M BTX produced an 18-mV depolarization. The time required to reach one-half of this steady state value ($t_{1/2}$) was 33 min. For comparison, 10 $\mu$M VTD produced a 12-mV depolarization with a $t_{1/2}$ of 20 sec.

**Effects of stimulation.** Previous studies have described an increase in the rate of onset of the effects of BTX on excitable membranes rendered by stimulation. Bartels-Bernal *et al.* (4) showed that stimulation of the eel electroplax in the presence of BTX led to a more rapid depolarization than in the absence of stimulation. Khodorov (3) and his colleagues have studied extensively the effects of BTX on the voltage-clamped amphibian node of Ranvier and have shown that repetitive depolarizations lead to a more rapid appearance of BTX-modified Na channels.

We observed the effects of 1 Hz stimulation on activator-induced depolarization (Fig. 2). For both BTX and VTD, stimulation did not alter the steady state depolarization but did enhance the rate of depolarization. Fig. 2A shows the effects of stimulation on the depolarization by 0.1 $\mu$M BTX. The depolarization proceeded in two phases. The first phase was very rapid and complete after only three to four CAPs had been elicited. The depolarization then proceeded in a second phase at a rate only slightly greater than that in the absence of stimulation. The $t_{1/2}$ was greatly reduced compared to the unstimulated nerve (i.e., the rate was enhanced), because of the early rapid depolarization. This change of the $t_{1/2}$ corresponded to a 15-fold increase in the depolarization rate compared to the unstimulated nerve (see Table 1).

Stimulation at 1 Hz also enhanced the rate of depolarization

[1] Our inability to reverse the effects of BTX by washing does not necessarily mean that BTX binds to a receptor irreversibly. It may be that a nerve exposed to BTX, which is highly lipophilic, contains stores of BTX in the dense myelin membranes. As the nerve is washed, BTX may be slowly dissociating from its receptor and removed, but there may be a continuous supply of the toxin from adjacent membranes. Thus, the reason for the irreversibility remains a mystery. Nevertheless, the following two points are contrary to the assignment of BTX irreversibility to membrane stores: 1) the irreversibility is not observed with VTD, which is also highly lipophilic, even though in most experiments it is used at more than 100 times the concentration of BTX, and 2) BTX also acts essentially irreversibly in squid axons which have far less membrane than frog sciatic nerves for the storage of drug.

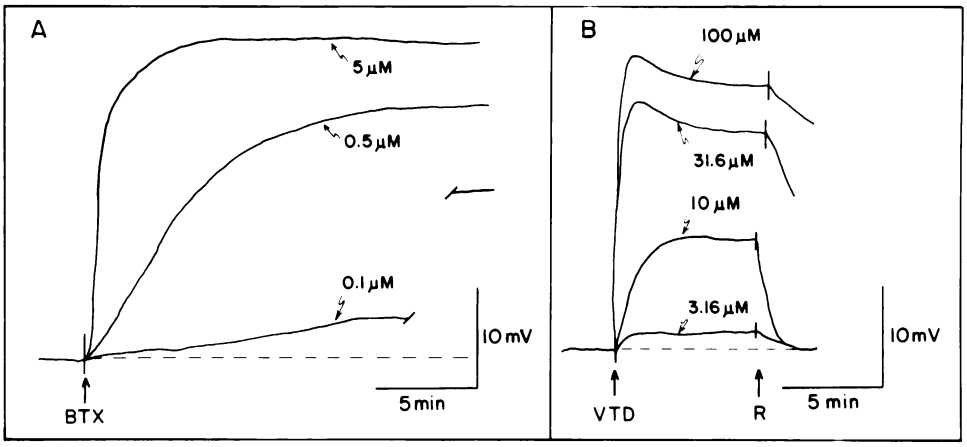



**Fig. 1.** Effect of BTX and VTD on the CRP of frog sciatic nerves: depolarization versus time at different activator concentrations. The baseline (– – –) represents the CRP in Ringer's solution (see Material and Methods for explanation of the CRP). Depolarizations are deflections upward; hyperpolarizations are deflections downward. A. Superimposed traces of the depolarization produced by three different concentrations of BTX on separate nerves. The gap in the trace for the depolarization by 0.1 $\mu$M BTX represents 60 min. B. Superimposed traces of the VTD-induced depolarization at various concentrations. After 7 min of VTD treatment, the nerves were each washed with Ringer's solution (*R*), and the nerves completely repolarized although much more slowly after higher VTD concentrations.

TABLE 1

**Effects of stimulation and of inhibitors of Na channel inactivation on BTX- and VTD-induced depolarizations**

| | BTX (0.1 μM) | | (n) | VTD (10 μM) | | (n) |
|---|---|---|---|---|---|---|
| | $\Delta V_{ss}$[a] | $t_{½}$[b] | | $\Delta V_{ss}$[a] | $t_{½}$[b] | |
| | mV | min | | mV | sec | |
| Control | 18 ± 0.6 | 29 ± 7.0 | (4) | 10 ± 2.6 | 20 ± 6.3 | (6) |
| 1 Hz Stimulation[c] | 19 ± 1.5 | 1.9 ± 0.6[d] | (3) | 12 ± 3.5 | e | (3) |
| Inhibitors of Na channel inactivation[f] | | | | | | |
| Leiurus toxin | 33 ± 2.0[d] | 1.0 ± 0.2[d] | (3) | 28 ± 4.2[d] | 24 ± 3.6 | (4) |
| Chloramine T | 20 ± 3.0 | 6.0 ± 1.5[d] | (3) | 12 ± 2.1 | 15 ± 2.5 | (3) |
| Ni | 20 ± 3.6 | 12 ± 1.7[g] | (3) | 10 ± 2.0 | 18 ± 2.5 | (3) |
| Inhibitor + one stimulus[h] | | | | | | |
| Chloramine T | 22 ± 3.9 | e | (4) | 13, 15 | e | (2) |
| Ni | 19, 21 | 1.5, 2 | (2) | 13 ± 2.6 | e | (3) |

[a] Steady state depolarization.
[b] Time to reach 0.5 $\Delta V_{ss}$.
[c] Stimulation was begun within 10 sec of BTX application and within 1 sec of VTD application.
[d,g] Statistical significance of differences between test and control parameters was evaluated by a modified Student's unpaired *t* test, the Bonferroni *t* test, and is indicated by *d* for $p < 0.01$ and *g* for $p < 0.02$. No letter means $p > 0.3$. See also Footnote *e*.
[e] In these cases, the depolarization by the activator overlapped with the time course of the CAP. Thus, the maximum value of $t_{½}$ is less than the duration of the CAP (see Fig. 3), not including the delay between the time of application of the activator and the beginning of stimulation. Significantly different from control with $p < 0.001$.
[f] Concentrations and durations of treatment were as in Fig. 6.
[h] Same as Footnote *f*; single stimulus was given within 1 sec of addition of activator.

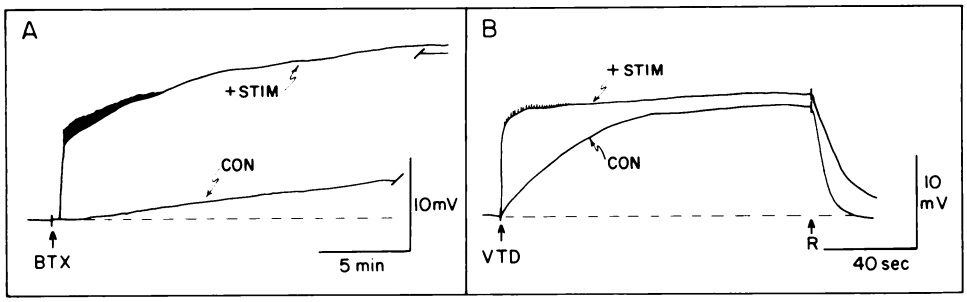



**Fig. 2.** Effect of stimulation on the rate and steady state level of depolarization by BTX and VTD. Within 10 sec of application of 0.1 μM BTX (A) and within 1 sec of application of 10 μM VTD (B), the nerves were stimulated continuously at 1 Hz. The effect of the same concentration of the activators in the absence of stimulation is shown for comparison. Note the difference in the time scale between A and B, and between B in this figure and Fig. 1B. As in Fig. 1, the gap in the record for 0.1 μM BTX represents 60 min. The small vertical deflections superimposed on the depolarizations of the compound resting potentials are the strip chart records of the CAP, truncated because of the response time of the recorder. They are not well resolved from one another at the slow sweep speed of panel A, but they are in panel B.

by 10 μM VTD (Fig. 2B). There appeared to be only a single phase of depolarization when a VTD-treated nerve was stimulated, and this depolarization was nearly complete after a single CAP. Thus, it is impossible to measure a $t_{½}$, since the VTD-induced depolarization overlaps with the depolarizing phase of the CAP. However, a maximum estimate of the $t_{½}$ is the duration of the CAP (~2 msec, not including the delay between VTD addition and the first stimulus) and, thus, a minimum estimate of the rate enhancement by stimulation would be ~$10^4$-fold (20 sec/<2 msec; see Table 1).

**Pharmacological modification: Inhibition of Na channel inactivation.** Based on such evidence that stimulation enhances the rate of onset of the effects of BTX, it was postulated that BTX interacts preferentially with open Na channels (3, 4). Indirect evidence in support of that hypothesis came from studies of Na channel activators in the presence of a toxin from the venom of the scorpion, *L. quinquestriatus*. When applied to frog nerves under voltage clamp conditions, this toxin inhibits Na channel inactivation and thus produces a maintained Na current in response to a step depolarization (16). When the scorpion toxin is applied to either neuroblastoma cells (5) or rat brain synaptosomes (6) in the presence of BTX or VTD, the potency of each activator in stimulating Na uptake is enhanced. That is, in the presence of *Leiurus* toxin, the activators appear to interact with Na channels with higher affinity. A simple hypothesis was advanced by Krueger and Blaustein (6) that, by inhibiting Na inactivation, *Leiurus* toxin potentiates the activators by increasing the fraction of channels which are in the open state and thus available to interact with the activators. A more general hypothesis was proposed by Catterall (5), in which the potentiation by *Leiurus* toxin occurs because of the toxin's ability to shift the equilibrium of the Na channel between two unspecified configurations. In the presence of the toxin, the equilibrium would be shifted toward that configuration which binds the activators with higher affinity.

We used three inhibitors of Na channel inactivation to test the specific, first hypothesis (6). In addition to *Leiurus* toxin, we used two other treatments that have been shown to inhibit Na channel inactivation in voltage-clamped nodes of Ranvier. The first is the chemical reagent chloramine T (17), and the second is the divalent metal cation $Ni^{2+}$ (18).

The effects of all three treatments on the CAP are shown in Fig. 3. The upper traces in the figure show the oscilloscope traces of the CAPs and display only the first 8 msec. The lower

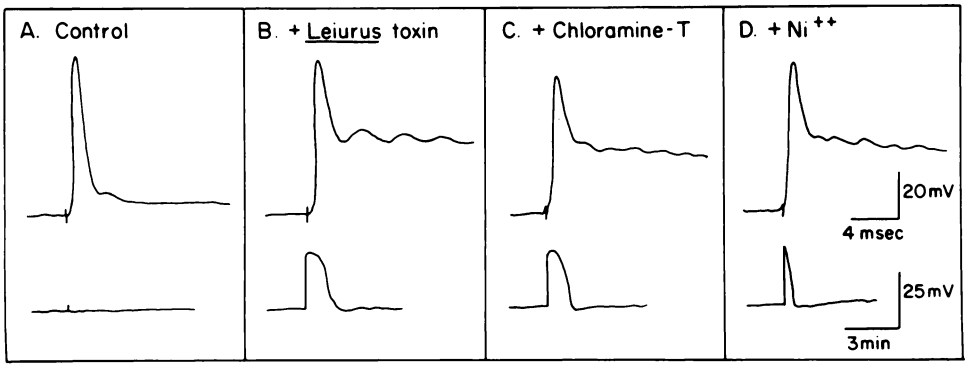



**Fig. 3.** Modification of the CAP by inhibitors of Na channel inactivation. The *upper trace* in each panel shows the oscilloscope record and the *lower trace* shows the strip chart record of CAPs under various conditions. The action potentials were prolonged compared to control (A) when nerves were treated with (B) 100 nM *Leiurus* toxin for 5 min, (C) 100 μg/ml of chloramine T for 10 min, or (D) 0.5 mM $Ni^{2+}$ for 1 min.

traces show the strip chart record of the CAP which, as in Fig. 2, is truncated because of the response time of the recorder. For the control (Fig. 3A), the CAP is too rapid to obtain useful information from the strip chart record, but when the CAP is prolonged by inhibiting Na channel inactivation (Fig. 3, B–D), the strip chart record gives an accurate measure of the duration of the CAP.

The oscilloscope traces of Fig. 3 do not distinguish the various treatments: all show a maintained depolarization of about 30 mV immediately following an initial peak of ~60 mV. The chart record, however, shows that the effects of the treatments on the duration of the CAP were not identical. The CAP durations (not including the small undershoots seen here) were increased to 1.5 min by *Leiurus* toxin, 1.0 min by chloramine T, and 20 sec by $Ni^{2+}$. Continued treatment with *Leiurus* toxin or $Ni^{2+}$ had no additional effect, whereas continued exposure to chloramine T resulted in a gradual diminution of the CAP amplitude. Raising the $Ni^{2+}$ concentration to 5 mM prolonged the CAP even further, without measurably reducing its amplitude. The prolongation of the CAP by *Leiurus* toxin or chloramine T was not reversed by washing with Ringer's solution, but that by $Ni^{2+}$ (0.5 mM) was immediately reversed by washing. Of importance is the fact that none of the treatments with these modulators of inactivation affected the CRP of the sciatic nerve.

Superimposed in Fig. 4A are traces from three separate nerves showing the effects of 0.1 μM BTX after the inhibition of inactivation by each of the three inhibitors. As in neuroblastoma cells and synaptosomes, *Leiurus* toxin potentiated the steady state effects of BTX. This depolarization is equivalent to the response of an unmodified nerve to 1 μM BTX in terms of both rate of onset and steady state level. Complete dose-response curves for BTX in control and *Leiurus* toxin-treated nerves are shown in Fig. 5A. Clearly, a potentiation of BTX of about 5-fold occurs in the presence of *Leiurus* toxin.

Inhibition of Na channel inactivation by chloramine T or $Ni^{2+}$ resulted in no change of the steady state depolarizing response to BTX (Fig. 4A, Table 1). The dose-response curves of Fig. 5A show clearly that BTX action was not potentiated by either of these treatments. Repeated comparisons of the steady state response to 0.1 μM BTX after the various treatments shows that only *Leiurus* toxin produced a statistically significant potentiation (Table 1). The steady state levels in the presence of the other inactivation modulators fell within the control range of 15–20 mV. There was, however, a large increase in the rate of depolarization by both treatments. In the chloramine T-treated nerves, the rate was enhanced 5-fold over control; in the $Ni^{2+}$-treated nerves the rate was enhanced 2.5-fold (Table 1).

As with BTX, the depolarization by 10 μM VTD was potentiated by *Leiurus* toxin but not by chloramine T or 0.5 mM $Ni^{2+}$ (Figs. 4B and 5B). The effect of *Leiurus* toxin on the steady state VTD response corresponded to a 10-fold increase of the potency of VTD (Fig. 5B). In addition, in the presence of *Leiurus* toxin, VTD-induced depolarizations attained maximal values at steady state of about 35 mV at high VTD concentrations, equivalent to the maximal depolarization by BTX. The transient depolarizing response to VTD at submaximum doses was also potentiated by *Leiurus* toxin, but not significantly more so than the steady state change. At 50 μM VTD, the ratio of peak potential change to steady state response was 1.10 ± 0.01 ($n = 2$) without the scorpion toxin and 1.38 ± 0.29 ($n = 4$) with the toxin.

Modification of the nerves with either chloramine T or 0.5 mM $Ni^{2+}$ did not significantly increase the rate of action of VTD, as occurred with BTX (Table 1), nor did treatment by these agents affect the maximal VTD-induced depolarization. (In two experiments we observed a strong inhibitory effect by

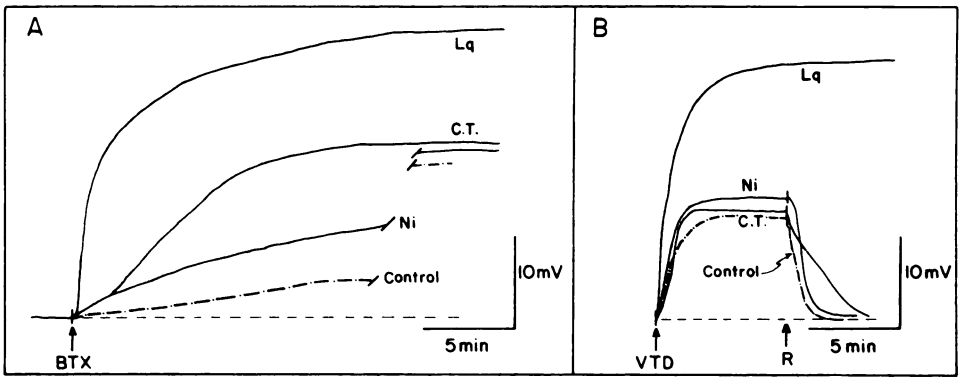



**Fig. 4.** The effect of BTX and VTD on the CRP of nerves pretreated with inhibitors of Na channel inactivation. The four superimposed traces in each panel are from four different nerves. Nerves were pretreated for 15 min with 100 nM *Leiurus* toxin (*Lq*), 100 μg/ml of chloramine T (*C.T.*), or 0.5 mM $Ni^{2+}$ (*Ni*), also present with the activator; then either 0.1 μM BTX (A) or 10 μM VTD (B) was applied. The gap in the trace from the $Ni^{2+}$-treated nerve in A represents 20 min, that for the BTX Control nerve represents 60 min. –·–·–, Control curve showing the effects of these concentrations of BTX and VTD on untreated nerves.

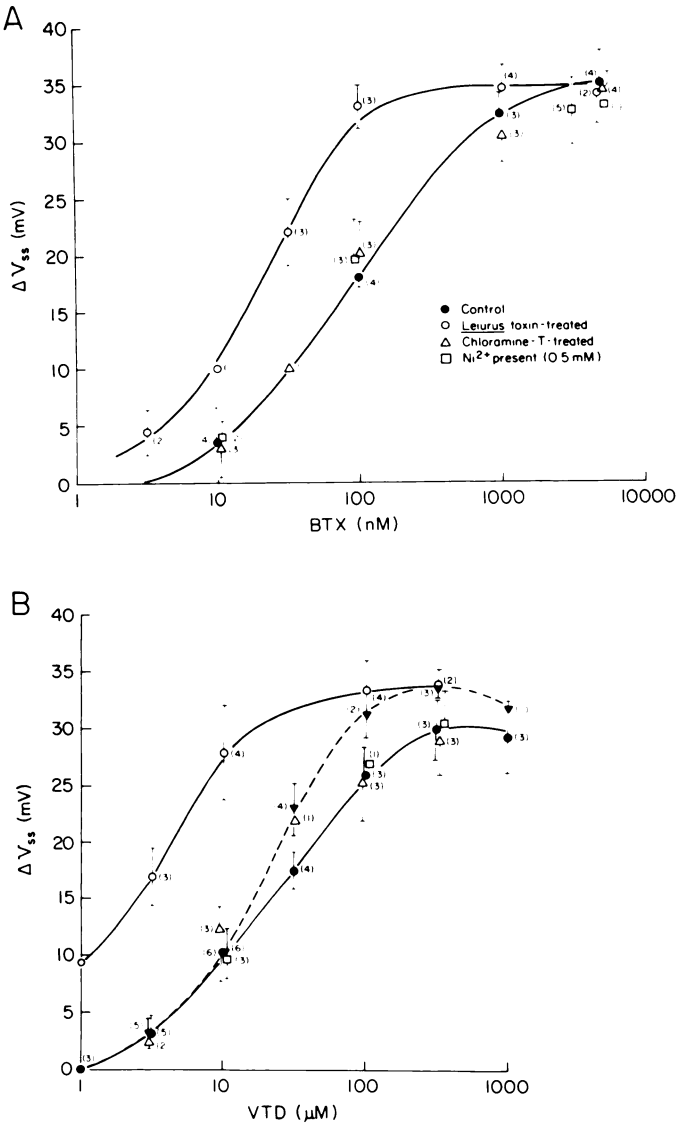



**Fig. 5.** Dose-response curves for the steady state depolarization (—) produced by different concentrations of BTX (A) and VTD (B), in nerves with normal inactivation (●) and with inactivation modified by *Leiurus* toxin (○), chloramine T (△), or 0.5 mM $Ni^{2+}$ (□). ▼, peak transient response to VTD in control Ringer alone (– – –). Data are derived from experiments like those illustrated in Figs. 1 and 4. The *error bars* show the standard deviation about the mean value, with the number of experiments noted in *parentheses*. Curves were drawn by eye.

higher concentrations of $Ni^{2+}$. At 5 mM, a $Ni^{2+}$ concentration that prolonged the CAP beyond its duration in 0.5 mM $Ni^{2+}$, there was no detectable depolarization from 10 μM VTD. The effects of $Ni^{2+}$ are complex and may follow from binding at multiple sites, one of which results in slowed inactivation and another which prevents the binding of VTD or the subsequent channel activation.)

Although chloramine T inhibits Na channel inactivation without potentiating the activators, it does not abolish the *Leiurus* toxin-activator interaction. In nerves treated with chloramine T, *Leiurus* toxin was still able to potentiate the steady state depolarizations by BTX ($n = 3$) and VTD ($n = 4$).

Finally, we observed that the repolarization of the VTD response on removing this activator from the bathing solution was significantly slower in *Leiurus* toxin-treated nerves than in controls, although this same repolarization was not consistently altered by chloramine T or $Ni^{2+}$ treatment. Therefore, potentiation of the reversibly acting agonist by scorpion α-toxin appears to be accompanied by a slower rate of reversal but not by a faster rate of action. The relatively irreversible activator, BTX, shows onset kinetics enhanced by all three modulators of inactivation although only the *Leiurus* α-toxin results in potentiation of the steady state response.

From these data we conclude that inhibition of Na channel inactivation is not sufficient to account for the potentiation of the activators by *Leiurus* toxin. Clearly, it is possible to inhibit Na channel inactivation by chloramine T or $Ni^{2+}$ and leave the potency of activators unchanged in terms of the steady state depolarizations they produce. This and the rate enhancement by chloramine T and $Ni^{2+}$ of the BTX-induced depolarization (but not that of VTD) are considered in the Discussion.

**Pharmacological modification: Stabilization of non-activating Na channels by local anesthetics.** Local anesthetics such as lidocaine have diverse effects on membrane properties. Of central importance in their impulse-blocking action is their ability to reduce the voltage-dependent Na permeability. One model for this action is that local anesthetics bind more tightly to the inactivated form of the Na channel and stabilize the channel in that configuration (19, 20). However, recent experiments show that local anesthetics are just as potent on channels where inactivation has been abolished, by application of *Leiurus* toxin, or reaction with chloramine T (21) and, thus, support an alternative hypothesis, that it is the activated (pre-open) or open form of channels which binds local anesthetics more tightly than the resting form (22). The kinetic consequence of either mechanism of action is that anesthetic-bound channels cannot activate in response to depolarization and thus mimic inactivated channels (23). If such drugs are in fact stabilizing channels in an inactivated-like state, one would expect them to alter the effects of activators which seem to bind preferentially to the open state.

The interaction between BTX and local anesthetics has been studied previously in several systems and has been shown to be one of mutual inhibition (1, 3, 7–9, 24). We used the local anesthetic, lidocaine, to study the interaction between activators and local anesthetics. Lidocaine reduced the CAP by 35 ± 8% at a concentration of 1 mM ($n = 4$), and by 100% at 10 mM ($n = 4$). This reduction was reversed by washing with Ringer's solution. At concentrations up to 10 mM, lidocaine usually produced no change in the CRP, although occasionally at 1–3-mV hyperpolarization was observed at the higher concentrations.

We tested the effect of lidocaine on a nerve depolarized by 0.5 μM BTX (Fig. 6). When the depolarization had reached a steady state at 25 mV, 1 mM lidocaine was applied but there was no change in the membrane potential. When 10 mM lidocaine was applied, there was a slight repolarization of 2 mV. This concentration of lidocaine will completely block the CAP, yet it had almost no effect in reversing the depolarization by 0.5 μM BTX, both of which are produced by $Na^+$ current through the voltage-dependent Na channel.

When 0.5 μM BTX was applied to a nerve that had been previously and continuously treated with 10 mM lidocaine, no depolarization was observed, even after 15 min (Fig. 7A). The nerve was next washed with lidocaine-containing Ringer's solution to remove all free BTX, then with Ringer's solution to

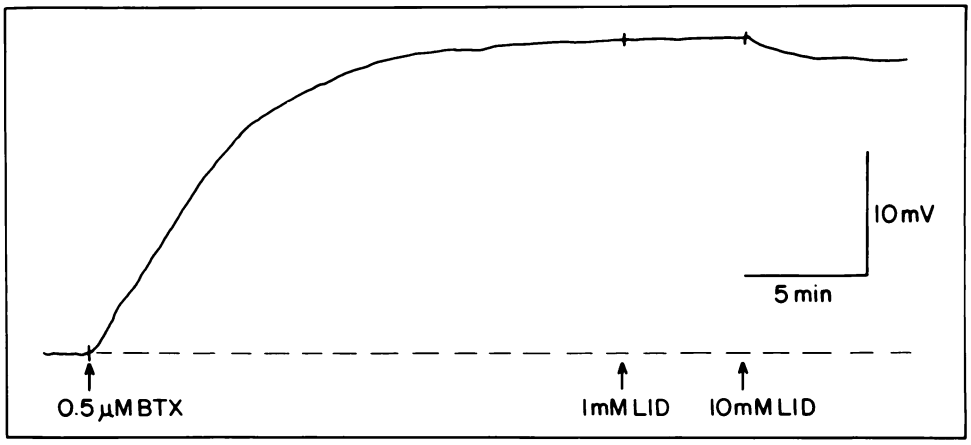



**Fig. 6.** Effect of lidocaine on a nerve depolarized by BTX. BTX (0.5 μM) was applied to a nerve and lidocaine (*LID*) was applied after a steady state depolarization had been reached. There was no effect of 1 mM lidocaine, and 10 mM lidocaine caused only a 2-mV repolarization.

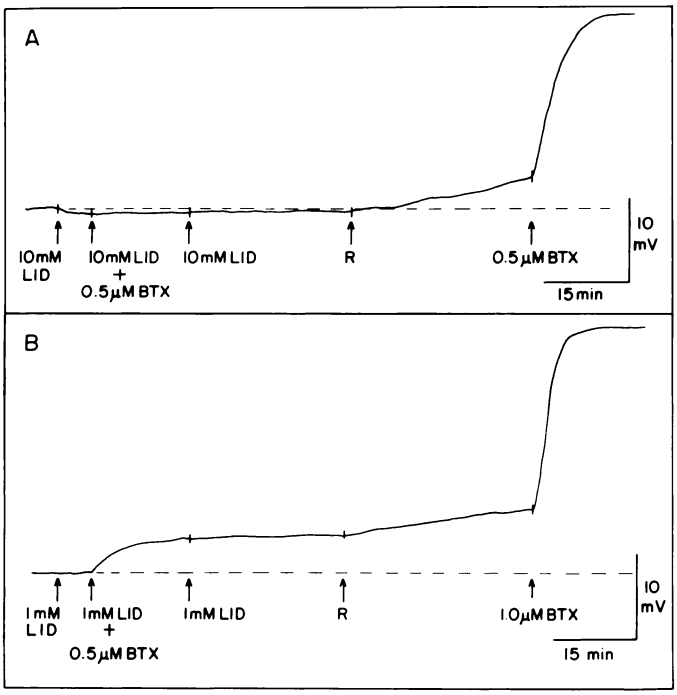



**Fig. 7.** Lidocaine inhibition of the BTX-induced depolarization. A. BTX (0.5 μM) was applied to a nerve in the presence of 10 mM lidocaine (*LID*). No depolarization ensued, and after 15 min the BTX was washed out with Ringer's solution containing 10 mM lidocaine. The lidocaine was then washed out with Ringer's solution (*R*). The nerve began depolarizing (presumably due to remaining BTX), and a subsequent application of 0.5 μM BTX produced a normal depolarization. B. BTX (0.5 μM) was applied to a nerve in the presence of 1 mM lidocaine. A partial depolarizing response was observed. When BTX and lidocaine were washed out as in A, it was observed that the nerve was fully responsive to BTX.

remove the lidocaine. After washing out the lidocaine, the nerve slowly depolarized but was responsive to a subsequent addition of BTX.

If, instead of 10 mM lidocaine, the nerve was continuously exposed to 1 mM lidocaine, then 0.5 μM BTX produced a slow depolarization which leveled off at a lower steady state value than in the absence of lidocaine (Fig. 7B). Identical experiments were carried out with 0.5 μM and 5 μM BTX on nerves pretreated with various lidocaine concentrations, and similar results were obtained—the continuous presence of lidocaine reduced the rate and the steady state potency of BTX as a depolarizing agent (Table 2).

It is therefore clear that the lidocaine-BTX interaction is dependent on the order of addition. If BTX has already depolarized a nerve, then lidocaine is quite ineffective in repolarizing that nerve. Yet lidocaine is very effective in inhibiting the depolarization of a nerve by subsequently added BTX.

The effect of lidocaine on the response of the nerve to VTD was to shift the dose-response curve in the direction of higher VTD concentrations (Fig. 8). That is, at low concentrations of lidocaine (less than 1 mM), a higher VTD concentration was required to achieve the same steady state depolarization as in the absence of lidocaine. At higher lidocaine concentrations, the maximal depolarization by VTD was reduced so that inhibition by lidocaine was not strictly competitive, in agreement with the results of Catterall (25) on inhibition of Na flux in neuroblastoma. Since VTD is readiy reversible, the order of addition of the two drugs proved not to be important as it was with BTX. Fig. 8A shows the response of a nerve to VTD with 0.316 mM lidocaine present, and Fig. 8B shows the shift in the dose-response relationship of VTD caused by lidocaine. These curves have been normalized by the maximum depolarization in the presence of lidocaine at the concentration noted and, therefore, present only the competitive interactions between VTD and lidocaine. From the two shifts in the VTD dose-response function we calculate an inhibitory dissociation constant of 91 and 95 μM for 0.316 mM and 1 mM lidocaine, respectively. This $K_i$ value is between those reported for the block of resting and "inactivated" (depolarized) sodium channels in frog node (150 μM and 30 μM, respectively; Ref. 26). It is quite close to the $K_i$ for lidocaine's competitive inhibition of VTD-activated Na flux in neuroblastoma cells, 55–60 μM, measured at 36° with zero $Ca^{2+}$ present (25).

As a final test of the interaction of Na channel activators

TABLE 2

**Effect of lidocaine on the BTX-induced depolarization**

Lidocaine was applied to the nerves at the indicated concentrations 5 min before BTX was applied

| BTX | Lidocaine | $\Delta V_{ss}$[a] | $t_{½}$[b] |
|---|---|---|---|
| μM | mM | mV | min |
| 0.5 | 0 | 25 | 3 |
| | 1 | 8 | 8 |
| | 10 | 0 | |
| 5.0 | 0 | 35 | 0.5 |
| | 1 | 23 | 5 |
| | 10 | 10 | 12 |

[a] Steady state depolarization.
[b] Time to reach 0.5 $\Delta V_{ss}$.

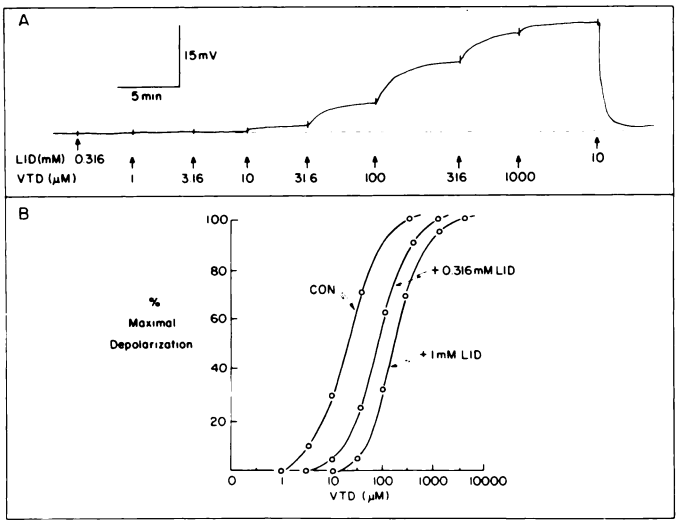



**Fig. 8.** Interaction between VTD and lidocaine. A. Effect of increasing concentrations of VTD on the CRP of a nerve in the continued presence of 0.316 mM lidocaine. B. Relationship between nerve depolarization and VTD concentration for a control nerve (*CON*) and a nerve treated with 0.316 mM or 1 mM lidocaine (*LID*).

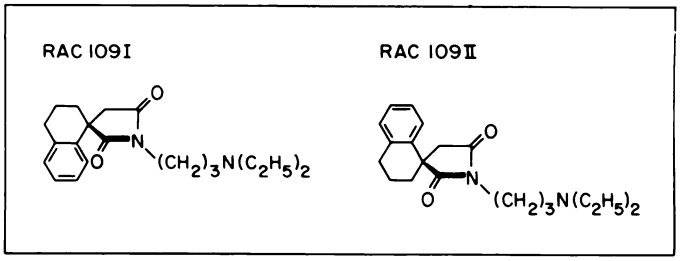



**Fig. 9.** Chemical structures of stereoisomeric local anesthetics RAC 109I and RAC 109II.

TABLE 3

**Effect of stereoisomeric local anesthetics, RAC 109I and RAC 109II, on the CAP amplitude and the VTD-induced depolarization**

| | $K_{\frac{1}{2}}$ (CAP)[a] | $K_{\frac{1}{2}}$ (VTD)[b] |
|---|---|---|
| | mM (n) | μM (n) |
| RAC 109I | 0.67 ± 0.15 (3) | 96 ± 18 (4) |
| RAC 109II | 1.90 ± 0.30 (3) | 344 ± 49 (4) |

[a] Concentration to reduce the CAP by 50%.
[b] Concentration to reduce the VTD-induced depolarization by 50% (VTD = 30 μM).

with local anesthetics we used the stereoisomeric anesthetics RAC 109I and RAC 109II (Fig. 9). These two isomers have been shown to differ in their potency in reducing the voltage-dependent Na current in squid axon (11) and in the amphibian node of Ranvier (12). In the node, RAC 109I is about 3 times as potent as RAC 109II in inhibiting sodium currents (12) and about 4 times as potent in blocking CAPs in the frog sciatic nerve, not under sucrose-gap conditions (27). Using the reduction of amplitude of the CAP as an assay, we found a similar potency difference: RAC 109I was about 2.8 times as potent as RAC 109II (Table 3).

When these two local anesthetics were applied to nerves depolarized by VTD, the results were qualitatively similar to those with lidocaine and VTD—there was a shift in the dose-response relationship of VTD. In this assay, RAC 109I was about 3.6 times as potent as its stereoisomer (Table 3). If the inhibition of VTD action by the RAC 109 isomers is treated as a purely competitive interaction, then the values calculated for the respective anesthetic inhibitory dissociation constants are 48 μM for RAC 109I and 172 μM for RAC 109II. By comparison, the respective dissociation constants calculated from the inhibition of sodium currents in voltage-clamped frog nerve are 222 μM and 676 μM (12). We see that, although the potency ratio is about the same for both measures of anesthetic action, the VTD-induced depolarization is much more sensitive to these drugs than is the infrequently elicited Na current. This continuing correspondence between the ratio of potencies for CAP reduction, for inhibition of Na currents, and for antagonism of the VTD-induced depolarization is consistent with the hypothesis that the site(s) where local anesthetics bind to the Na channel and antagonize veratridine shares some structural features with the site where they block voltage-gated channels (8, 9, 24, 25).

## Discussion

We have described the actions of BTX and VTD on the frog nerve by the use of the sucrose-gap technique. This technique is a useful complement to studies of activator-induced Na flux into excitable cells because one can monitor changes that occur on the millisecond time scale (i.e., the time scale of channel gating) as well as changes on the second-to-minute time scale as detected in the flux studies. The technique also has some advantages over the voltage clamp technique—what it loses in rigor it gains in ease, stability (preparations regularly last 5–6 hr with very little drift, even after many changes of solution), and reproducibility (nerve-to-nerve variations are small).

Many of the responses of this preparation to pharmacological and electrical procedures parallel the effects seen in voltage-clamped frog nerve and, although the amplitude of the steady state depolarization and the reduction of the CAP are not proportional to the fraction of activated or inhibited sodium channels, respectively, there is nevertheless a strong quantitative correlation between these measures and the modified channels. Based on this correlation, we can directly address the hypotheses concerning the general effects on activator drugs on inactivation removal and of local anesthetic inhibition that were stated in the introduction.

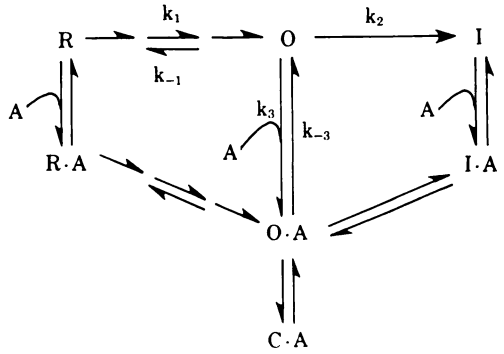
**The role of inactivation in activator potentiation.** The hypothesis that *Leiurus* toxin potentiates steady state activator action specifically by providing more open channels for binding is entirely inconsistent with our results. Neither chloramine T nor $Ni^{2+}$, both of which inhibit inactivation and thus would provide more open channels at depolarized potentials, potentiates the action of either activator. The simple scheme proposed by Krueger and Blaustein (6) thus cannot account for the results.

It seems likely to us that the potentiation of the activators by *Leiurus* toxin is due to a unique modification of the channel by this toxin that is not conferred by chloramine T or $Ni^{2+}$. Such a unique modification would not be surprising since the changes in channel structure produced by a 7000-Da polypeptide are probably unlike those produced by an oxidizing agent or a divalent metal cation, although all three have the ultimate effect of inhibiting channel inactivation. Until more detailed information on channel structure can be obtained, differentiation between these pharmacological effects may not be possible. However, correlates of drug-specific modification may be found

in physiological assays, for example, the voltage-dependent binding of *Leiurus* toxin (28, 29) which may have no analogue in the actions of chloramine T or $Ni^{2+}$.

**A model for the different actions of VTD and BTX.** Nerve stimulation enhances the rate of BTX action on voltage-clamped nodes of Ranvier (3), and, indeed, we observed that repetitive stimulation enhanced the rates of depolarization induced by both BTX and VTD in these studies. The steady state depolarization levels were unaltered by stimulation. The rate enhancement is consistent with the published hypothesis that activators bind preferentially to open channels (3, 4), although the difference between the kinetics of BTX and VTD on the node requires a more detailed analysis. An interesting observation is that both chloramine T and $Ni^{2+}$ enhanced the rate of BTX-induced depolarization, but neither enhanced the rate of VTD-induced depolarization. Diffusion is clearly not the limiting step in the rate of activator action since nerve stimulation enhances the depolarization rates from both BTX and VTD. This is consistent with the notion that the drugs are available at their site of action, but the site itself is unavailable for binding until the channels open.

The experimental observations can be explained for the most part by a simple modulated receptor hypothesis, as shown in the following scheme.




Sodium channels normally exist in several different states, here condensed into resting ($R$), open ($O$), and inactivated ($I$), each of which can potentially bind an activator molecule ($A$). The rate of depolarization is assumed to be limited largely by the reaction of $A$ with open channels. For the purpose of calculating relative rates of activator action, we assume that the $\Delta V_m$ is proportional to the modified sodium permeability and, therefore, to $O{\cdot}A$. Time constants for the depolarization process are then given by:

$$\tau_{\Delta V} = \frac{1}{(k_3 \cdot [A] + k_{-3})} \qquad (1)$$

Rate constants for activator binding ($k_3$) can be calculated using Eq. 1 while setting $k_{-3}$ equal to zero for the irreversible action of BTX and to 0.02 $sec^{-1}$ for VTD (see Fig. 1B); the calculated values are $3.8 \times 10^3$ $M^{-1}sec^{-1}$ for BTX and $1.3 \times 10^3$ $M^{-1}sec^{-1}$ for VTD. The similarity between these on rate constants shows that the 300-fold difference in potency for the steady state responses of BTX and VTD (see Fig. 5) is due almost entirely to the extremely slow off-rate of BTX.

The observed rates of depolarization from these two activators are probably limited by different factors under these experimental conditions. Veratridine is present at a relatively high concentration and the binding reaction will be fast compared to the open state lifetime (determined by $k_2$ and $k_{-1}$). Stimulation will increase the population of $O$, thereby decreasing $\tau_{\Delta V}$, but treatments that increase the lifetime of $O$ (by inhibiting fast inactivation) will not measurably change the rate. In contrast, at 0.1 $\mu$M BTX, the binding reaction to $O$ will be rate limiting and both stimulation (to open channels) and inhibition of activation (to keep channels open longer) should increase the rate. These predictions are supported by two experimental observations. The first is that neither chloramine T nor $Ni^{2+}$ enhances the rate of depolarization from higher BTX concentrations (>0.5 $\mu$M). The model predicts that, at increased concentrations, BTX is more likely to bind to an open channel before it can inactivate, so that the slowing of inactivation would have correspondingly less effect on the rate of depolarization. By the same logic, it would be expected that, at lower VTD concentrations, the inactivation inhibitors would enhance the rate of VTD action since the open channel duration would become a rate-limiting factor. However, the inhibitors of inactivation had no effect on the depolarization rate caused by 3 $\mu$M VTD which was the lowest VTD concentration which produced a consistent depolarization response. This concentration may already be too high for the open channel duration to be a limiting factor in the on-rate.

The second supporting observation is given in Table 1. When a nerve is treated with an inhibitor of inactivation, a single stimulus elicits a CAP that is seconds to minutes in duration (see Fig. 3, B–D). Therefore, many channels are driven to the open state by the stimulation and maintained there by inactivation inhibitors. Under these conditions, the rate of depolarization by 0.1 $\mu$M BTX is further enhanced over that arising from either treatment alone, suggesting that both the rate of channel opening and the open channel duration are independent limiting factors in the rate of BTX action. Since the rate of VTD action in a stimulated nerve is already too fast to resolve, we cannot draw any conclusion as to the combined effects of stimulation and inhibition of inactivation on the rate of VTD action. Nonetheless, it seems likely that channel opening is the rate-limiting step of VTD action since the VTD effect nearly reaches steady-state during the time course of a single CAP (~2 msec) in a nerve whose inactivation has not been modified. On the basis of these arguments, we propose that inhibitors of inactivation will enhance the rate of action of Na channel activators only under conditions where activator binding to the open channel (and not the frequency of channel opening) limits the rate of activator action.

The data do show that the effectiveness of VTD in producing a transient but not a steady state response equals that of BTX; the difference in agonist effectiveness is due to the partial reversal of the VTD-induced depolarization (Fig. 5B). The lower potency of VTD is due to its faster off-rate and that of the partial agonist character is due to a slow partial conversion of modified channels to a non-conducting form, a reaction not apparent in BTX-activated channels.

[Although the transient depolarization response to VTD has a decay component reminiscent of channel inactivation, it is not affected by *Leiurus* scorpion toxin sufficient to prevent 70–80% of fast inactivation from occurring (30). Therefore, we have introduced a step in the modulated receptor scheme for the conversion of activator-bound channels to a closed state ($C{\cdot}A$) unrelated to normal inactivation. Since the relative amplitude of the transient VTD response increases with in-

creasing VTD concentrations as well as with increased depolarization, the conversion to $C \cdot A$ may follow from binding of VTD to additional sites which block or close open channels, or may be a response to membrane depolarization per se.]

**Activator inhibition by local anesthetics.** The data on activator-local anesthetic interactions are qualitatively consistent with previous studies of BTX-local anesthetic interactions (1, 3, 7–9, 24). Although BTX acts essentially irreversibly in the frog sciatic nerve, its antagonism by local anesthetics is revealed by the slower rate of BTX-induced depolarization and the smaller steady state effect in nerves pretreated with lidocaine (Table 2). Veratridine and local anesthetics are more directly competitive inhibitors of one another since both are reversible. There is, however, some non-competitive action of local anesthetics in inhibiting the VTD-induced depolarization at high local anesthetic concentrations. This non-competitive action of local anesthetics probably also accounts for the small inhibition of the BTX-induced depolarization by 10 mM lidocaine (Fig. 6).

Recent voltage clamp studies of frog nerve show that chloramine T-modified channels, opened during constant depolarization, were blocked by benzocaine as rapidly as were unmodified, transiently open channels, whereas veratridine-modified open channels were blocked at a 70-fold slower rate (31). These observations were interpreted as the mutually exclusive binding of VTD and benzocaine to a sodium channel. However, a different conclusion was reached on the basis of a kinetic study of activator binding to synaptosomes (9). The binding of a derivative of BTX to sodium channels was slowed and its dissociation accelerated in the presence of local anesthetics requiring the presence of a tertiary anesthetic/channel/activator complex. The results with BTX that we report here are more consistent with the hypothesis of mutually exclusive binding, but those with VTD can be explained by either hypothesis. The difference may depend as much on the nature of the local anesthetic as on the degree of reversibility of the activator ligand, for there appear to be multiple binding sites for different forms of local anesthetics inhibiting BTX-modified sodium channels (24).

The stereoselectivity of the RAC 109 enantiomers is relatively constant for the measures of anesthetic action, despite the almost 10-fold range in apparent potencies among these different measures. As shown in Table 3, the relative potency of RAC 109I to RAC 109II is 3.6 for inhibition of the VTD response and 2.8 for the inhibition of the CAP. The results suggest that some structural feature of the anesthetic receptor is common to the various binding sites that account for the different actions of local anesthetics. Alternatively, one anesthetic binding site may account for all of these actions, and the structural rearrangements which correspond to affinity differences between resting and activated channel states would not involve regions of the anesthetic binding site that interact with the chiral carbon-centered moiety of RAC 109 enantiomers.

**Multiple meanings and mechanisms of Na channel inactivation.** BTX and VTD both inhibit Na channel inactivation themselves, their depolarizing actions at steady state are potentiated by one inhibitor of inactivation (*Leiurus* toxin) but not by others (chloramine T and $Ni^{2+}$), and both drugs are inhibited by stabilizers of nonactivatable channels (local anesthetics). What is the relationship between the site of action of the activators and Na channel inactivation? The inactivation process is complex. First of all, there are multiple inactivation processes in nodal Na channels with very different time courses—two phases of fast inactivation (32), a slow inactivation (33), and an ultra-slow inactivation (34). The precise relationship between these processes is unclear. It is not known, for example, whether channels must pass through a fast inactivated state in order to reach the slow inactivated states (33). Second, the inactivation process is inhibited by a plethora of vastly different treatments—enzymes (e.g., Pronase, trypsin), polypeptide toxins (e.g., *Leiurus* toxin, *Centruroides sculpturatus* $\alpha$-toxin V, sea anemone toxins), alkaloid toxins (e.g., BTX, VTD, aconitine), reactive chemicals (e.g., chloramine T, *N*-bromoacetamide, iodate), small ions (e.g., $Ni^{2+}$, $Zn^{2+}$), etc. (see Ref. 35 for references). Therefore, a process that is susceptible to such a variety of agents, some externally and some internally applied, is likely to involve a large part of the protein that constitutes the Na channel. This lends further support to the notion that inactivation does not occur through a single "site" on the channel but is a process that may be coupled to various pharmacologically defined sites. We hypothesize that the modulation of the activators by the inactivation process occurs at a site that is strongly coupled to the sites of action of *Leiurus* toxin and local anesthetics but weakly coupled to the sites of action of chloramine T and $Ni^{2+}$. Current views of Na channel pharmacology involve segregated sites of action for various classes of drugs; it may be that a view of a more interactive and dynamic pharmacology is required.

**References**


1. Albuquerque, E. X., I. Seyama, and T. Narahashi. Characterization of batrachotoxin-induced depolarization of the squid giant axons. *J. Pharmacol. Exp. Ther.* **184**:308–314 (1973).
2. Ulbricht, W. The effects of veratridine on excitable membranes in nerve and muscle. *Egreb. Physiol. Biol. Chem. Exp. Pharmakol.* **61**:17–71 (1968).
3. Khodorov, B. I. Chemicals as tools to study nerve fiber sodium channels; effects of batrachotoxin and some local anesthetics, in *Membrane Transport Processes* (D. C. Tosteson, Y. A. Ovchinnikov, and R. Latorre, eds.), Vol 2. Raven Press, New York, 153–174 (1978).
4. Bartels-Bernal, E., T. L. Rosenberry, and J. W. Daly. Effect of batrachotoxin on the electroplax of electric eel: evidence for voltage-dependent interaction with sodium channels. *Proc. Natl. Acad. Sci. USA* **74**:951–955 (1977).
5. Catterall, W. A. Activation of the action potential $Na^+$ ionophore by neurotoxins: an allosteric model. *J. Biol. Chem.* **252**:8669–8676 (1977).
6. Krueger, B. K., and M. P. Blaustein. Sodium channels in presynaptic nerve terminals: regulation by neurotoxins. *J. Gen. Physiol.* **76**:287–313 (1980).
7. Huang, L.-Y. M., G. Ehrenstein, and W. Catterall. Interaction between batrachotoxin and yohimbine. *Biophys. J.* **23**:219–232 (1978).
8. Creveling, C. R., E. T. McNeal, J. W. Daly, and G. B. Brown. Batrachotoxin-induced depolarization and [$^3$H]batrachotoxin-A 20$\alpha$-benzoate binding in a vesicular preparation from guinea pig cerebral cortex. *Mol. Pharmacol.* **23**:350–358 (1983).
9. Postma, S. W., and W. A. Catterall. Inhibition of binding of [$^3$H]batrachotoxin-A 20$\alpha$-benzoate to sodium channels by local anesthetics. *Mol. Pharmacol.* **25**:219–227 (1984).
10. Khodorov, B. I., E. Peganov, S. Revenko, nd L. Shishkova. Sodium currents in voltage clamped nerve fiber of frog under the combined action of batrachotoxin and procaine. *Brain Res.* **84**:541–546 (1975).
11. Yeh, J. Z. Blockage of $Na^+$ channels by stereoisomers of local anesthetics, in *Progress in Anesthesiology* (B. R. Fink, ed.), Vol. 2. Raven Press, New York, 35–44 (1980).
12. Hille, B., K. Courtney, and R. Dum. Rate and site of action of local anesthetics in myelinated nerve fibers, in *Progress in Anesthesiology* (B. R. Fink, ed.), Vol. 1. 13–24 (1975).
13. Stampfli, R. A new method for measuring membrane potentials with external electrodes. *Experientia* **12**:508–509 (1954).
14. Strong, P. N., J. T. Smith, and J. F. W. Keana. A convenient bioassay for detecting nanomolar concentrations of tetrodotoxin. *Toxicon* **11**:433–438 (1973).
15. Hahin, R., and G. R. Strichartz. Effects of deuterium oxide on the rate and dissociation constants for saxitoxin and tetrodotoxin action. *J. Gen. Physiol.* **78**:113–139 (1981).
16. Wang, G. K., and G. R. Strichartz. Purification and physiological characterization of neurotoxins from venoms of the scorpions *Centruroides sculpturatus* and *Leiurus quinquestriatus*. *Mol. Pharmacol.* **23**:519–513 (1983).

17. Wang, G. K. Irreversible modification of sodium channel inactivation in toad myelinated nerve fibers by the oxidant chloramine T. *J. Physiol.* (*Lond.*) **346**:127–141 (1984).
18. Dodge, F. A. Ionic permeability changes underlying nerve excitation, in *Biophysics of Physiological and Pharmacological Actions* (A. M. Shanes, ed.). American Association for the Advancement of Science, Washington, D. C., 119 (1961).
19. Hille, B. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* **69**:497–515 (1977).
20. Courtney, K. R. Mechanism of frequency-dependent inhibition of sodium currents in frog myelinated nerve by the lidocaine derivative GEA-968. *J. Pharmacol. Exp. Ther.* **171**:45–51 (1975).
21. Strichartz, G., and G. K. Wang. The kinetic basis for phasic local anesthetic blockade of neuronal sodium channels, in *Molecular and Cellular Mechanisms of Anesthetics* (K. W. Miller and S. Roth, eds.). Plenum Publishing Corp., New York, 217–226 (1986).
22. Strichartz, G. R. The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J. Gen. Physiol.* **62**:37–57 (1973).
23. Cahalan, M., B. I. Shapiro, and W. Almers. Relationship between inactivation of sodium channels and block by quaternary derivatives of local anesthetics and other compounds, in *Progress in Anesthesiology* (B. R. Fink, ed.), Vol. 2. Raven Press, New York, 17–33 (1980).
24. Huang, L.-Y. M., and G. Ehrenstein. Local anesthetics QX-572 and benzocaine act at separate sites on the BTX-activated sodium channel. *J. Gen. Physiol.* **77**:137–153 (1981).
25. Catterall, W. A. Inhibition of voltage-sensitive sodium channels in neuroblastoma cells by antiarrhythmic drugs. *Mol. Pharmacol.* **20**:356–362 (1981).
26. Courtney, K. R. Structure-activity relations for frequency-dependent sodium channel block in nerve by local anesthetics. *J. Pharmacol. Exp. Ther.* **213**:114–119 (1980).
27. Akerman, B. Uptake and retention of the enantiomers of a local anaesthetic in isolated nerve in relation to different degrees of blocking of nervous conduction. *Acta Pharmacol. Toxicol.* **32**:225–236 (1973).
28. Catterall, W. A. Binding of scorpion toxin to receptor sites associated with sodium channels in frog muscle: correlation of voltage-dependent binding with activation. *J. Gen. Physiol.* **74**:375–391 (1979).
29. Strichartz, G. R., and G. K. Wang. Rapid voltage-dependent dissociation of scorpion $\alpha$-toxins coupled to Na channel inactivation in amphibian myelinated nerves. J. Gen. Physiol., in press.
30. Wang, G. K., and G. Strichartz. Kinetic analysis of the action of *Leiurus* scorpion $\alpha$-toxin on ionic currents in myelinated nerve. *J. Gen. Physiol.* **86**:739–762 (1985).
31. Ulbricht, W., and M. Stoye-Herzog. Distinctly different rates of benzocaine action on sodium channels of Ranvier nodes kept open by chloramine-T and veratridine. *Pfluegers Arch. Eur. J. Physiol.* **402**:439–445 (1984).
32. Chiu, S. Y. Inactivation of sodium channels: second order kinetics in myelinated nerve. *J. Physiol.* (*Lond.*) **273**:573–596 (1977).
33. Peganov, E. M., B. I. Khodorov, and L. D. Shishkova. Slow sodium inactivation in the Ranvier node membrane: role of extracellular potassium. *Bull. Exp. Biol. Med.* (*Engl. Transl. Byull. Eksp. Biol. Med.*) **76**:1014–1017 (1973).
34. Fox, J. M. Ultra-slow inactivation of the ionic currents through the membrane of myelinated nerve. *Biochim. Biophys. Acta* **426**:232–244 (1976).
35. Khodorov, B. I. Some aspects of the pharmacology of $Na^+$ channels in nerve membrane. Process of inactivation. *Biochem. Pharmacol.* **28**:1451–1459 (1979).

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