J. Membrane Biol. 110, 199–207 (1989)

The Journal of
Membrane Biology


# Polypeptide Neurotoxins Modify Gating and Apparent Single-Channel Conductance of Veratridine-Activated Sodium Channels in Planar Lipid Bilayers

Adrian M. Corbett and Bruce K. Krueger
Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

**Summary.** The effects of scorpion and sea anemone polypeptide toxins on partially purified veratridine (VER)-activated Na channels from rat brain were studied at the single-channel level in planar lipid bilayers. The probability of the VER-activated channel being open ($P_o$) increased with depolarization; $P_o$ was 0.5 at −40 to −50 mV. Saxitoxin (STX) blocked VER-activated channels with an apparent dissociation constant of about 1 nM at −45 mV. The apparent single-channel conductance was approximately 9 pS, similar to that seen in VER-activated Na channels from skeletal muscle transverse tubules. Addition of sea anemone or scorpion polypeptide toxins to VER-activated Na channels resulted in a 19% increase in apparent single-channel conductance and a hyperpolarizing shift in the $P_o$ *vs.* $V_m$ relation such that the channels were more likely to be open at potentials <40 mV. These effects of the polypeptide toxins on the single-channel properties of VER-activated Na channels may account for the previously described potentiation of VER action by polypeptide toxins.

**Key Words** sodium channels · polypeptide toxins · veratridine · single-channel analysis · planar bilayer · reconstitution

## Introduction

Voltage-dependent Na channels from excitable tissues can be modulated by several classes of naturally occurring neurotoxins (Catterall, 1988). Saxitoxin (STX) and tetrodotoxin (TTX) are potent blockers of open Na channels, while the steroidal alkaloid neurotoxins, batrachotoxin (BTX) and veratridine (VER), activate the channels, causing them to remain open for long periods of time. Certain polypeptide toxins from scorpions (generally described as α-toxins) and sea anemones inhibit channel inactivation (Meves, Simard & Watt, 1986; Strichartz, Rando & Wang, 1987) and potentiate the effects of the activators, BTX and VER (Catterall, 1976). The α-scorpion and sea anemone polypeptide toxins share a common site of action (Catterall & Beress, 1978), but the scorpion toxins are about an order of magnitude more potent than the sea anemone toxins (Catterall, 1977*a*,*b*; Catterall & Beress, 1978; Krueger & Blaustein, 1980). Some scorpion venoms also contain β-polypeptide toxins, which act at a different site than the α-toxins (Rack, Richter & Rubly, 1987) and primarily affect channel activation rather than inactivation (Meves et al., 1986).

When reconstituted in artificial planar lipid bilayers, BTX-activated Na channels have a maximal single-channel conductance of 21–30 pS and show a 9 to 12-fold selectivity for $Na^+$ over $K^+$ (Krueger, Worley & French, 1983; Moczydlowski, Garber & Miller, 1984). VER-activated channels have a considerably smaller single-channel conductance (10 pS) and a selectivity for $Na^+$ over $K^+$ of less than threefold (Garber & Miller, 1987).

Studies with neuroblastoma cells and rat brain synaptosomes have shown that VER acts as a partial agonist and BTX as a full agonist (Catterall, 1977*a*; Krueger & Blaustein, 1980; Tamkun & Catterall, 1981); maximal concentrations of VER activate only a fraction of the available Na channels, whereas BTX can activate all available channels. Tracer $^{22}Na$ influx measurements in neuroblastoma cells, cultured cardiac myocytes and rat brain synaptosomes had indicated that polypeptide toxins from sea anemones (*Anemonia sulcata*; *Anthopleura xanthogrammica*) and scorpion (*Leiurus quinquestriatus*) potentiated the effects of the activators such that the $K_a$s for VER and BTX activation were lowered and maximally effective concentrations of VER were able to produce nearly full activation of the Na channel (Catterall, 1975, 1977*a*; Jacques, Fosset & Lazdunski, 1978; Krueger & Blaustein, 1980; Romey et al., 1980; Tamkun & Catterall, 1981).

In these studies, we describe the effects of the α-polypeptide toxin, LqTx, from the scorpion *L. quinquestriatus quinquestriatus*, and AxTx, from the sea anemone *A. xanthogrammica*, on single VER-activated Na channels from rat brain incor-

porated into planar lipid bilayers. Although their effects are similar, these two polypeptide toxins have no sequence homologies (Tanaka et al., 1977; Kopeyan, Martinez & Rochat, 1978; Strichartz et al., 1987). Both AxTx and LqTx primarily affected the probability of the channel being open, but also caused a small increase in apparent single-channel conductance. Some of these results have appeared in abstract form (Corbett, Zinkand & Krueger, 1987).

## Materials and Methods

### Materials

Lipids were obtained from Avanti Polar Lipids (Birmingham, AL). Decane was repurified before use by passage over acidic, basic and neutral alumina. STX (paralytic shellfish poison standard) was obtained from the Food and Drug Administration (Cincinnati, OH). Anemone toxin (AxTx or Anthopleurin A) was purified from whole anemones (*A. xanthogrammica*) by the method of Norton et al. (1976). Scorpion venom from *L. quinquestriatus quinquestriatus* was obtained from Sigma or Latoxan (Rosans, France). $\alpha$-scorpion toxin (LqTx) was purified as described by Catterall (1976). This preparation showed only $\alpha$-toxin-like activity in patch-clamped $GH_3$ cells (M.E. O'Leary & B.K. Krueger, *unpublished observations*) and no measurable $\beta$-toxin activity (*cf.* Rack et al., 1987).

### Preparation of Brain and Muscle Membranes

Crude membrane vesicles were prepared from rat brain as described by Krueger et al. (1979). Briefly, rat brains were homogenized in isotonic sucrose using a cavitating tissue disrupter and the homogenate was subjected to differential centrifugation. The $1{,}000 \times g$ and the $10{,}000 \times g$ pellets were discarded and the $100{,}000 \times g$ pellets (P3) were resuspended in 0.4 M sucrose at approximately 10 mg protein/ml. This material had a specific activity of 5 pmol $^3$H-STX binding sites/mg. Transverse tubules were prepared from rat skeletal muscle by the method of Rosemblatt et al. (1981).

### Purification and Reconstitution of Na Channels

The P3 membrane preparation from rat brain was solubilized in 1% Triton X-100, 0.1% egg phosphatidylcholine (PC), 100 mM KCl, 10 mM HEPES, and the protease inhibitors 1 $\mu$M pepstatin, 0.1 mM phenylmethylsulfonyl fluoride and 1 mM 1,10-phenanthroline (modified from Hartshorne & Catterall, 1984), centrifuged at $146{,}000 \times g$, and the supernatant retained. $CaCl_2$ was added to a final concentration of 10 mM, the pH was adjusted to 6.5 and the sample was loaded onto a DEAE fast-flow Sepharose column, which was batch eluted with 0.3 M KCl, 20 mM histidine, 10 mM $CaCl_2$, 0.1% Triton X-100, 0.025% egg PC, and protease inhibitors, pH 6.5. Fractions having detectable protein (Bradford, 1976) were pooled; the pH was adjusted to 7.0, free $Ca^{2+}$ was chelated with excess EDTA, and K-phosphate added to a final concentration of 0.1 M. The adjusted pooled fractions were loaded onto a column containing immobilized wheat germ agglutinin (WGA), washed with a NaCl buffer (0.4 M NaCl, 20 mM HEPES, 10 mM $CaCl_2$, 0.1% Triton X-100, 0.025% egg PC, and protease inhibitors) and eluted with the same buffer containing 60 mM N-acetyl-glucosamine. Peak protein fractions were pooled and had an average specific activity of 400–450 pmol $^3$H-STX binding sites/mg, as determined by rapid gel filtration through 2-ml Sephadex G-50 columns as described by Agnew et al. (1978).

The pooled fractions from the WGA column were concentrated approximately 12-fold (Centricon 30; Amicon, MA), and loaded onto a HPLC size exclusion column (TSK 4000 SW, $21.5 \times 600$ mm), which had been equilibrated with 0.1 M KCl, 20 mM histidine, 10 mM $CaCl_2$, 0.1% Triton X-100, 0.025% PC, and protease inhibitors, pH 6.5. The $^3$H-STX binding site eluted at 165 ml at a flow rate of 2 ml/min. The fractions with the highest STX binding activity ($\approx$1000 pmol $^3$H-STX binding sites/mg) were pooled for reconstitution.

Partially purified Na channel fractions from the WGA or size exclusion steps were reconstituted as follows. Initially, the pH of the pooled samples was adjusted to 7.0 following the chelation of free $Ca^{2+}$ by addition of excess EDTA. Three volumes pooled fractions were then mixed with one volume lipid solution (containing 12 mg/ml egg PC, 3% Triton X-100, 20 mM histidine, 100 mM KCl, 4 mM $CaCl_2$, and protease inhibitors, pH 7.0) such that the final concentrations were 3 mg/ml PC, 0.75% Triton X-100 and 1 mM $CaCl_2$. This mixture was gently shaken with polystyrene beads for 3 hr at 4°C, then poured through a sintered glass filter to remove the beads.

### Planar Bilayer Formation and Channel Incorporation

Planar bilayers were formed according to the method of Mueller et al. (1963) across a 250-$\mu$m hole in a polycarbonate partition separating identical solutions containing 250 mM NaCl, 75 $\mu$M $CaCl_2$, 50 $\mu$M EGTA, 2 mM MgCl, 10 mM HEPES-Tris, and 100 $\mu$M VER, pH 7. The membrane forming solution contained 39 mg/ml 1-palmitoyl-2-oleoyl phosphatidylethanolamine (PE) and 26 mg/ml bovine brain phosphatidylserine (PS) in decane.

Incorporation of reconstituted Na channels was achieved using a modification of the fusion technique (Miller, 1978; Krueger et al., 1983; Hanke, 1986). Reconstituted vesicles were diluted with an equal volume of 1.2 M sucrose, 20 mM histidine (pH 7.0) and subjected to two rapid freeze-thaw cycles. This material was then taken up in a glass volumetric pipette (25 $\mu$l) and gently blown on the bilayer membrane from the *cis* side. Upon incorporation of channels, the conductance of the membrane increased in steps, each level indicating the incorporation of at least one channel.

### Data Acquisition and Analysis

The current across the bilayer was measured and command voltages applied to the bathing solutions via a pair of Ag/AgCl electrodes connected to a homemade voltage-clamp circuit (French et al., 1986). The side to which the reconstituted vesicles were added was designated the *cis* side, and the opposite (*trans*) side was held at virtual ground. Data were recorded on videotape (Neurodata Inst., NY); stored records were filtered at 50–60 Hz

when played back onto a strip chart recorder or oscilloscope for manual determination of single-channel current and open durations.

The probability of the channel being open ($P_o$) is the total time each channel remained open, regardless of the subconductance state, divided by the total recording time. In Figs. 7 and 8, the $P_o$ *vs.* $V_m$ curves were fit to a Boltzmann relation of the form:

$$P_o = P_o(\max)/[1 + \exp\{(V_{0.5} - V_m)Fq/RT\}] \quad (1)$$

where $V_m$ is the membrane potential, $V_{0.5}$ is the $V_m$ at which $P_o$ = 0.5, and $P_o$(max) is the maximum $P_o$ obtained (assumed to be 1.0). The apparent gating charge, $q$, describes the steepness of the $P_o$ *vs.* $V_m$ relation. A program developed by Dr. Bruce Simon called FitA was used to fit the curves using a nonlinear least squares Marquardt algorithm.

A paired Student's $t$ test was employed to determine the significance of the difference between the apparent single-channel conductance in the absence and presence of polypeptide toxins. For each of seven experiments, the apparent single-channel conductance was determined from linear regression analyses ($r > 0.995$) of the single-channel current-voltage relations in the absence and presence of AxTx or LqTx. The increase in apparent single-channel conductance induced by either polypeptide toxin was significant ($P < 0.001$).

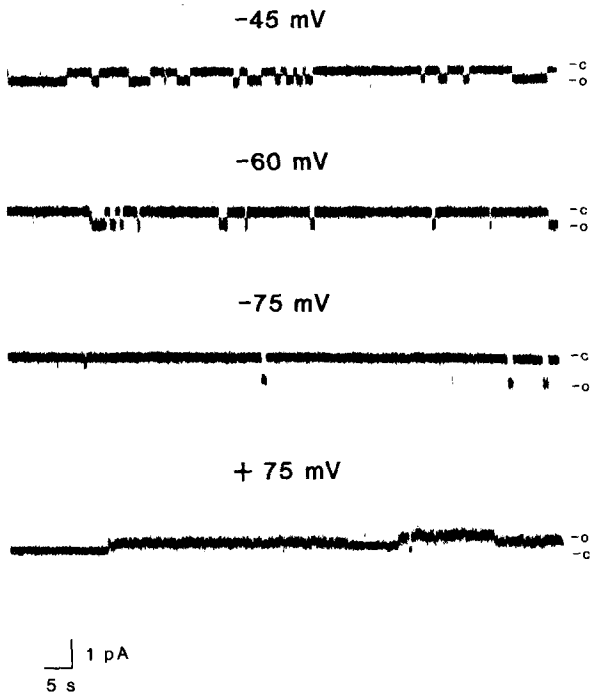


**Fig. 1.** Voltage-dependent gating of VER-activated Na channels. VER-stimulated Na channels from the size exclusion column step (specific activity ≈1,000 pmol/mg) were incorporated into a planar lipid bilayer (56%PE, 44%PS) with symmetrical 250 mM NaCl, 25 μM $Ca^{2+}$, 2 mM $Mg^{2+}$, 10 mM HEPES and 100 μM VER. Single Na channel current records are shown at −45 mV ($P_o$ = 0.33), −60 mV ($P_o$ = 0.08), and −75 mV ($P_o$ = 0.03) and at +75 mV ($P_o$ = 0.83). Each $P_o$ was determined for 2- to 3-min recordings, only portions of which are shown. For determination of $P_o$, any channel opening was considered as open whether the opening was to a subconductance state or to the fully open state. Records were filtered at 50 Hz

## Results

### Single-Channel Properties of Purified VER-Activated Na Channels

Figure 1 shows single-channel current records illustrating the voltage-dependent gating of rat brain Na channels in the presence of 100 μM VER. Although not clear in these heavily filtered records, each open event appeared to be a burst of openings with incompletely resolved closings. At −45 mV, the VER-activated channels were open 16 to 50% of the time, with bursts of openings generally lasting 1 to 7 sec and interburst, closed, intervals of 0.5 to 10 sec. The channels displayed a decreased probability of opening with hyperpolarization, with shorter openings and longer closed periods. At −75 mV, where the VER-activated channels were open less than 5% of the time, the burst duration was on the order of 1 to 3 sec.

At depolarized potentials, VER-activated channels opened in long bursts (30 sec to several minutes long), with occasional closings between bursts of approximately 15- to 45-sec duration. Rapid flickering to the closed state (manifested as increased open-channel noise) was usually seen throughout the depolarized range, causing a reduction in the apparent single-channel conductance. In contrast, BTX-activated rat brain channels showed no unresolved high frequency flickering at depolarized potentials. The flickering and the greatly increased incidence of subconductance states (*see below*) made analysis of these records difficult, so most of our studies were limited to the hyperpolarized range (<−40 mV).

VER-activated Na channels were blocked by STX from the extracellular side (Fig. 2). At +60 and −45 mV, the apparent dissociation constants ($K_i$) for STX block were estimated to be about 8 and 1 nM, respectively. Partial channel openings to less-than-maximal apparent single-channel conductance (substates) were observed at both depolarized and hyperpolarized potentials (Fig. 2). This is characteristic of VER-activated Na channels from rat brain: the channels spontaneously enter substates with conductances $\frac{1}{3}$ to $\frac{2}{3}$ that of the normal channel conductance. These less-than-maximal conductance states may be due to an increase in rapid flickering.

Na channels purified from rat brain and reconstituted into planar lipid bilayers displayed an apparent single-channel conductance of approximately 9 pS in the hyperpolarizing range (−45 to

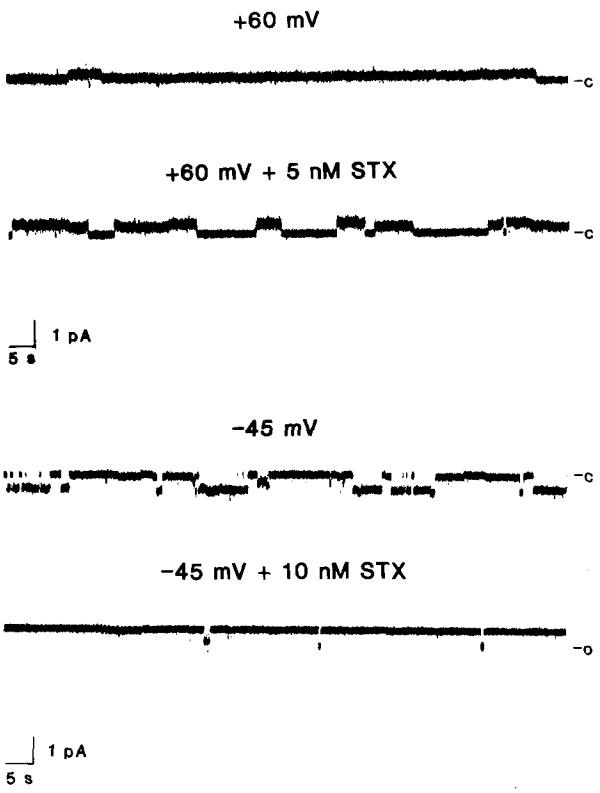


**Fig. 2.** STX block of VER-activated Na channels. Na channels were reconstituted from the HPLC size exclusion column pooled fraction (specific activity ≈1000 pmol/mg) into a planar lipid bilayer as described in Methods. +60 mV: $P_o$ = 0.98 and 0.59 in the absence and presence of external 5 nM STX ($K_i$ = 8 nM). −45 mV: $P_o$ = 0.31 and 0.02 in the absence and presence of 10 nM STX ($K_i$ = 1 nM). Records were filtered at 50 Hz

−90 mV), while the apparent conductance at depolarized potentials was lower due to flickering (Fig. 3*A*). We have also observed this flickering at depolarized potentials in VER-activated Na channels from crude rat brain membranes prior to solubilization and purification. Thus, this effect is not an artifact of solubilization. In contrast, although the skeletal muscle transverse tubule Na channel also displayed more open-channel noise at positive potentials, it was possible to resolve the full 10-pS conductance over the entire voltage range examined (−90 to +90 mV, Fig. 3*B*). We believe that the brain and muscle channels differ primarily with respect to the degree of flickering at depolarized potentials and that the conductance (9–10 pS) is similar for both channels at all potentials.

## Effects of Polypeptide Toxins

The effects of AxTx and LqTx on VER-activated channels are shown in Figs. 4 and 5. In the absence of polypeptide toxin, the channels opened rarely at hyperpolarized potentials and the probability of being open ($P_o$) decreased with hyperpolarization. Following the addition of the polypeptide toxins to the extracellular side, the channels opened in bursts that were much longer than the brief isolated openings seen in the absence of polypeptide toxins (Figs. 4 and 5). In the experiment shown in Fig. 4, in the presence of VER but in the absence of polypeptide toxin, only one channel was open at a time. In con-

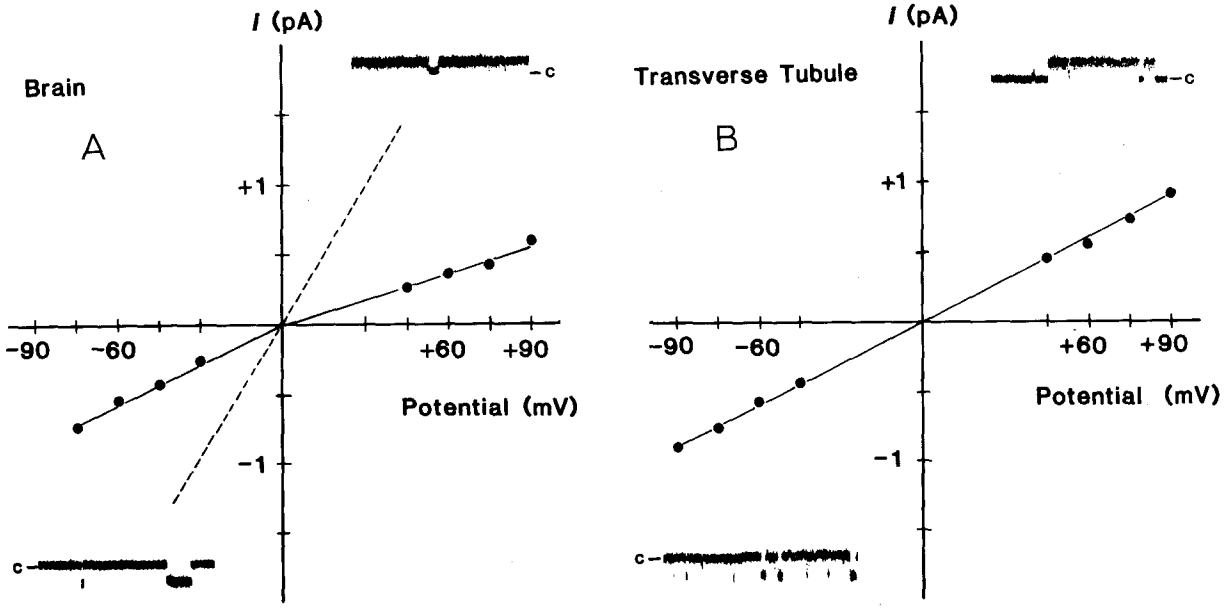


**Fig. 3.** Current-voltage relationships for VER-activated Na channels from (*A*) rat brain and (*B*) rat skeletal muscle transverse tubules. (*A*) The dashed line shows the corresponding relation for BTX-activated rat brain Na channels. Insets show single-channel current records of VER-stimulated rat brain Na channels at +60 mV (upper right) and −60 mV (lower left). (*B*) Insets show muscle transverse tubule Na channel openings at +75 mV (upper right) and −75 mV (lower left). Records were filtered at 50 Hz. Rat brain Na channels were reconstituted from the HPLC size exclusion column pooled fraction

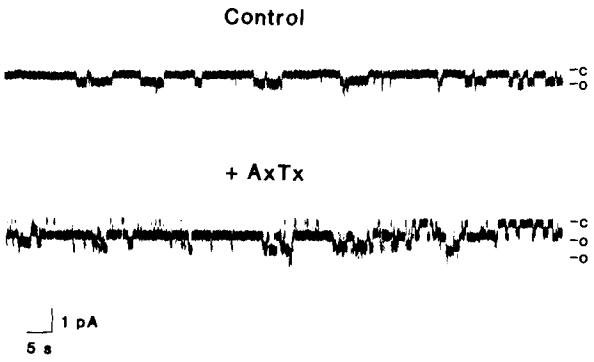


**Fig. 4.** Effect of sea anemone (*Anthopleura xanthogrammica*) toxin (AxTx) on VER-stimulated Na channels. $V_m = -65$ mV. Control $P_o = 0.11$; following addition of 200 nM AxTx, $P_o = 0.32$. Na channels were reconstituted from WGA column pooled fractions (specific activity ≈400 pmol/mg). Records filtered at 60 Hz

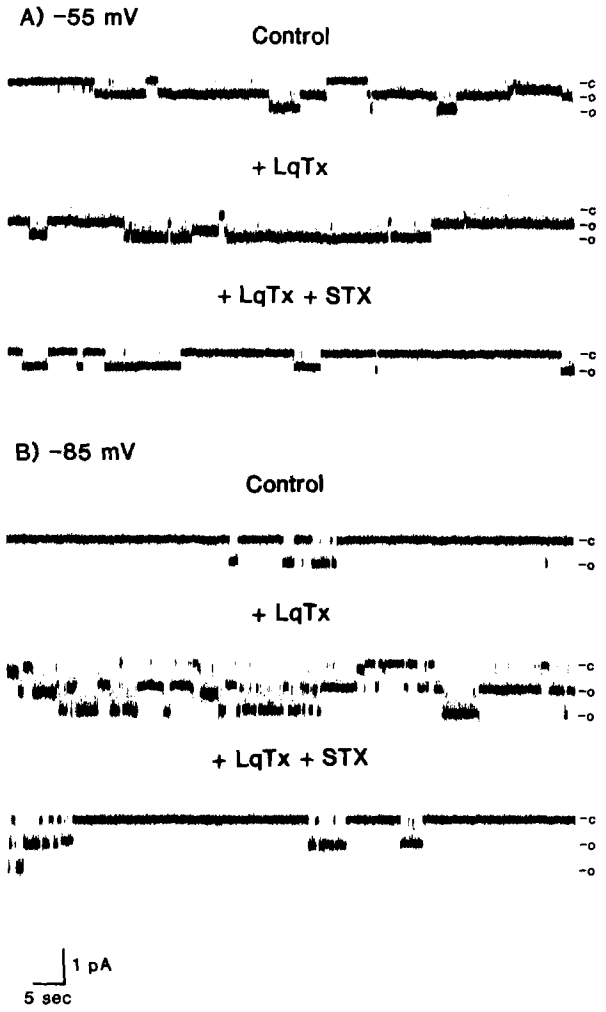


**Fig. 5.** Effect of scorpion (*Leiurus quinquestriatus quinquestriatus*) toxin (LqTx) on VER-stimulated Na channels. Single-channel current records from VER-activated Na channels are shown in the absence and presence of 500 nM LqTx at −55 mV (control $P_o = 0.39$, +LqTx $P_o = 0.78$) and −85 mV (control $P_o = 0.03$; + LqTx $P_o = 0.49$). STX block following the addition of the LqTx is shown at both potentials with 3 nM external STX. STX inhibited by 60% at −55 mV ($K_i = 2$ nM) and by 84% at −85 mV ($K_i = 1$ nM). Na channels were reconstituted from WGA column pooled fractions. Records were filtered at 50 Hz

trast, we observed two, and occasionally three, open-channel levels in the presence of AxTx. In the presence of LqTx (Fig. 5), the channels remained open 88 and 49% of the time at −55 and −85 mV, respectively. Figure 5 also shows block of the LqTx-modified Na channels by 3-nM STX. We estimate the $K_i$ to be approximately 2 nM at −55 mV and 1 nM at −85 mV.

There was a small increase in the apparent single-channel conductance of VER-activated Na channels following the addition of polypeptide toxins. In the presence of AxTx (Fig. 6*A*), the conductance increased from 8.7 to 10.8 pS, while in the presence of LqTx (Fig. 6*B*) it increased to 10.4 pS. Overall, both toxins increased the apparent single-channel conductance by 19 ± 1% ($n = 7$). This increase was highly significant ($P < 0.001$, paired Student's $t$ test).

Figures 7 and 8 show the effects of AxTx and LqTx on the probability of the channel being open ($P_o$) as a function of membrane potential. In the absence of polypeptide toxins, the probability of channel opening decreased with hyperpolarization, with the channel rarely open ($P_o$ of 0.01 to 0.05) at potentials below −70 mV. Upon the addition of extracellular AxTx (Fig. 7) or LqTx (Fig. 8), $P_o$ increased at each potential with the result that more negative potentials were required to fully close the channels. The data were fit to a Boltzmann relation with the assumption that at depolarized potentials, $P_o$ approached 1. In Fig. 7, the control data were fit with $V_{0.5} = -40$ mV and a gating charge of approximately 2; addition of AxTx shifted the curve such that $V_{0.5} = -52$ mV, while the gating charge remained constant. In Fig. 8, the $V_{0.5}$ for the control curve was −49 mV and the gating charge was approximately 1.4. Following addition of LqTx, the $V_{0.5}$ was shifted to −79 mV with no apparent effect on $q$. The gating charge determination should be considered a rough estimate since the data in our control curves did not exceed a $P_o$ of 0.5. Due to the small single-channel conductance under these experimental conditions (symmetrical 250 mM NaCl), we were not able to study channel properties between ±40 mV, where the driving force on $Na^+$ is small.

## Discussion

### Comparison with Previous Results

In the absence of polypeptide toxin but in the presence of 100 μM VER, we observed an apparent sin-

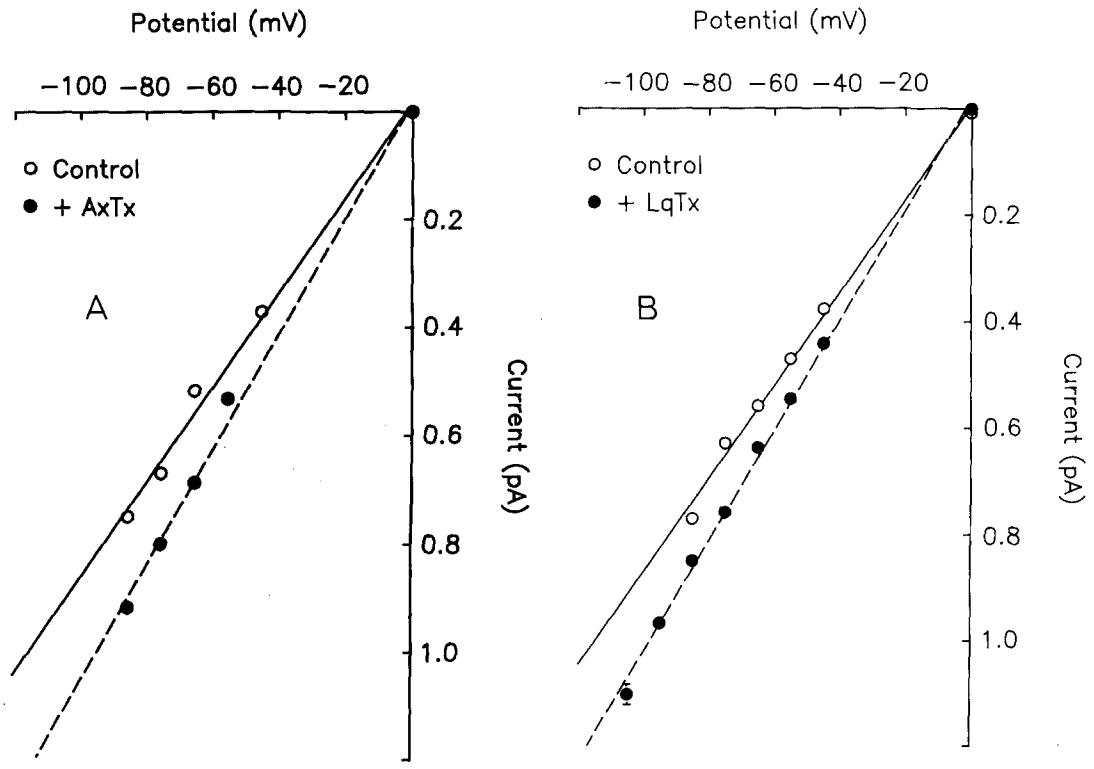


**Fig. 6.** Effect of polypeptide toxins on apparent single-channel conductance of VER-stimulated channels. Control single-channel current-voltage relation (O) corresponds to a conductance of approximately 9 pS. (*A*) Following the addition of 200 nM AxTx, the conductance (●) increased to 10.8 pS. (*B*) After addition of 500 nM LqTx, the apparent single-channel conductance (●) increased to 10.4 pS. Conditions are the same as in the legend to Fig. 1. Data were fit with linear regression with correlation coefficients >0.995. Na channels were reconstituted from WGA pooled fractions

gle-channel conductance of about 9 pS in symmetrical 250 mM NaCl and <100 μM calcium. This is nearly identical to that observed by Garber and Miller (1987) for muscle sarcolemmal Na channels reconstituted into planar lipid bilayers under similar conditions. Two other studies reported lower single-channel conductances (4–6 pS) in cell-attached patches of *Xenopus* oocytes injected with avian mRNA (Sigel, 1987) and neuroblastoma cells (Barnes & Hille, 1988). The lower conductance observed in these two cases was probably due to the high $K^+$ concentration on the intracellular side (*cf.* Garber & Miller, 1987). Thus, the single-channel conductance we observed is consistent with that found for Na channels in other excitable tissues. We occasionally observed STX-blockable Na channels with a smaller single-channel conductance (4–6 pS) under our standard experimental conditions using both crude brain membrane vesicles and partially purified and reconstituted STX-binding proteins (*unpublished*). We believe that these represent a distinct, less prevalent subtype of Na channels.

In the absence of polypeptide toxins, the single-channel open probability ($P_o$) in the presence of 100 μM VER was voltage dependent. Openings were brief and very rare ($P_o < 0.1$) at −90 mV and the $V_{0.5}$ was typically −40 to −50 mV. We were not able to study the voltage dependence of gating in the range of ±40 mV because, in symmetrical NaCl, the single-channel current is too small (<0.4 pA) to measure reliably. The $V_{0.5}$ of VER-activated Na channels was substantially depolarized compared to that estimated for VER-activated Na channels in frog muscle (≪−100 mV; Leibowitz, Sutro & Hille, 1986) and raises the possibility that the voltage dependence of $P_o$ we observe reflects the voltage-dependent binding and unbinding of VER to unmodified channels, rather than the voltage-dependent opening of VER-modified channels. This is consistent with the finding that, unlike BTX, the action of VER is rapidly reversible as demonstrated by a greatly reduced probability of opening when the VER concentration is lowered (Garber & Miller, 1987). Also, in our experiments, the typical burst duration of 1 to 5 sec at −85 mV is consistent with the estimated dissociation time constant of 3 sec (−90 mV) for VER in frog muscle (Sutro, 1986) as determined from macroscopic tail current decay.

Leibowitz et al. (1986) and Barnes and Hille (1988) have proposed that VER binds primarily, but

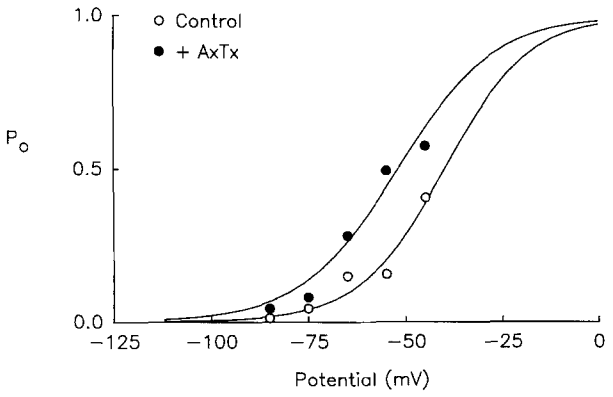


**Fig. 7.** Effect of AxTx on the voltage-dependent gating of VER-activated channels. $P_o$ is shown for VER-activated channels in the absence (○) and presence (●) of 200 nM AxTx. The data were fit to a Boltzmann relation (Eq. 1) with the assumption that the maximal $P_o$ was 1. The values for $V_{0.5}$ and gating charge ($q$) were −40 mV and 2.0 in the absence and −52 mV and 2.3 in the presence of AxTx. Na channels were reconstituted from HPLC size exclusion column pooled fractions

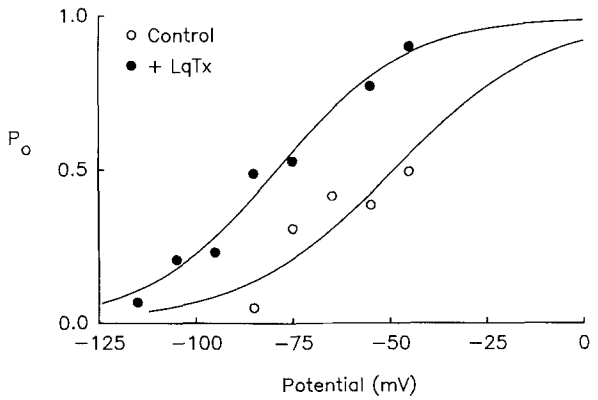


**Fig. 8.** Effect of LqTx on the voltage-dependent gating of VER-activated channels. $P_o$ is shown for VER-activated channels in the absence (○) and presence (●) of 500 nM LqTx. Data were fit as described in Materials and Methods and in the legend to Fig. 7. The values for $V_{0.5}$ and gating charge were −49 mV and 1.3 in the absence and −79 mV and 1.4 in the presence of LqTx. Na channels were reconstituted from WGA column pooled fractions

not exclusively, to open Na channels; binding to closed or inactivated channels is much less likely. Our experiments were conducted in the steady state where unmodified channels would be expected to be nearly always in the closed (at hyperpolarized potentials) or inactivated (depolarized potentials) states and, thus, would have only a low probability of activation by VER. There should be a narrow voltage "window" within which a channel will have a finite probability of being open and a non-zero probability of being non-inactivated. Occasional brief openings in this voltage "window" may allow VER to bind and the voltage dependence of these openings may account for the apparent voltage dependence of opening we observe in the presence of VER. We have never observed single Na channel currents in the absence of VER or BTX probably because any opening of unmodified channels would be too brief to detect with our instrumentation.

## Effects of Polypeptide Toxins on VER-Activated Na Channels

α-Scorpion and sea anemone polypeptide toxins act primarily to slow Na channel inactivation in the absence of VER or other activating toxin (Meves et al., 1986; Strichartz et al., 1987). They also act synergistically with activating toxins, increasing the apparent potency of BTX and increasing both the apparent potency and maximal effect of VER as measured by $^{22}Na$ tracer influx into neuroblastoma and rat brain synaptosomes (Catterall, 1977*a*). The effects of the polypeptide toxins on VER-activated Na channels have not previously been studied at the single-channel level. In the present study, we found that both sea anemone and scorpion polypeptide toxins altered two single-channel parameters of VER-activated Na channels in planar bilayers, *viz.*, the open probability ($P_o$) was increased at all hyperpolarized potentials studied and the apparent single-channel conductance underwent a small but significant increase from 9 to 10.5 pS.

The polypeptide toxin-induced increase in $P_o$ appears to be due both to an increase in the opening rate (shorter closed intervals between openings, *cf.* Fig. 5*B*) and to a decrease in the closing rate (longer open or burst times, in some cases lasting 30 sec or more, *cf.* Fig. 5*A*). We cannot unequivocally distinguish between the kinetics of polypeptide toxin binding and the kinetics of VER binding and unbinding; however, the off-rate for $^{125}I$-LqTx is less than 0.2 $min^{-1}$ (Catterall, 1977*b*), suggesting that the polypeptide toxin stays bound to the Na channel for relatively long periods of time compared to the mean open time of Na channels in the presence of VER and either LqTx or AxTx. Thus, in the presence of VER and polypeptide toxins, the opening and closing events (Figs. 4 and 5) are probably due to binding and unbinding of VER or to the gating of VER + polypeptide-modified channels rather than to binding and unbinding of the polypeptide toxin.

The slowing of inactivation induced by polypeptide toxins may account, in part, for the increased open probability ($P_o$) observed in the presence of LqTx or AxTx if, like N-bromoacetamide (Patlak & Horn, 1982), the polypeptide toxins increase the duration of channel openings and, thus, the probability of VER binding (Nagy, 1988). In ad-

dition, in the presence of VER, the open-channel lifetimes are increased by polypeptide toxins, indicating either a lower probability of VER unbinding or a decreased closing probability of VER + polypeptide toxin-modified channels. We have never observed single Na channel openings in the presence of polypeptide toxins without VER or BTX, probably because, even with a decreased inactivation rate, openings in the steady state are still rare and too brief to detect at our recording bandwidth.

Regardless of the mechanism, both LqTx and AxTx cause hyperpolarizing shifts in the $P_o$ *vs.* $V_m$ relation for VER-activated Na channels. Polypeptide toxins appear to primarily affect the midpoint ($V_{0.5}$) rather than the steepness ($q$) of the gating curve. Thus, polypeptide toxins do not affect the apparent gating charge required to move to open the channel. Instead, the channels behave as if they are exposed to a depolarizing bias potential of 10 to 30 mV. We have observed considerable channel-to-channel variation in both $V_{0.5}$ and $q$ for VER-activated channels (e.g., $V_{0.5}$ and $q$ were −45 mV and 2.2 in Fig. 7 and −50 mV and 1.4 in Fig. 8). These values for $q$ are lower than those reported for unmodified and BTX-activated Na channels, however, comparable measurements in the presence of VER have not previously been reported. The variation among individual channels has also been observed for both purified (Hartshorne et al., 1985) and unpurified (Cukierman et al., 1988) BTX-activated Na channels in planar bilayers and, thus, is not an artifact of solubilization and purification.

A second effect of polypeptide toxins was to increase the apparent single-channel conductance of VER-activated Na channels by about 20%. This small increase was reproducible and highly significant, and may be due to a change in the ion permeation pathway through the channel or to an alteration in the kinetics of a fast gating process that is heavily filtered at our recording bandwidth. An example of the latter mechanism is the decrease in the apparent single-channel conductance of VER-activated rat brain Na channels we observed at depolarized potentials (Fig. 3*A*) that is accompanied by increased open-channel noise, suggesting rapid flickering that is too fast to resolve. Barnes and Hille (1988) also reported increased open-channel flickering in single VER-activated Na channel recordings from neuroblastoma cells. Clarification of the mechanism for this polypeptide toxin-induced increase in apparent single-channel conductance will have to await the capability of experiments at considerably higher bandwidth.

It is of interest that in the presence of both VER and LqTx or AxTx, the voltage-dependent gating is similar to that of BTX-activated Na channels. However, in the presence of AxTx or LqTx, VER-activated channels still differ from BTX-activated channels in that the apparent single-channel conductance (10–11 pS) is less than half that of purified BTX-activated channels (22–25 pS, *see* Fig. 3*A*).

The effects of polypeptide toxins on the single-channel properties of VER-activated Na channels are, in general, consistent with previously reported effects on VER-stimulated tracer Na influx in synaptosomes and neuroblastoma cells and on macroscopic Na current inactivation in several excitable cells. Polypeptide toxin binding to the Na channel may result in a longer VER residency in its binding site and longer burst durations as observed in our experiments. The decreased probability of inactivating may provide a greater opportunity for VER binding and thus shorter, interburst intervals. Overall, the net result is a hyperpolarizing shift in the $P_o$ *vs.* $V_m$ relation (Figs. 7 and 8) and an increased apparent single-channel conductance (Fig. 6) induced by polypeptide toxins, resulting in a potentiation of VER action.

We thank Bill Zinkand and Dan Brougher for technical assistance and Bruce Simon for curve fitting software. This work was supported by NIH grants NS16285 and NS20106, and a U.S. Army Medical Research and Development Command contract DAMD17-85-C-5283.

## References

Agnew, W.S., Levinson, S.R., Brabson, J.S., Raftery, M.A. 1978. Purification of the tetrodotoxin-binding component associated with the voltage-sensitive Na channel from *Electrophorus electricus* electroplax membranes. *Proc. Natl. Acad. Sci. USA* **75**:2606–2610

Barnes, S., Hille, B. 1988. Veratridine modifies open Na channels. *J. Gen. Physiol.* **91**:421–443

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein dye binding. *Anal. Biochem.* **72**:248–254

Catterall, W.A. 1975. Activation of the action potential $Na^+$ ionophore of cultured neuroblastoma cells by veratridine and batrachotoxin. *J. Biol. Chem.* **250**:4053–4059

Catterall, W.A. 1976. Purification of a toxic protein from scorpion venom which activates the action potential $Na^+$ ionophore. *J. Biol. Chem.* **251**:5528–5536

Catterall, W.A. 1977*a*. Activation of the action potential $Na^+$ ionophore by neurotoxins. An allosteric model. *J. Biol. Chem.* **252**:8669–8676

Catterall, W.A. 1977*b*. Membrane potential-dependent binding of scorpion toxin to the action potential $Na^+$ ionophore. *J. Biol. Chem.* **252**:8660–8668

Catterall, W.A. 1988. Structure and function of voltage-sensitive ion channels. *Science* **233**:653–661

Catterall, W.A., Beress, L. 1978. Sea anemone toxin and scorpion toxin share a common receptor site associated with the action potential sodium ionophore. *J. Biol. Chem.* **253**:7393–7396

Corbett, A.M., Zinkand, W.C., Krueger, B.K. 1987. Activation of single neuronal sodium channels by veratridine and polypeptide neurotoxin in planar lipid bilayers. *Biophys. J.* **51**:435*a* (*Abstr.*)

Cukierman, S., Zinkand, W.C., French, R.J., Krueger, B.K. 1988. Effects of membrane surface charge and calcium on the gating of rat brain sodium channels in planar bilayers. *J. Gen. Physiol.* **92**:431–447

French, R.J., Worley, J.F., Blaustein, M.B., Romine, W.O., Tam, K.K., Krueger, B.K. 1986. Gating of batrachotoxin activated sodium channels in lipid bilayers. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 363–383. Plenum, New York

Garber, S.S., Miller, C. 1987. Single $Na^+$ channels activated by veratridine and batrachotoxin. *J. Gen. Physiol.* **89**:459–480

Hanke, W. 1986. Incorporation of ion channels by fusion. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 141–153. Plenum, New York

Hartshorne, R.P., Catterall, W.A. 1984. The sodium channel from rat brain. Purification and subunit composition. *J. Biol. Chem.* **259**:1667–1675

Hartshorne, R.P., Keller, B.U., Talvenheimo, J.A., Catterall, W.A., Montal, M. 1985. Functional reconstitution of the purified brain sodium channel in planar bilayers. *Proc. Natl. Acad. Sci. USA* **82**:240–244

Jacques, Y., Fosset, M., Lazdunski, M. 1978. Molecular properties of the action potential $Na^+$ ionophore in neuroblastoma cells. *J. Biol. Chem.* **253**:7383–7392

Kopeyan, C., Martinez, G., Rochat, H. 1978. Amino acid sequence of neurotoxin V from the scorpion *Leiurus quinquestriatus quinquestriatus*. *FEBS Lett.* **89**:54–58

Krueger, B.K., Blaustein, M.P. 1980. Sodium channels in presynaptic nerve terminals: Regulation by neurotoxins. *J. Gen. Physiol.* **76**:287–313

Krueger, B.K., Ratzlaff, R.W., Strichartz, G.R., Blaustein, M.P. 1979. Saxitoxin binding to synaptosomes, membranes, and solubilized binding sites from rat brain. *J. Membrane Biol.* **50**:287–310

Krueger, B.K., Worley, J.F., French, R.J. 1983. Single sodium channels from rat brain incorporated into planar lipid bilayer membranes. *Nature (London)* **303**:172–175

Leibowitz, M.D., Sutro, J.B., Hille, B. 1986. Voltage dependent gating of veratridine-modified Na channels. *J. Gen. Physiol.* **87**:25–46

Meves, H., Simard, J.M., Watt, D.D. 1986. Interactions of scorpion toxins with the sodium channel. *Ann. NY Acad. Sci.* **479**:113–132

Miller, C. 1978. Voltage-gated cation conductance channel from fragmented sarcoplasmic reticulum: Steady-state electrical properties. *J. Membrane Biol.* **40**:1–23

Moczydlowski, E., Garber, S.S., Miller, C. 1984. Batrachotoxin-activated $Na^+$ channels in planar lipid bilayers. Competition of tetrodotoxin block by $Na^+$. *J. Gen. Physiol.* **84**:665–686

Mueller, P., Rudin, D.O., Tien, H.T., Wescott, W.C. 1963. Methods for the formation of single bimolecular lipid membranes in aqueous solution. *J. Phys. Chem.* **67**:534–535

Nagy, K. 1988. Mechanism of inactivation of single sodium channels after modification by chloramine-T, sea anemone toxin and scorpion toxin. *J. Membrane Biol.* **106**:29–40

Norton, T.R., Shibata, S., Kashiwagi, M., Bentley, J. 1976. Isolation and characterization of the cardiotonic polypeptide anthopleurin-A from the sea anemone *Anthopleura xanthogrammica*. *J. Pharm. Sci.* **65**:1368–1374

Patlak, J., Horn, R. 1982. Effect of N-bromoacetamide on single sodium channel currents in excised membrane patches. *J. Gen. Physiol.* **79**:333–351

Rack, M., Richter, D., Rubly, N. 1987. Purification and characterization of a $\beta$-toxin from the venom of the African scorpion *Leiurus quinquestriatus*. *FEBS Lett.* **214**:163–166

Romey, G., Renaud, J.F., Fosset, M., Lazdunski, M. 1980. Pharmacological properties of the interaction of a sea anemone polypeptide toxin with cardiac cells in culture. *J. Pharmacol. Exp. Ther.* **213**:607–615

Rosemblatt, M., Hidalgo, C., Vergara, C., Ikemoto, N. 1981. Immunological and biochemical properties of transverse tubule membranes isolated from rabbit skeletal muscle. *J. Biol. Chem.* **256**:8140–8148

Sigel, E. 1987. Effects of veratridine on single neuronal sodium channels expressed in *Xenopus* oocytes. *Pfluegers Arch.* **410**:112–120

Strichartz, G., Rando, T., Wang, G.K. 1987. An integrated view of the molecular toxinology of sodium channel gating in excitable cells. *Ann. Rev. Neurosci.* **10**:237–267

Sutro, J.B. 1986. Kinetics of veratridine action on Na channels of skeletal muscle. *J. Gen. Physiol.* **87**:1–24

Tamkun, M.M., Catterall, W.A. 1981. Ion flux studies of voltage-sensitive sodium channels in synaptic nerve ending particles. *Molec. Pharmacol.* **19**:78–86

Tanaka, M., Haniu, M., Yasanobu, K.T., Norton, T.R. 1977. Amino acid sequence of the *Anthopleura xanthogrammica* heart stimulant, Anthopleurin A. *Biochemistry* **16**:204–208

Received 13 January 1989; revised 3 April 1989