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NEUROTOXINS THAT ACT ON VOLTAGE-SENSITIVE SODIUM CHANNELS IN EXCITABLE MEMBRANES

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William A. Catterall

Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington 98195

INTRODUCTION

A large number of biological toxins exert their toxic effects by modifying the properties of voltage-sensitive sodium channels involved in action potential generation in nerve, heart, and skeletal muscle. These include the water-soluble heterocyclic guanidines, tetrodotoxin and saxitoxin, the lipid-soluble polycyclic compounds, veratridine, aconitine, batrachotoxin, and grayanotoxin, and the low-molecular weight, basic polypeptide toxins isolated from scorpion venoms and sea anemone nematocysts. The isolation, chemical characterization, and structure determination of many of these toxins as well as their systematic and comparative pharmacology have been the subject of previous excellent reviews. These aspects are, therefore, not treated in detail here. This review focuses on experiments that have given insight into the cellular and molecular mechanisms of action of these toxins and have revealed common features of their interaction with voltage-sensitive sodium channels.

EXPERIMENTAL APPROACHES

Most of the results to be considered in this review have been obtained with three experimental approaches: electrophysiologic analysis of membrane voltage and membrane current, isotopic flux measurements of ion permeability, and direct radioligand binding studies of toxin-receptor interaction.

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Each of these approaches has strengths and weaknesses. The classical approach to analysis of electrical excitability has been through electrophysiologic techniques. These methods have the time resolution necessary to resolve the rapid changes in membrane voltage and current which constitute the action potential. However, because membrane voltage is related to ion permeability in a highly nonlinear way, recordings of membrane voltage alone are seldom sufficient to resolve the mechanism of action of drugs and toxins on electrical excitability. Determination of membrane currents at constant membrane voltage under voltage clamp is usually required to determine the mechanism by which toxins and other agents affect electrical excitability. This method, pioneered by Hodgkin & Huxley (1), allows direct measurement of ion permeabilities as a function of time and membrane voltage. Some of the essential features of sodium channel function as revealed by the voltage clamp technique are considered here to facilitate subsequent discussion of the effects of neurotoxins. These topics have been extensively reviewed (2–6).

Hodgkin & Huxley showed that the changes in membrane voltage during an action potential in squid giant axon were due to an increase in membrane permeability to sodium followed by a delayed increase in permeability to potassium (1). Subsequent work (reviewed in 2–6) has established that separate permeability pathways are involved in the increases in sodium and potassium permeability. Using the voltage clamp method, Hodgkin & Huxley (1) showed that the change in sodium permeability during a maintained depolarization is biphasic. The permeability increases for a few msec and then returns to the resting level. These changes can be described in terms of two voltage-dependent processes: *activation,* which controls the rate and voltage dependence of sodium permeability increase following depolarization, and *inactivation,* which controls the rate and voltage dependence of the subsequent return of the sodium permeability to the resting level during a maintained depolarization. Subsequent work has shown that these concepts can also be applied in studies of vertebrate myelinated nerve and skeletal muscle. Hodgkin & Huxley viewed activation and inactivation as separate processes. However, recent voltage clamp work has shown that they are highly interdependent (7). Nevertheless, the operational definitions of activation and inactivation introduced by Hodgkin & Huxley have proven useful in describing the effects of toxins and pharmacologic agents that alter sodium channel function and are used throughout this review.

Transport of ions by the sodium channel is selective. Among monovalent inorganic cations, the order of permeability is $Na^+ \simeq Li^+ > K^+ > Rb^+ > Cs^+$ (8, 9). Many organic cations have also been studied (10). These data on ion selectivity have been used to construct a detailed model of the ion

selectivity filter, the region of the sodium channel responsible for ion selectivity (10).

The voltage clamp method measures three properties of sodium channels: voltage-dependent activation, voltage-dependent inactivation, and selective ion transport. In the sections below, the effects of neurotoxins on each of these properties are considered. The main advantage of the voltage clamp method is the ability to monitor the complete time course of sodium permeability change at constant voltage. Unfortunately, only a few experimental systems are amenable to voltage clamp analysis and these are often not optimal for other more biochemical experiments on toxin action and sodium channel function.

Measurements of isotopic flux provide an alternative approach to estimation of ion permeabilities. This approach has not been widely used in studies of electrical excitability because it lacks the time resolution required to quantitate rapid changes in ion permeability during an action potential. The alterations of sodium permeability caused by neurotoxins are slow, however, and can be conveniently measured by isotopic flux techniques (11–13). Isotopic flux measurements provide a useful measure of ion permeability only when the driving force for ionic movements is constant. The driving force consists of both a chemical component, the ion gradient, and an electrical component, the membrane potential. Both must be considered and carefully controlled if meaningful results are to be obtained (14). The advantages of the ion flux approach are the convenience of studies of concentration-effect relationships, the ease of direct correlations with ligand binding and other biochemical data under identical experimental conditions, and the ability to study a number of experimental systems (small diameter nerves, small cells, membrane vesicles) which cannot be analyzed by voltage clamp methods. The clear disadvantage of this approach is lack of time resolution.

Electrophysiologic and isotopic flux methods measure the effect of neurotoxins on the ion transport properties of sodium channels. The interaction of neurotoxins with receptor sites associated with sodium channels has also been studied directly in radioligand binding experiments. The major obstacles in this approach are preparation of a radiolabeled derivative which retains biological activity, characterization of the radiolabeled derivative with respect to purity and specific radioactivity, and determination of the specific and nonspecific components of ligand binding. The approaches used to solve these problems with specific neurotoxins are considered in subsequent sections of this review.

Radioligand binding assays can provide important information that cannot be obtained from studies of ion permeability alone. The densities of toxin receptor sites, and therefore of sodium channels, in excitable mem-

branes can be estimated. The cellular and subcellular localization of sodium channels can be studied. Finally, since binding of ligands to components of the sodium channel can, in principle, be measured after disruption of the excitable membrane, ligand binding methods can be used to monitor isolation of components of the sodium channel from the excitable membrane.

TOXINS THAT INHIBIT SODIUM CHANNELS

Tetrodotoxin

Tetrodotoxin is a heterocyclic guanidine originally found in the ovaries and liver of puffer fish of the suborder *Gymnodontes* and more recently in some species of newt, octopus, frog, and goby (15–19). The isolation, chemistry, structure determination, and systematic and comparative aspects of the pharmacology of the toxin have been reviewed in detail (20–22). The structure of tetrodotoxin is illustrated in Figure 1. In the intact animal, tetrodotoxin has toxic effects on the neuromuscular, cardiovascular, and respiratory systems (20–22). It is probable that all of these effects derive from the inhibition of action potential generation by the toxin. It has been known since the turn of the century that tetrodotoxin blocks impulse conduction in motor axons (20). The mechanism of this inhibition was first revealed by voltage clamp studies in squid and lobster giant axons (23, 24). These experiments showed that a low concentration of tetrodotoxin (10^{-7} M) selectively blocks the increase in sodium permeability caused by depolarization with no effect on the ion permeability of the unstimulated axon or on the delayed potassium permeability increase caused by depolarization. This selective action of tetrodotoxin has been demonstrated in myelinated nerve, in skeletal muscle, and in a number of other excitable cells in which inward Na^+ currents are important in generation of action potentials (20–22). The inhibition of sodium currents is reversible after washing (23). Equilibrium concentration-effect curves are hyperbolic with half-maximal inhibition at 1 to 5 nM in different nerve preparations (25–27). The toxin

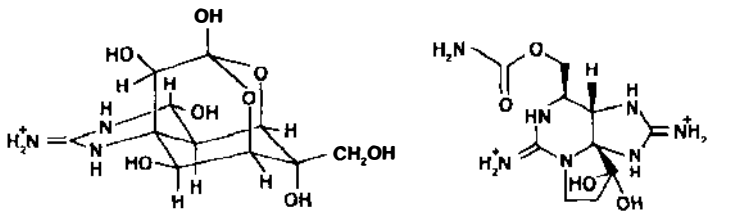


Figure 1 The structures of tetrodotoxin and saxitoxin.

is inactive when perfused inside the squid giant axon, indicating that the receptor is available only from the outside (28). These findings are consistent with reversible interaction of tetrodotoxin at a specialized receptor site located on the extracellular aspect of the sodium channel. The hyperbolic inhibition curves indicate that binding of one toxin molecule is sufficient to inhibit one sodium channel.

Saxitoxin

Saxitoxin, like tetrodotoxin, is a heterocyclic guanidine. It is produced by dinoflagellates of the genus *Gonyaulax* and is found in large concentration in clams, mussels, and other shellfish that feed on these organisms. The isolation and chemistry of saxitoxin have been reviewed previously (20–22). The definitive structural determination (Figure 1) has been achieved only recently, however, by X-ray crystallographic methods (29, 30). A naturally occurring, biologically active derivative, 11-hydroxysaxitoxin sulfate, has also been described (31).

Studies of the mechanism of action of saxitoxin began later than those on tetrodotoxin. Experiments with frog nerve showed that the toxin blocked action potentials without depolarization (32). Voltage clamp studies demonstrated a selective block of inward sodium current without effect on the ion permeability of the unstimulated nerve or on the outward potassium current (25, 33). As with tetrodotoxin, this action of saxitoxin is reversible and the concentration-dependence of inhibition is hyperbolic with half-maximal inhibition at 1–5 nM (25).

Detection of the Receptor Site for Tetrodotoxin and Saxitoxin

Since tetrodotoxin and saxitoxin block sodium currents at very low concentrations, it seemed likely that the toxins acted at a small number of discrete sites in the excitable membrane. Moore et al (34) first demonstrated this by using a bioassay to estimate the amount of tetrodotoxin removed from the bathing medium when the action potential in lobster nerve was blocked. Their studies suggested an upper limit of only 13 toxin receptor sites per μm^2 of axonal membrane. Other studies using the bioassay approach suggested somewhat higher densities (35), but confirmed the essential finding that the sites of toxin action are very sparsely distributed.

More quantitative studies of the binding of tetrodotoxin and saxitoxin to their receptor sites in excitable membranes have been possible using ^3H-labeled toxin preparations. A detailed review of these studies has appeared (36). Hafemann (37) introduced the use of the Wilzbach gas discharge method for labeling tetrodotoxin. This approach was subsequently employed to label both tetrodotoxin and saxitoxin (37–41) and study their

binding to excitable membranes. In nerve preparations, a saturable binding component was observed with a K_D of 3–10 nM and an apparent site density of 3–30 sites/μm^2 of nerve membrane (37–41). No saturable binding was observed in nonexcitable cells (39). The hyperbolic binding curves for the saturable binding component were consistent with the hyperbolic inhibition curves observed in physiological experiments (38–41) and the values for binding constants agreed with those estimated in electrophysiological experiments. These results supported the conclusion that the saturable component of binding of ^3H-labeled toxin represented binding to receptor sites associated with sodium channels.

The binding of [^3H]-tetrodotoxin to its receptor site is blocked by unlabeled saxitoxin (39, 41), and the binding of [^3H]-saxitoxin is blocked by tetrodotoxin (40). These experiments provide strong evidence that these two toxins inhibit sodium channels by binding at a common receptor site. This conclusion has been confirmed by kinetic analysis of the rates of onset and offset of toxin inhibition of sodium currents in myelinated nerve (42).

Since the concentration-dependence of both inhibition and binding is hyperbolic, it is probable that binding of a single toxin molecule is sufficient to inhibit a sodium channel. In view of this, measurements of the densities of toxin receptor sites in excitable membranes provide an estimate of the density of sodium channels. The initial estimates of sodium channel densities in unmyelinated nerves ranged from 3 to 30 sites per μm^2 (37–40). Although the [^3H]-labeled toxin preparations used in these studies appeared pure by chemical criteria, subsequent work showed that a large fraction of the ^3H cpm could not bind to excitable membranes and, thus, could not be biologically active saxitoxin or tetrodotoxin (43). Correction of the specific radioactivity for labeled contaminants results in higher estimates of binding site densities.

The difficulties of obtaining highly purified preparations of saxitoxin and tetrodotoxin labeled by the Wilzbach method led Ritchie et al to develop a specific ^3H-exchange method for labeling saxitoxin (44). Using saxitoxin labeled in this manner (44–48) or using carefully purified and characterized preparations of [^3H]-tetrodotoxin (49–51), more precise estimates of sodium channel density in different excitable tissues have been made. Values of K_D are 1 to 10 nM for all tissues studied (44–51). The site density for nonmyelinated nerve and for cultured neuroblastoma cells is in the range of 30 to 100 μm^{-2} (44, 47). Skeletal muscle fibers have somewhat greater site densities—from 170 to 400 sites per μm^2 (45, 49, 50). Giant axons from two different species of squid have 170 and 550 sites/ μm^2 (48, 51). Studies of myelinated nerves suggested very high densities in the range of 12,000 sites per μm^2 in the node of Ranvier (46). However, this estimate required a number of assumptions that cannot be verified at present [see discussion

in reference (43)]. Voltage clamp data suggest a lower density of 2000 sodium channels per μm^2 in the nodal membrane (52).

In addition to providing the first estimates of sodium channel density in excitable membranes, these studies of tetrodotoxin binding also provided estimates of the ion transport capacity of an individual sodium channel by comparison of site densities with ionic current measurements. These estimates are in the range of 1×10^7 ions/sec per site, or a conductance of 2 to 8 pS per site (43, 39, 52). This high rate of ion transport is inconsistent with a mobile carrier mechanism of transport and, thus, represents the main experimental support for the generally accepted view that the sodium channel functions as a selective pore.

The Mechanism of Inhibition of Sodium Channels by Tetrodotoxin and Saxitoxin

Guanidinium groups are prominent features of the structure of both tetrodotoxin and saxitoxin (Figure 1). Guanidine is one of the few cations that is an effective substitute for sodium in action potential generation (10). Kao & Nishiyama (32) first proposed that the guanidinium moieties of tetrodotoxin or saxitoxin might enter the sodium channel like guanidine but then the bulky toxins would bind tightly within the ion channel blocking further passage of ions. This hypothesis is consistent with many aspects of the action of the toxins.

Tetrodotoxin and saxitoxin inhibit sodium channels without altering the rate or voltage dependence of activation or inactivation (23–25, 42). The binding of these toxins to their receptor site is not modified by voltage (49, 53, 54) or by other toxins (see below) that modify activation or inactivation (39, 40, 47, 53–55). These results indicate that tetrodotoxin and saxitoxin bind equally well to resting, active, or inactivated sodium channels and suggest that the toxin receptor site is not associated with the voltage-sensitive component(s) of the sodium channel. These results, therefore, support the hypothesis that tetrodotoxin and saxitoxin block ion transport directly.

The hypothesis of Kao & Nishiyama (32) was extended by Henderson et al (56), who proposed that the toxin receptor site was a specific ion coordination site in the sodium channel, and by Hille (10, 57), who proposed that the receptor site was the ion selectivity filter, the coordination site which determines the ion selectivity of the sodium channel. This site is thought to contain an acid group with a pKa of 5.4 (58). Protonation of this group blocks sodium channel function. The selectivity filter also is thought to bind cations causing saturation of transport at high concentrations of permanent ions (59). Both the pKa and the apparent binding constants for cations are voltage-dependent, suggesting that the acidic group at the selectivity filter

is located part way through the sodium channel in the membrane-electric field (58, 59). The proposal that the toxins bind at the selectivity filter predicts that the properties of the selectivity filter measured physiologically and of the receptor site measured by toxin binding should be identical.

The major experimental evidence in favor of locating the toxin receptor site at the ion selectivity filter is the correlation between the block of sodium currents and of toxin binding by H^+. Tetrodotoxin and saxitoxin binding are blocked by protonation of a group with a pKa of approximately 5.4 in a number of experimental systems (40, 55, 56, 60, 61). In neuroblastoma cells, the pKa's for block of sodium influx and for block of tetrodotoxin binding have been measured in companion experiments and shown to be essentially identical (62). These experiments demonstrate that acid groups with similar pKa's are essential for both toxin binding and ion transport. Since most carboxyl groups have similar pKa's, however, this cannot be considered rigorous evidence that the same acid group is involved in both processes.

Correlations between apparent K_D values for saturation of the sodium channel by cations and for block of toxin binding by cations have also been presented as evidence supporting the hypothesis that tetrodotoxin and saxitoxin bind at the ion selectivity filter. The data presently available in the literature do not show a strong quantitative correlation, however. The data for apparent K_D of cations at the selectivity filter from the work of Hille (59) are not in quantitative agreement with the values for K_D at the toxin receptor site from direct binding measurements (56, 60, 61), nor is the rank order of K_D values identical. There are wide variations in species, preparations, and incubation conditions among the different experiments and perhaps a close correlation is too much to expect. Nevertheless, it seems clear that the data on cation binding presently available cannot be taken as support for locating the toxin receptor site at the selectivity filter.

Experiments with carboxyl-modifying reagents have provided evidence for the involvement of separate carboxyl groups in toxin binding and ion selectivity. Toxin binding is irreversibly blocked by treatment of excitable membranes with carboxyl-modifying reagents such as carbodiimides followed by a nucleophile (63, 64) or trialkyloxonium salts (60, 65). The chemical modifications seem to be specific since irreversible block is prevented if the reactions are carried out in the presence of saturating concentrations of tetrodotoxin. These reactions most likely result in modification of carboxyl groups and, thus, the results implicate a carboxyl group in the toxin receptor site. Sodium channels that are made tetrodotoxin-insensitive by these reactions are still active in generating action potentials (64, 65). Spalding has studied sodium channels made saxitoxin- and tetrodotoxin-resistant by treatment with trimethyloxonium in frog muscle under volt-

age clamp (66). These modified channels have normal voltage dependence of activation and inactivation, normal ion selectivity, and normal pKa for block by H^+ (66). Similar treatment reduces the unit conductance of sodium channels in myelinated nerve by 65% (67) but does not reduce the number of functional sodium channels. Taken together, the experiments with carboxyl reagents show that there is a carboxyl group that is essential for toxin binding but is not involved in determination of ion selectivity or block of sodium channels by H^+. This carboxyl group must not be part of the selectivity filter.

Some other experiments also suggest that different functional groups are involved in determining ion selectivity and toxin affinity. Batrachotoxin (see below) alters ion selectivity (62, 68) but has no effect on toxin binding (47). Conversely, neuroblastoma cell lines with 100-fold different affinity for tetrodotoxin have nearly identical ion selectivity (62).

The current status of the experimental evidence on the site of toxin action can be summarized as follows. There is strong evidence for an essential acidic group, probably a carboxyl, that is required for ion transport by the sodium channel and is located at the selectivity filter. There is also strong evidence, mainly from chemical modification studies, for a different carboxyl group which is essential for toxin binding but not for ion transport or ion selectivity. Finally, there is suggestive, but inconclusive, evidence that the carboxyl group at the ion selectivity filter that is essential for ion transport may also be required for toxin binding. Further supporting evidence is needed on this last point before the hypothesis that saxitoxin and tetrodotoxin bind at an ion coordination site within the sodium channel can be considered proven.

Structure-Activity Relationships

The guanidinium moieties of saxitoxin and tetrodotoxin (Figure 1) give the toxins a positive charge at neutral pH. Each toxin has an unusually acidic hydroxyl group with a pKa of 8.2 for saxitoxin and 8.8 for tetrodotoxin (20–22). Studies of the pH dependence of their inhibition of sodium channels indicate that both toxins are more active at neutral pH when these hydroxyls are protonated (25, 69).

A number of derivatives of tetrodotoxin and saxitoxin were prepared in the course of chemical studies. All the derivatives tested pharmacologically lack any biological activity (20–22). These studies lead to the conclusion that the biological activity of the toxins requires that most, if not all, of the toxin structure remain intact. Inspection of the structures of the toxins reveals two common features: the guanidinium moieties and the unusually acidic hydroxyls. Hille has pointed out that these functional groups have a similar stereochemical orientation in the two toxins (57). Recent efforts

at preparation of derivatives have focused on functional groups distant from these moieties which are probably required for biological activity. The hydroxymethyl group of tetrodotoxin can be cleaved from the ring structure by mild periodate oxidation (70) and the resulting carbonyl group can be coupled with methoxyamine (70) or with primary amines by reductive amination (71) to yield active derivatives. Tetrodotoxin can be esterified at this same hydroxymethyl group with retention of biological activity (72). Naturally occurring derivatives of tetrodotoxin have been described which have modifications of this same hydroxymethyl group (17). Decarbamyl saxitoxin prepared by acid hydrolysis retains biological activity (73). All of these active derivatives involve reactions at the positions most distant from the guanidine and acidic hydroxyl functional groups that are common to tetrodotoxin and saxitoxin. The success of chemical modifications at these positions reinforces the conclusion that the guanidine and acid hydroxyl groups common to the two toxins represent the points of attachment to the receptor site. Toxin derivatives modified to covalently attach to their receptor site (71, 72) should prove valuable in future biochemical studies of the toxin receptor.

The Chemical Nature of the Toxin Receptor

Since it is probable that the receptor for saxitoxin and tetrodotoxin is an important functional component of the sodium channel, it is of great interest to identify, isolate, and characterize the receptor. Radioactively labeled toxin preparations make an approach to this problem feasible. The first steps in this direction were taken by Henderson & Wang (74) and Benzer & Raftery (75) who showed that the receptor from garfish olfactory nerve could be solubilized by detergents with retention of toxin binding activity. The solubilized material had a molecular weight in the range of 250,000 to 500,000 (70, 71) and was sensitive to proteases and lipases (75), suggesting that the receptor is a large lipoprotein complex. An independent estimate of receptor size made by irradiation inactivation methods indicates a molecular weight of 230,000 (76).

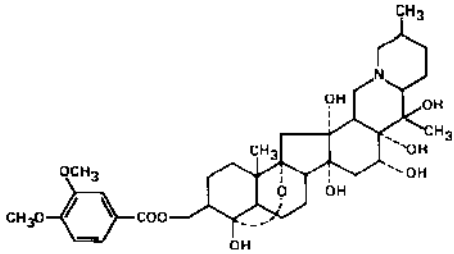
Purification of the receptor has proven difficult because of its instability after solubilization. Recently, Agnew et al have shown that the solubilized tetrodotoxin receptor from eel electroplax can be stabilized by addition of phospholipids in detergent solution and have achieved substantial purification by conventional methods (77). It is likely that the toxin receptor in mammalian systems has similar properties since the receptor from mammalian brain and skeletal muscle can be solubilized by nonionic detergents (53, 54, 78) and stabilized by phospholipid (53, 78), and has a molecular weight in the range of 250,000 to 500,000 (53, 78). It seems likely that further work using these approaches will result in purification and characterization of an important component of the sodium channel.

LIPID-SOLUBLE TOXINS THAT MODIFY ACTIVATION AND INACTIVATION

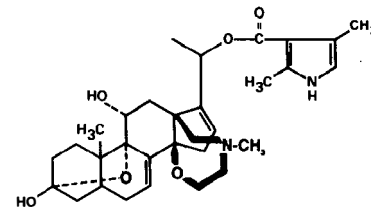
Lipid-soluble compounds isolated from various species of plants and from tropical frogs have been shown to have dramatic effects on excitable membranes. The most thoroughly studied of these compounds are veratridine, batrachotoxin, aconitine, and grayanotoxin (Figure 2). The properties of each of these toxic compounds and the evidence for a common site and mechanism of action are considered in the following sections.

Veratridine

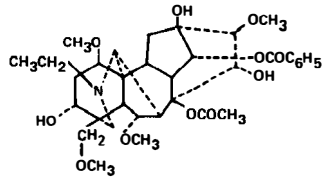
The steroidal alkaloid veratridine is the most potent of the veratrum alkaloids, a mixture of compounds produced by plants of the suborder *Melanthaceae* of the family *Lilaceae.* The chemistry, structure (Figure 2), and early pharmacology of these alkaloids have been reviewed (79, 80). Veratridine has a broad spectrum of pharmacological activity causing muscle contracture, repetitive firing of nerves, and irregular heart rhythms (79). Most, if not all, of these effects can be ascribed to alteration of sodium channel properties leading to hyperexcitability and depolarization of the excitable membrane (81). Straub showed that veratridine causes depolarization of nerve due to increased sodium permeability (82). In voltage clamp studies of myelinated nerve, Ulbricht showed that veratridine modified the properties of a fraction of the voltage-sensitive sodium channels (81). These



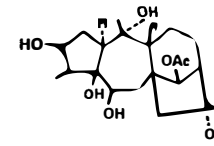
VERATRIDINE



BATRACHOTOXIN



ACONITINE



GRAYANOTOXIN I

Figure 2 The structures of the lipid-soluble toxins.

modified ion channels were activated 1000 times more slowly following a depolarization and did not inactivate. The voltage-dependence of activation was less steep and was shifted to more negative membrane potentials so that a fraction of the modified ion channels was active at the resting membrane potential. Thus, the depolarization of excitable cells by veratridine is caused by two effects: block of inactivation and shift of activation to more negative potentials. Veratridine-induced sodium permeability is blocked by tetrodotoxin (11, 81, 83) supporting the conclusion that veratridine modifies the properties of a fraction of the sodium channels normally involved in action potential generation in nerve.

Concentration-effect relationships for increase of sodium permeability by veratridine have been studied in electrically excitable neuroblastoma cells (84). The dependence on veratridine concentration is hyperbolic with half-maximal activation at 80 μM, suggesting that binding of a single toxin molecule is sufficient to activate a sodium channel. Tetrodotoxin inhibits veratridine activation noncompetitively, indicating that veratridine and tetrodotoxin act at separate receptor sites (84).

Batrachotoxin

Batrachotoxin is a steroidal alkaloid isolated from the skin of the Columbian frog *Phyllobates aurotaenia* and related species. The isolation of the pure alkaloid and determination of chemical properties and structure were carried out by Daly, Witkop, and collaborators (reviewed in 85). Batrachotoxin and veratridine have some common structural features including a steroid ring nucleus and an unusual oxygen bridge between the A and B rings of the steroid skeleton (Figure 2). Albuquerque, Daly, Witkop, and co-workers have described the pharmacologic effects of the toxin on nerve axons, Purkinje fibers and papillary muscle of the heart, brain slices, superior cervical ganglion, and both presynaptic and postsynaptic aspects of the neuromuscular junction. These studies have been reviewed in detail (85). All of these diverse pharmacologic effects of batrachotoxin result from depolarization of nerve and muscle due to increased Na^+ permeability of the excitable membrane (86). Batrachotoxin depolarizes the squid giant axon in normal bathing medium, but has no effect if both the intracellular and extracellular compartments are perfused with sodium-free solution (87). Depolarization of excitable membranes by batrachotoxin is completely blocked by tetrodotoxin (84, 86–88). This observation indicates that batrachotoxin depolarizes excitable cells by causing voltage-sensitive sodium channels to become persistently active at the resting membrane potential.

The concentration-dependence of batrachotoxin depolarization has been measured in a number of excitable tissues. It is probable, however, that these studies do not provide an accurate measure of the concentration-effect

relationships for batrachotoxin action because of the highly nonlinear relationship between membrane potential and sodium permeability. Ion flux measurements of the increase in sodium permeability caused by batrachotoxin indicate a hyperbolic concentration-effect relationship with half-maximal effect at 0.4 μM (84). Tetrodotoxin is a noncompetitive inhibitor of batrachotoxin action (84). These results indicate that batrachotoxin acts at a different receptor site from that of tetrodotoxin and saxitoxin.

Detailed voltage clamp analysis of batrachotoxin action in myelinated nerve by Khodorov and colleagues (68, 89) has further defined the mechanism of toxin action. They showed that batrachotoxin has two effects on the voltage-sensitive sodium channels in the node of Ranvier. It blocks inactivation completely and shifts the voltage dependence of activation more than 30 mV to more negative membrane potentials. These two effects combine to cause a fraction of the ion channels to be persistently activated at the resting membrane potential. The persistent activity of these sodium channels depolarizes excitable cells and causes the various pharmacologic effects of batrachotoxin. In addition, batrachotoxin alters the ion selectivity of the sodium channel (62, 68), increasing the permeability of the ion channel toward larger cations.

Studies of batrachotoxin derivatives reveal substantial dependence of physiological activity on details of the chemical structure (90). The oxygen bridge between the A and B rings and the dimethyl pyrrole carboxylate ester moiety are essential. Changes in the pyrrole ring substituents have marked effects on activity. These observations suggest an interaction with a specific receptor site. Batrachotoxin acts more rapidly at alkaline pH, suggesting that the unprotonated form is more active. Quaternary batrachotoxin methiodide is permanently charged and retains substantial activity, however. These results may indicate that interaction of the free base with the receptor is preferred, but could also be due to the higher permeability of the free base through biological membranes.

Aconitine

Aconitine is an alkaloid produced by the plant *Aconitum napellus.* Its structure (Figure 2), reported by Wiesner et al (91), does not closely resemble either veratridine or batrachotoxin. It causes arrhythmia in ventricular and Purkinje fiber preparations from heart (92, 93), and repetitive afterpotentials and oscillations following stimulation of nerve (94). These effects are caused by prolonged depolarization following action potentials which prevent complete repolarization of the excitable membrane. These prolonged depolarizations are blocked by tetrodotoxin (93, 95), suggesting that they result from prolonged activation of voltage-sensitive sodium channels.

Voltage clamp analysis of aconitine action in myelinated nerve showed that the alkaloid modifies the voltage dependence of activation of sodium

channels and blocks inactivation (95). Two populations of sodium channels were observed in aconitine-treated nodes of Ranvier: one having nearly normal properties but with the voltage-dependence of activation and inactivation shifted about 10 mV to more negative membrane potentials, and a second having the voltage-dependence of activation shifted nearly 50 mV to more negative membrane potentials and inactivation completely blocked (95). Aconitine-modified sodium channels have altered ion selectivity such that they are substantially more permeable to larger cations than unmodified channels (96). These observations suggest underlying similarity in the mechanisms of aconitine, veratridine, and batrachotoxin action.

Grayanotoxin

Grayanotoxins I, II, and III are the toxic principles contained in the leaves of rhododendron and other members of the plant family *Ericaceae.* Their chemical structures have been determined [(97, 98) and references therein]. Toxins II and III are derivatives of grayanotoxin I illustrated in Figure 2. These toxins depolarize the excitable membranes of skeletal muscle (99), heart (100), and squid giant axon (101, 102) by increasing sodium permeability. Concentration-effect curves for the increase in sodium current under voltage clamp are hyperbolic with half-maximal effect at 50 μM (101), suggesting that binding of a single toxin molecule is sufficient to activate a sodium channel. Tetrodotoxin inhibition of grayanotoxin-induced Na^+ current under voltage clamp conditions is noncompetitive showing that tetrodotoxin and grayanotoxin act at separate receptor sites (101).

The effect of grayanotoxin on the rate and voltage-dependence of activation and inactivation has not been studied in detail. Seyama & Narahashi (101) observed a small (6 mV) shift in the voltage-dependence of activation to more negative membrane potentials. Grayanotoxin-activated sodium channels are more permeable to large cations (103) than voltage-sensitive sodium channels in untreated axons.

The Site of Action of the Lipid-Soluble Toxins

Ulbricht (81) first concluded that veratridine depolarized excitable cells by altering the voltage-sensitivity of sodium channels so that a significant fraction was active at the resting membrane potential. A substantial body of evidence now supports extension of that conclusion to all four classes of the lipid-soluble toxins. In each case, the increase in sodium permeability is inhibited by tetrodotoxin at concentrations similar to those that block sodium currents (11, 81, 83, 84, 86, 88, 93, 95, 101, 102). Since the action of tetrodotoxin is highly specific, this constitutes strong evidence that the same ion channels are involved in action potential generation and toxin-induced depolarization. With rare exceptions the toxins have little or no

effect on the sodium permeability of inexcitable cells or excitable cells having no sodium currents (11, 14, 104, 105). This is most convincingly demonstrated in studies of closely related clones of mouse neuroblastoma cells that differ in electrical activity. Only those clones that generate sodium-dependent action potentials are responsive to neurotoxins (11, 14, 105). Closely related clones that retain neuronal properties including delayed rectification (K^+ channels) do not respond (11, 14). These studies provide a second piece of strong evidence that the lipid-soluble toxins act directly on sodium channels involved in action potential generation in most excitable tissues. Finally, the voltage clamp experiments of Khodorov et al (68) show that, as batrachotoxin-modified sodium channels appear, normal sodium channels are lost. All the voltage-activated sodium channels in the node of Ranvier are modified by the toxin. Taken together, these three kinds of evidence provide strong support for the conclusion that the lipid-soluble toxins act directly on sodium channels involved in action potential generation in most excitable tissues and modify both their voltage-sensitivity and ion selectivity.

Some exceptions have been described (105). Schwann cells in the squid giant axon are depolarized by veratridine and grayanotoxin but are not electrically excitable (106). In contrast, glia and Schwann cells in other species seem not to have sodium channels (11, 105, 107). Cultured embryonic heart cells (108–112) and certain nonspiking neurons (113) have sodium channels that are not activated by depolarization but are activated by veratridine and other toxins. In each case, these actions are blocked by tetrodotoxin. These experiments indicate that, under special circumstances, sodium channels closely related to those involved in action potential generation can be electrically silent but responsive to neurotoxins.

While these four toxins seem superficially to cause different combinations of depolarization, repetitive firing, and after-potentials in excitable tissues, voltage clamp analysis has revealed important similarities in their mechanism of action. In each case, the voltage dependence of activation is shifted to more negative membrane potentials (68, 81, 95, 101). Veratridine, batrachotoxin, and aconitine block inactivation of modified sodium channels completely (68, 81, 95). Batrachotoxin, grayanotoxin, and aconitine alter the ion selectivity of the sodium channel (68, 96, 103). Concentration-effect relationships conform to a Langimuir isotherm in each case, suggesting that binding of a single toxin molecule is sufficient to cause persistent activation of a sodium channel (14, 84, 102). These similarities suggest the possibility of a common site of action. A common receptor site for the four toxins has been demonstrated directly in ion flux experiments with neuroblastoma cells (14, 114). These experiments demonstrated competitive interactions among the four lipid-soluble toxins and showed that batrachotoxin is a full

agonist activating all the sodium channels in neuroblastoma cells, whereas veratridine, aconitine, and grayanotoxin are partial agonists (14, 114). These experiments define a specific chemically sensitive site at which these toxins bind and cause persistent activation of sodium channels. Thus, although these four toxins have different chemical structures, they exert their effects by interaction with a common site. Determination of the common structural features that are required for toxin action is an important area for further work.

Although ion flux experiments have defined a common receptor site for these toxins, it has not yet proven possible to resolve binding to this site directly using a radioligand binding assay. Studies with veratridine (55), grayanotoxin (115), and batrachotoxin derivatives (J. W. Daly et al, unpublished results) have found values for nonspecific binding in excess of those expected for specific binding to sodium channels. These difficulties are due to the relatively low affinity and high lipid solubility of these toxins.

The Mechanism of Action of the Lipid-Soluble Toxins

An allosteric model has been proposed by Catterall (14, 114) that provides a quantitative fit of data on the concentration-dependence of persistent activation of sodium channels. This model is based on the ideas introduced by Monod, Wyman & Changeux to explain heterotropic cooperativity in allosteric enzymes (116). The principal assumption is that the lipid-soluble toxins bind with a higher affinity to an active state of the sodium channel and thereby shift a preexisting voltage-dependent equilibrium between active states and inactive states (14, 114). The efficacy of the toxins in activating sodium channels depends on the selectivity of binding to the active vs inactive states. High selectivity gives high efficacy. The energy of binding is translated into shifts in the voltage-dependence of activation, causing sodium channels to be active at the resting membrane potential. This simple model is sufficient to explain many aspects of toxin action.

Reexamination of electrophysiologic experiments in light of this proposed mechanism of action reveals additional evidence supporting the model. Consistent with the idea that batrachotoxin is a full agonist while the other toxins are partial agonists, voltage clamp studies in myelinated nerve show that batrachotoxin modifies all of the sodium channels (68), while aconitine and veratridine modify only a fraction (81, 95). In support of the idea that the toxins have high affinity for the active state, Khodorov et al (68) have shown that the rate of batrachotoxin action under voltage clamp is greatly enhanced by repetitive voltage pulses to activate sodium channels, and repetitive stimulation is required for batrachotoxin to modify any sodium channels in eel electroplax (117). Older work on aconitine and veratridine effects on action potentials shows that repetitive stimulation

enhances toxin action at low concentration, producing summation effects and bursting after-potentials consistent with enhanced binding of the toxin during repetitive stimulation (81, 94). Thus, an allosteric model of toxin action provides a quantitative fit of data on persistent activation of sodium channels by toxins and a qualitative explanation for many other observations.

POLYPEPTIDE TOXINS THAT ACT ON SODIUM CHANNELS

Two classes of polypeptide toxins that act on sodium channels have been purified and characterized chemically and pharmacologically: scorpion toxins and sea anemone toxins. Many of these toxins have been purified, sequenced, and analyzed for activity in a wide range of pharmacological preparations. Space does not permit detailed coverage of the work on protein sequence or the analysis of effects in various pharmacological preparations. The sections below focus on the underlying mechanism responsible for the pharmacological activity of these toxins, the modification of voltage-sensitive sodium channels.

Scorpion Toxins

Toxins from North African scorpions were first purified, characterized, and sequenced by Rochat, Miranda, Lissitzky, and colleagues (118–120). These toxins are basic polypeptides with molecular weights of approximately 7000. Each scorpion species studied contained multiple toxins having extensive sequence homology (119). Polypeptides that are specifically toxic to either insects or to mammals have been described (121). Polypeptide toxins from American scorpions have also been purified (122, 122a, 122b) and their sequence determined (122). They are basic polypeptides with molecular weights of approximately 7000. Substantial sequence homology between American and African scorpion toxins is observed (122).

Scorpion venoms and toxins cause secretion of neurotransmitters, arrhythmias in heart, repetitive firing and depolarization in nerve, and a number of other pharmacologic effects. The effects on excitable tissues probably all result from modification of the properties of voltage-sensitive sodium channels. Adam et al (123) showed that scorpion venom caused depolarization and spontaneous activity of myelinated nerve due to increased sodium permeability and prolonged the duration of action potentials. Analysis of the effect of venom of the North African scorpion *Leiurus quinquestriatus* under voltage clamp showed that inactivation of sodium channels was slowed greatly and was incomplete, and the voltage dependence of activation and inactivation was shifted to more negative membrane

potentials by a few mV (124, 125). Studies with venoms of other North African scorpions (126) and toxins purified from these venoms (127–129) have confirmed that the principal effect of scorpion toxin on excitable membranes is to slow, and in some tissues completely block, inactivation of sodium channels. Effects on voltage dependence of activation and inactivation have varied among the different preparations studied.

In contrast to these results with several North African venoms and toxins, the venom of the American scorpion *Centruroides sculpturatus* has no effect on inactivation but shifts the voltage-dependence of activation 40 to 50 mV to more negative membrane potentials (130). No voltage clamp study of purified toxins from American scorpions has yet appeared.

While scorpion venoms increase sodium permeability and depolarize excitable cells, purified toxins that slow inactivation have little or no effect on resting membrane sodium permeability (127–129, 131). Thus, purified scorpion toxins do not cause persistent activation of sodium channels in nerve or skeletal muscle. Purified scorpion toxin does increase ion flux into beating heart cells in culture (109), but this may reflect prolongation of the action potential of the beating cells, rather than direct activation by toxin.

Although scorpion toxins do not cause persistent activation of sodium channels, both scorpion venom and purified toxins from North African scorpions markedly enhance persistent activation of sodium channels by all four lipid-soluble toxins (14, 114, 132). Scorpion toxin increases the fraction of sodium channels activated by the partial agonists veratridine, aconitine, and grayanotoxin, and shifts the concentration-effect curves for toxin action as much as 20-fold to lower concentration (14, 114). These effects are quantitatively described by an allosteric model which assumes that scorpion toxin reduces the energy required for activation of sodium channels by the lipid-soluble toxins (14, 114). These assumptions are similar to those used to describe heterotropic cooperativity in allosteric enzymes (116). The cooperative interaction between the lipid-soluble toxins and scorpion toxin indicates that they act at separate receptor sites which interact allosterically. Under conditions where the parameters measured vary linearly with sodium permeability, concentration-effect curves for scorpion toxin action are hyperbolic (14, 128, 131), consistent with the conclusion that binding of a single toxin molecule is sufficient to slow inactivation and enhance activation of one sodium channel. Scorpion toxin action is blocked noncompetitively by tetrodotoxin indicating that these toxins act at separate sites (114, 132a).

Binding of scorpion toxin to its receptor site has been studied directly using [^{125}I]-labeled scorpion toxin derivatives (133). The toxin binds to a single class of sites in electrically excitable neuroblastoma cells (133–136), synaptosomes (137, 138), and frog sartorius muscle (129) with K_D's of

0.5–15 nM. No specific binding sites are observed in inexcitable clones of neuroblastoma cells, indicating specific interaction with receptor sites associated with voltage-sensitive sodium channels (134, 135). Binding is unaffected by tetrodotoxin and saxitoxin (134, 135, 137), indicating that the receptor site for scorpion toxin is separate from the receptor site for the inhibitory toxins. Scorpion toxin binding is enhanced by lipid-soluble toxins (134, 137) as expected from the cooperative interactions observed in sodium flux studies. Batrachotoxin is a full agonist in enhancing scorpion toxin binding, while veratridine and aconitine are partial agonists (134, 137). Competitive interactions are observed among the lipid-soluble toxins (134, 137). These binding studies confirm earlier conclusions derived from ion flux data on the mechanism of action of the lipid-soluble toxins (14, 114). Both the competitive interactions among lipid-soluble toxins and the allosteric interactions between scorpion toxin and lipid-soluble toxins are observed in broken membrane fractions, demonstrating that neither ion gradients nor membrane potential is required (53).

Catterall et al (129, 133–135, 137) have shown that the binding of scorpion toxin to its receptor site is highly voltage-dependent. The voltage-dependence detected in direct binding studies has been confirmed in voltage clamp experiments (139). In neuroblastoma cells, the K_D increases tenfold for each 31 mV depolarization (134). The voltage dependence of binding closely parallels the voltage dependence of activation of sodium channels measured in voltage clamp experiments with both neuroblastoma cells and frog sartorius muscle (129). These results suggest that the scorpion toxin receptor site is located on a component of the sodium channel which undergoes a conformational change on depolarization leading to activation of the sodium channel. This component may be the voltage sensor or activation gate of the sodium channel. It is surprising that scorpion toxin blocks or slows inactivation specifically (124–129), but the voltage-dependence of binding correlates with activation (129). Since recent experiments have shown that activation and inactivation are coupled processes (7), scorpion toxin may bind to a component of the sodium channel involved in activation and uncouple activation from subsequent inactivation.

Since saxitoxin and scorpion toxin bind to separate receptor sites that are associated with different functional components of the sodium channel, it is of interest to compare the density of their receptor sites in excitable membranes. The experimental systems in which this comparison can be made are limited, however, since high affinity scorpion toxin binding requires a membrane potential and the nonspecific component of binding to most intact tissues is very high. Comparative binding measurements have been carried out on neuroblastoma cells and synaptosomes (44, 50). The ratio of saxitoxin sites to scorpion toxin sites is 2.8 in neuroblastoma cells

and 3.7 in synaptosomes. Thus, in these two excitable membranes there are approximately 3 saxitoxin receptor sites for each scorpion toxin site. Several interpretations of this finding have been considered (47, 53). One interesting possibility is that the 3 to 1 stoichiometry of receptor sites reflects a 3 to 1 stoichiometry of subunits in the sodium channel structure (47, 53).

Sea Anemone Toxins

Partially purified preparations of sea anemone nematocyst toxins from *Condylactis gigantea* were shown to prolong action potentials in crustacean axons by Shapiro (140). Subsequent voltage clamp studies of this material in crayfish giant axon showed that this effect was due to slowing the rate of inactivation of sodium channels (141, 142). The toxin also caused a 12 mV shift in the voltage-dependence of inactivation to more negative membrane potentials and made it less steep (142).

Anemone toxins were first isolated in pure form from *Anemonia sulcata* (143, 144), shown to be basic polypeptides of 2500 to 5000 molecular weight (143, 144), and sequenced (145, 146) by Beress, Wunderer, and their colleagues. The sequences of three toxins from *Anemonia sulcata* and one from *Anthopleura xanthogrammica* have now been determined (145–149). Toxins I and II from *A. sulcata* and anthopleurin A from *A. xanthogrammica* consist of 47 to 49 amino acid residues and have substantial sequence homology (145, 148, 149). Toxin III from *A. sulcata* is only 27 residues long but 7 residues between positions 7 and 17 are homologous to those in positions 25 to 36 of the other three toxins (146, 147). None of the anemone toxins has detectable sequence homology with the scorpion toxins.

As with the partially purified extracts, all the pure toxins prolong action potentials in crayfish axons (150, 151). The long peptides, *A. sulcata* toxins I and II and anthopleurin A, are much more potent than the shorter toxin III (150, 151). Toxin II and anthopleurin A slow sodium channel inactivation in crayfish giant axons (151, 152) and toxin II also has this effect in frog myelinated nerve fibers (152, 153).

Toxin II from *A. sulcata* enhances persistent activation of sodium channels by veratridine and other lipid-soluble toxins as measured in ion flux studies of neuroblastoma cells (154, 155). The cooperative interaction can be quantitatively described by the simple allosteric model proposed for scorpion toxin action (154), although more complicated schemes have been considered (155). The cooperative effect of toxin II is inhibited by depolarization, but the effect is much smaller than for scorpion toxin (154).

Sea anemone toxins and scorpion toxins have similar actions on sodium channels: inhibition of inactivation, enhancement of persistent activation by lipid-soluble toxins, and voltage-dependent binding. Direct binding experiments in neuroblastoma cells (136, 154), synaptosomes (137, 138), and frog

skeletal muscle (129) show that toxin II from *A. sulcata* competitively inhibits scorpion toxin binding. These two classes of polypeptide toxins, therefore, act at a common receptor site associated with sodium channels despite their lack of sequence homology. Presumably, aspects of the tertiary structure of the toxins allow them to interact with the same or overlapping receptor sites.

Saturation of the toxin receptor sites by *A. sulcata* toxin II measured in binding studies follows a simple Langmuir isotherm (136, 154). In contrast, the concentration-effect curves for enhancement of veratridine activation of sodium channels are less steep than a simple Langmuir isotherm (154, 155). The mechanism responsible for this different behavior is not clear at present, but could involve either multiple classes of receptor sites or negative cooperativity. Thus, while *A. sulcata* toxin II and scorpion toxin interact with a common receptor site associated with sodium channels, toxin II may interact with additional receptor sites or induce negatively cooperative interactions not observed with scorpion toxin.

The Chemical Nature of the Polypeptide Toxin Receptor

It has not proven possible to solubilize the polypeptide toxin receptor with retention of binding activity (53). However, protein components of the receptor have been covalently labeled with photoactivable derivatives of scorpion toxin and sea anemone toxin (156, 157). Preliminary evidence for covalent attachment of sea anemone toxin I to a polypeptide of $M_r \sim 53{,}000$ in crayfish walking leg nerve has been reported (156). Scorpion toxin has been covalently attached to a polypeptide of $M_r \sim 250{,}000$ in both neuroblastoma cells and synaptosomes (157) and also labels a second polypeptide of $M_r \sim 32{,}000$ in synaptosomes (157). The covalent labeling with scorpion toxin derivatives is blocked by unlabeled toxin and by depolarization and is not observed in variant neuroblastoma clones that lack sodium channels (157). These results suggest that components of the sodium channel have been specifically labeled. This approach, along with purification of the tetrodotoxin/saxitoxin receptor, may allow identification and purification of all of the components of the sodium channel.

OTHER TOXINS

Two other toxins have been described which also seem to affect sodium channels. Lipid-soluble toxins of unknown structure from the dinoflagellate *Gymnodinium brevi* alter the kinetics of sodium currents in squid giant axon, depolarize the axon, and cause repetitive action potentials (158). These effects are blocked by tetrodotoxin (158). Palytoxin from soft corals of the genus *Palythoa* is a highly toxic nonprotein substance. It causes

irreversible depolarization of myelinated nerve fibers which is partially blocked by tetrodotoxin and shifts the voltage dependence of both activation and inactivation to more negative membrane potentials (159). Both of these toxins have effects that resemble those of other lipid-soluble toxins. It will be of interest to determine whether these new toxins of unknown structure act at the same receptor site in modifying activation and inactivation of sodium channels.

CONCLUSION

The main conclusions of the experiments reviewed here can be summarized with reference to the properties of the three separate toxin receptor sites associated with sodium channels (Table 1; 14, 114, 154). Receptor site I binds tetrodotoxin and saxitoxin which inhibit ion transport. This site may be located within or at the external end of the ion-conducting pore of the sodium channel. Receptor site II binds veratridine, batrachotoxin, aconitine, and grayanotoxin. Since these toxins alter both activation and inactivation of sodium channels, receptor site II may be located on a region of the sodium channel important for both of these voltage-dependent processes. Receptor site II must change conformation during activation and inactivation of sodium channels because ligands at this site modify activation, block inactivation, and cause persistent activation by binding more tightly to active state(s) of sodium channels. Receptor site III binds scorpion toxin and sea anemone toxin. While these toxins block inactivation, the voltage-dependence of scorpion toxin binding is correlated with activation. Receptor site III may be located on a region of the sodium channel which undergoes a voltage-dependent conformational change on depolarization leading to activation and subsequent inactivation of sodium channel ion transport.

Table 1 Properties of neurotoxin receptor sites associated with sodium channels

Toxin receptor site	Ligands	Physiologic effect
I	Tetrodotoxin Saxitoxin	Inhibit ion transport
II	Veratridine Batrachotoxin Aconitine Grayanotoxin	Alter activation and inactivation Cause persistent activation
III	Scorpion toxin Sea anemone toxin	Inhibit inactivation Enhance persistent activation by veratridine, batrachotoxin, aconitine, and grayanotoxin

Binding of toxins at receptor site I is independent of voltage and of ligand binding at sites II and III. In contrast, toxin binding at sites II and III is highly interdependent. These two sites interact allosterically such that binding of ligands at one of the two sites enhances binding at the second site. Voltage-dependent allosteric interactions between these two sites may play an important role in activation and inactivation of sodium channels.

While this review has focused on the mechanism of action of specific neurotoxins, it is clear that these studies of neurotoxin action have provided important new insight into sodium channel function. Tetrodotoxin and saxitoxin have provided the first evidence that Na^+ and K^+ channels are separate entities, the first estimates of sodium channel density and ion transport capacity in excitable membranes, and the first approaches to isolation of components of the sodium channel. The lipid-soluble toxins, which alter both activation and inactivation by binding to a single receptor site, have provided clear evidence of the coupling of these two processes at the molecular level and have provided the first evidence of the allosteric nature of sodium channel activation and inactivation. The polypeptide toxins have provided biochemical evidence of voltage-dependent conformational change associated with activation of sodium channels and of allosteric interactions between different functional regions of the sodium channel and have provided tools for identification of sodium channel components. These results represent important steps toward relating the physiologic events of electrical excitation to underlying molecular events. Specific neurotoxins should continue to be essential tools in understanding the molecular basis of electrical excitability.

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