

JGP 100th Anniversary

# The envenomation of general physiology throughout the last century

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Toxins are the poisonous products of organisms. Toxins serve vital defensive and offensive functions for those that harbor them: stinging scorpions, pesticidal plants, sanguinary snakes, fearless frogs, sliming snails, noxious newts, and smarting spiders. For physiologists, toxins are integral chemical tools that hijack life's fundamental processes with remarkable molecular specificity. Our understanding of electrophysiological phenomena has been transformed time and time again with the help of some terrifying toxins. For this reason, studies of toxin mechanism are an important and enduring facet of *The Journal of General Physiology* (JGP). This Milestone in Physiology reflects on toxins studied in JGP over its first 100 years, what they have taught us, and what they have yet to reveal.

## An introduction to toxin physiology

Toxins originate in all of life's kingdoms, take diverse chemical forms, and disrupt our physiology with deadly precision. Plants and animals harbor small molecules such as strychnine, ouabain, cocaine, caffeine, and tetrodotoxin that modulate ion channels, receptors, and transporters to wreak havoc in creatures who dare consider them for lunch. Toxins from infectious anthrax bacteria are large proteins that form their own ion channels in the process of ulcerating your skin. Venomous predators as diverse as anemones, cone snails, spiders, and snakes inject their prey with disulfide-knotted peptides that target specific conformations of ion channels. These toxins, and scores more, have been the subject of mechanistic studies in *The Journal of General Physiology* (JGP). The motive for their study is to exploit the molecular specificity and incredible potency with which toxins act on their targets. The integration of toxins that precisely perturb ion channels into electrophysiological investigations that precisely measure protein function has revealed especially intimate details of protein thermodynamics. Toxin physiology articles have shaped our conceptions of physiological mechanisms, and have earned nature's poisons a special place in the hearts of physiologists.

## What is JGP's toxin load?

While conducting research for this article, I turned to the JGP archive for a historical perspective on the intensity of toxin research, beginning with JGP's inception in 1918. A search for articles with toxin-related terms in titles, abstracts, and text returned 28% of all JGP arti-

cles; a remarkable fraction. To trim this list down to articles that study toxin mechanism, rather than ones that merely use a toxin as a reagent, I individually appraised each article that contained a toxin search string in the title or abstract. This yielded the curated collection of 274 toxin mechanism articles in the Appendix; these represent 3% of all JGP articles. A histogram of annual occurrence illustrates the variation of toxin article incidence over the years (Fig. 1 A). In the first decade of JGP, there were a modest number of toxin articles. This was followed by a great depression that persisted until the 1940s, when toxin articles again began to sporadically appear. The 1960s saw a significant uptick in toxin articles that continued for the next half-century. In the last decade, toxin articles have appeared somewhat less often. The recent reduction in toxin article frequency has been accompanied by a shift from simpler articles describing a toxin's action to ever-more-complex investigations of interactions with target proteins. Compare, for example, the simple elegance of a figure establishing tetrodotoxin block of  $\text{Na}^+$  conductance in the squid giant axon (Fig. 2; Nakamura et al., 1965) with the compound rigor of a recent figure detailing the how voltage-gated  $\text{Na}^+$  ( $\text{Na}_v$ ) channel pore residues alter the state-dependence of tetrodotoxin block (Fig. 3; Huang et al., 2012). Another telling illustration is to contrast the elegant plot that revealed conformation-dependent binding of a  $\text{Na}_v$  channel to a scorpion toxin (Fig. 4; Catterall, 1979) with the consummate analyses of  $\text{Na}_v$  channel mutations on revealing that scorpion toxin action (Fig. 5; Leipold et al., 2012). The latter articles are

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Abbreviations used: ASIC, acid-sensing ion channel; TRP, transient receptor channel.

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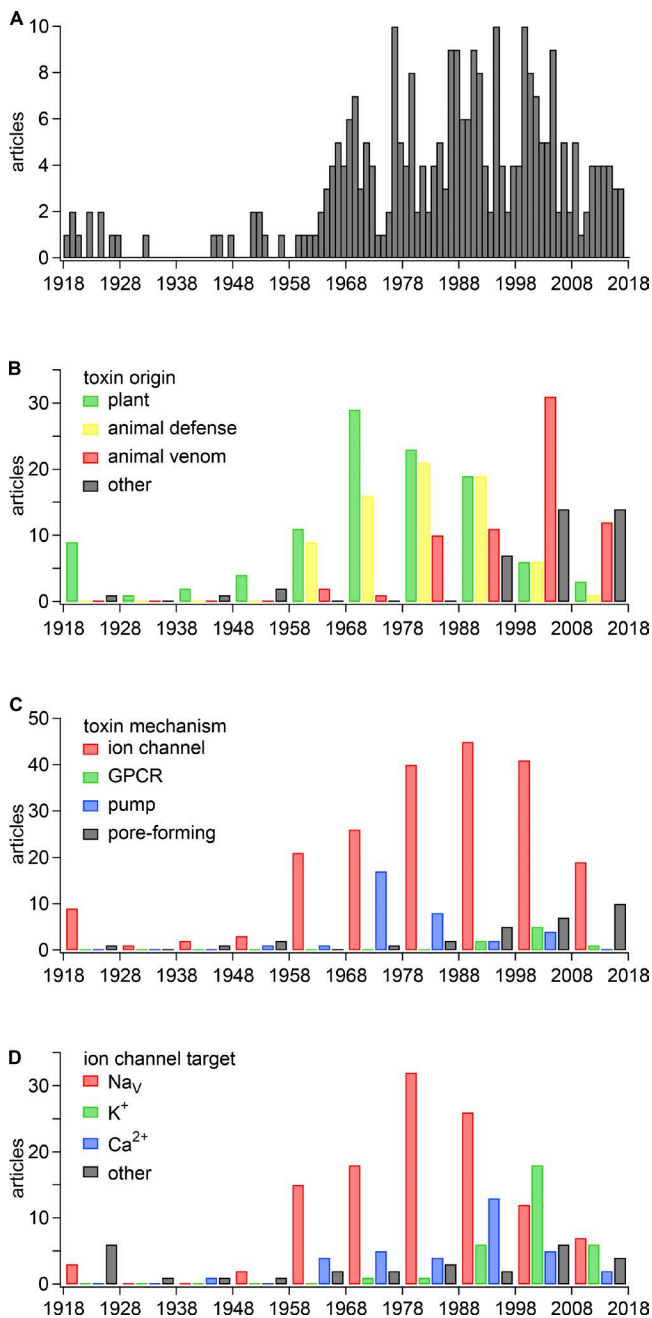


Figure 1. Frequency of articles in JGP that investigate toxin mechanism of action. (A) Number of articles per year. (B) Number of articles per 10-yr bin separated by toxin type and origin. Green, small molecule from a plant; yellow, defensive small molecule from an animal; red, peptide toxin from an animal venom; black, other sources including bacteria, fungi, protists, and anthozoans. (C) Number of articles per 10-yr bin separated by toxin target. Red, ion channels; green, G protein-coupled receptors; blue, pumps; black, toxins themselves are transmembrane pore-forming ion channels. (D) Number of articles per 10-yr bin separated by type of ion channel targeted. Red, voltage-gated Na<sup>+</sup> channels; green, K<sup>+</sup> channels; blue, Ca<sup>2+</sup> channels; black, other channels, including nonspecific ion channel inhibition.

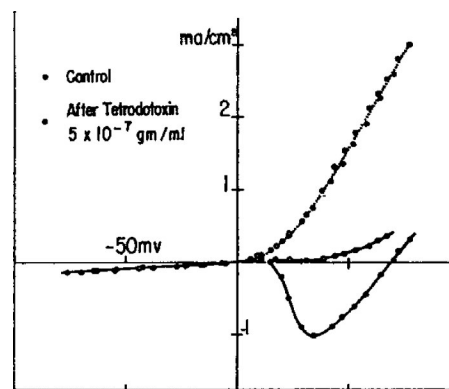


Figure 2. Tetrodotoxin block of Na<sup>+</sup> conductance in the squid giant axon. Figure 3 from Nakamura et al. (1965).

exemplars of the *tour de force* studies of toxin mechanism that are a staple of JGP today.

What toxins have general physiologists exploited?

As natural product isolations have improved over the last century, so has the diversity of toxins isolated. The sources and chemical nature of toxins studied in JGP have systematically changed over the years (Fig. 1 B). In the first decade of JGP, studies mostly examined defensive toxins from plants. These plant toxins elicit pleasure, death, or both in animals that ingest them: strychnine and curare from the genus *Strychnos*, nicotine from tobacco, atropine from nightshade, cocaine from the coca plant, and veratridine extracts from certain lilies. Known to profoundly disrupt human neurophysiology, they were used as tools to study the generality of nervous system function between vertebrates and invertebrates, and even general membrane permeability in plants (Macht and Livingston, 1922). The action of these toxins indicated commonality of electrical signaling mechanisms across animal phyla. Many of these toxins reemerged in later decades, as modulators of ionic currents.

Defensive animal toxins began to appear in JGP articles of the late 1950s. Smaller-scale toxin extractions, from sources such as pufferfish gonad and poison arrow frog skin, were enabled by advances in chemistry. These toxins were primarily small molecules that were stable enough to survive harsh chemical treatments. Some of them were extremely potent ion channel modulators. These small molecules from animals were often exquisitely selective for proteins. This contrasts with many plant toxins, such as strychnine, that modulate multiple protein targets, possibly because plants need to deter a wide range of menacing herbivores. Because of their potency and selectivity, defensive animal neurotoxins including tetrodotoxin, and later batrachotoxin, became a mainstay of electrophysiological studies.

The next toxin onslaught came from the venoms of hunting creatures. Obtaining individual peptides from

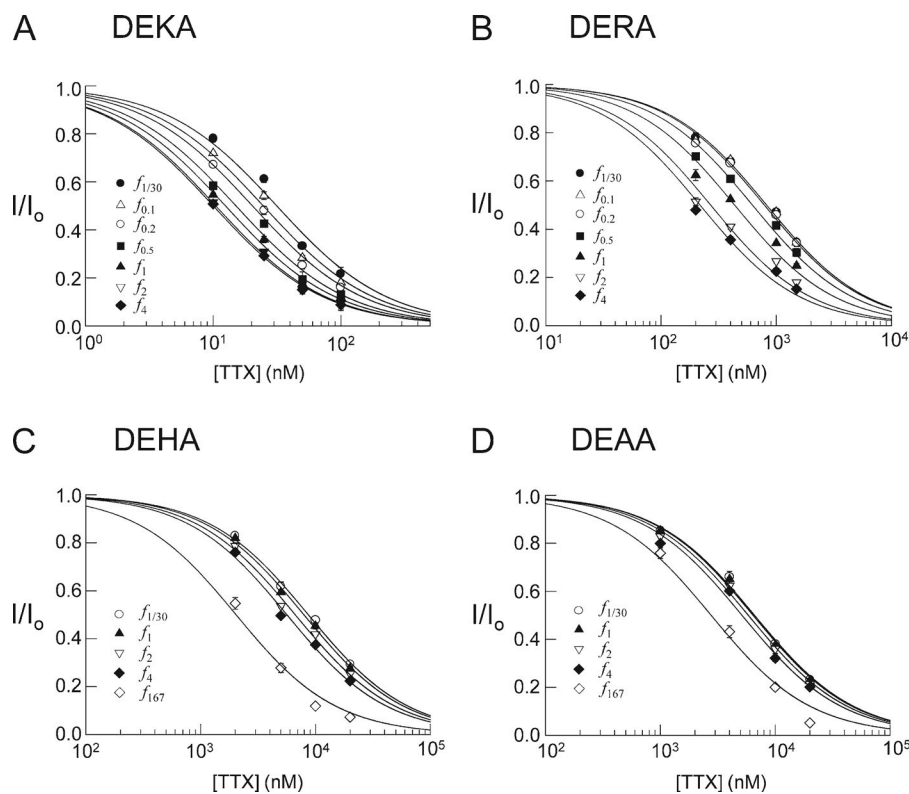


Figure 3.  $\text{Na}_v1.4$  pore residues alter the state-dependence of tetrodotoxin block. Figure 5 from Huang et al. (2012).

complex venoms became possible with further improvements in chemical separation techniques. Scorpion, snake, spider, sea anemone, and cone snail venoms were found to be rich sources of peptides that paralyze prey by hijacking their electrical signaling. Their peptide toxins are typically disulfide knotted, giving them a chemical stability rivaling that of small molecules. Their peptidyl nature has allowed eukaryotic organisms to evolve a formidable combinatorial library of toxins, which specifically target subtypes of proteins, especially ion channels. For the past several decades, peptide toxins have been intensively studied, due to their potency, selectivity, and amenability to chemical modification through mutagenesis and solid-phase synthesis. Peptide toxins are the largest, as well as fastest, growing class of toxin today.

#### How have general physiologists' toxins acted?

More interesting to physiologists than the ecology and structure of toxins are their molecular mechanisms. Of the 274 toxin mechanism articles surveyed from JGP, 12% study pore-forming toxins, 3% study G protein-coupled receptor-targeting toxins, 12% study  $\text{Na}^+/\text{K}^+$  ATPase-targeting toxins, and perhaps not surprisingly, 77% study ion channel-targeting toxins. Of the ion channel-targeting toxins studied, 55% were toxins selective for  $\text{Na}_v$  channels, 15% for  $\text{K}^+$  channels, and 16% for  $\text{Ca}^{2+}$  channels. Toxins affecting multiple ion channel types or other ion channels accounted for the remaining 13%. The chronology of toxin mechanism prominence

indicates several waves of toxin targets as a subject of study (Fig. 1, C and D). Overall, the historical distribution of toxin articles mirrors the general subject matter of JGP articles themselves, suggesting that toxins have played a prominent role across research areas. The next section provides an overview of how toxins have been used in these articles to poison channels and pumps, or form their own pores. This section was informed by the content of articles in the Supplemental material; it generalizes the content of numerous articles. For the sake of simplicity, it is devoid of individual references.

#### Toxins block, open, and shift the gating of ion channels.

Some of the earliest electrophysiological studies of toxins involved cocaine block of  $\text{Na}^+$  conductances. Studies of channel block by a cocaine-like mechanism were largely supplanted by local anesthetic derivatives such as lidocaine, which are easier to legally procure, but are not technically toxins. In the 1960s, the majority of toxin studies involved the  $\text{Na}_v$  blocker tetrodotoxin, or the similarly acting saxitoxin. Today, tetrodotoxin endures as the principal  $\text{Na}_v$  channel-blocking toxin in electrophysiological studies, as it is highly selective for a well-defined subset of  $\text{Na}_v$  channels.  $\text{Na}_v$  channel-opening toxins have a similarly long history in JGP. The first  $\text{Na}_v$  channel openers were veratrine plant extracts, most notably veratridine. The family of  $\text{Na}_v$  channel openers expanded to include other toxins such as aconitine, grayanotoxin, and batrachotoxin. Batrachotoxin, the most potent of these openers, proved effective

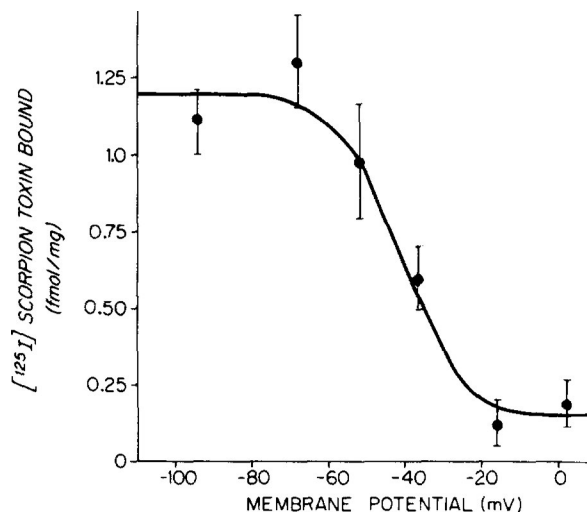


Figure 4. Depolarization of frog muscle  $\text{Na}_V$  channels alters binding of  $\alpha$ -scorpion toxin from *Leiurus quinquestriatus* venom. Figure 5 from Catterall (1979).

at lengthening the open times of ephemeral  $\text{Na}_V$  channels and fostered studies of conduction through single channels. When the study of venoms began burgeoning, physiologists found that scorpion and anemone peptides could modulate voltage sensor movements, and used them to dissect the steps in  $\text{Na}_V$  channel gating. The peptidyl  $\text{Na}_V$  channel toxins were soon complemented by  $\text{K}^+$  channel peptide toxins that block pores or alter gating. Detailed mechanistic studies of the interaction of charybdotoxin and  $\text{K}^+$  channels set the stage for a series of studies with derivatized pore-blocking toxins that investigated the molecular features of channels. Beginning with hanatoxin, studies of  $\text{K}^+$  channel voltage sensor movement have been aided by toxins that trap voltage sensors in key conformations. Present-day ion channel toxin studies primarily focus on gating mechanisms that are modulated by gating modifier toxins.

Studies of the interactions between toxins and many additional channel types have appeared in JGP. A few have involved voltage-gated  $\text{Ca}^{2+}$  channels, but most have involved intracellular channels affected by caffeine, ryanodine, or membrane-penetrating imperatoxin peptides from scorpion venom. Sporadically, mechanistic studies have appeared with toxins that target cyclic nucleotide gated channels or acid-sensing ion channels (ASICs).

**Toxins stop pumps and turn them into ion channels.** In the late 1960s and 1970s, studies of the  $\text{Na}^+/\text{K}^+$  ATPase with ouabain proliferated in JGP. Ouabain was the most heavily studied of the “cardiac glycoside” toxins from plants. With ouabain, the pump can be trapped in distinct conformations that allow the affinities of ions to be measured without interference from complicating en-

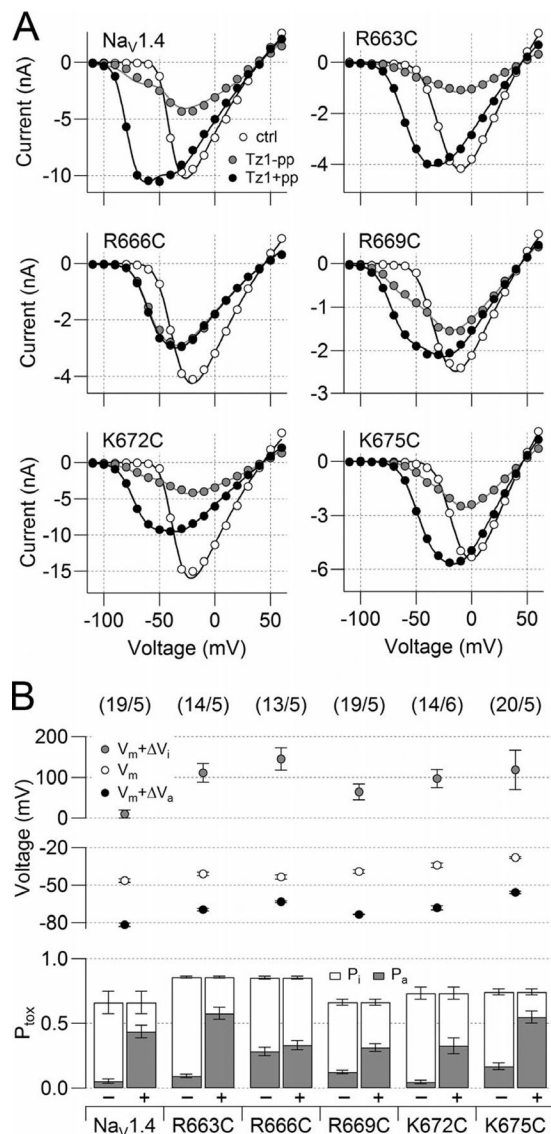


Figure 5. Charge neutralizations in the domain 2 voltage sensor of  $\text{Na}_V1.4$  differentially influence gating modification by the  $\beta$ -scorpion toxin Tz1. Figure 7 from Leipold et al. (2012).

zymatic activity of the pump. Ouabain inhibits pump activity at conformations distinct from those that manipulate ATP and played a critical role in revealing steps of the  $\text{Na}^+$  and  $\text{K}^+$  transport cycle. More recent studies have deployed the incredibly poisonous coral toxin, palytoxin, which induces a conformational change in the ion translocation machinery of the  $\text{Na}^+/\text{K}^+$  ATPase, rendering it an ion channel.

**Toxins make their own channels.** Toxins that form a pore in cell membranes have been an important area of research in JGP. Studies of pore-forming toxins, such as diphtheria toxin, have appeared since before their mechanism of action was known. Recent decades have seen in-depth electrophysiological studies of the

properties of pore-forming toxins, including anthrax toxin. This class of toxin creates a protein translocation pore that also allows ionic flux, enabling their pore properties to be probed by electrophysiological techniques. Pore-forming toxins are also found in black widow spider venom. The nonselective pore formed by the spider's latrotoxins allow calcium influx into the presynaptic terminals leading to uncontrolled synaptic vesicle release.

#### What have we learned from JGP's toxin studies?

Toxins have played in critical roles understanding physiology, especially the functioning of ion channels. The following discussion examines how toxin studies in JGP have contributed to our understanding of nervous system evolution, discerned that ionic currents arise from distinct molecules, identified structures important for ion conduction, revealed moving parts of channels, and exposed coupling between conformational changes.

Strychnine poisoning indicated that common molecular mechanisms control electrophysiological signaling across the animal kingdom. JGP's first issue in 1918 contained an article investigating the action of strychnine (Moore, 1918). This study discusses effects of strychnine on nervous signaling in starfish and flatworms. Strychnine's effects on these invertebrates were found to be similar to its effects on vertebrates. The article concluded that "strychnine acts upon some chemical component of the neuron which is always present in synaptic structures but which also occurs in the simpler neurons of lower forms." This was an impressively apt conclusion about general physiology based on toxin effects. We know now that strychnine inhibits pentameric Cys-loop ionotropic receptors for glycine, acetylcholine, and GABA neurotransmitters (Houamed et al., 1984; Matsubayashi et al., 1998), as well as voltage-gated  $\text{Na}^+$  and  $\text{K}^+$  channels (Shapiro et al., 1974; Shapiro, 1977a,b).

Tetrodotoxin revealed that the axonal  $\text{Na}^+$  conductance is independent of the  $\text{K}^+$  conductance. It was once debated whether the  $\text{Na}^+$  and  $\text{K}^+$  conductances underlying axonal action potentials arose from independent  $\text{Na}^+$  and  $\text{K}^+$  channels or from a single channel that switched ionic selectivity (Mullins, 1959, 1968). Careful experiments on isolated, voltage-clamped axons revealed that tetrodotoxin blocks  $\text{Na}_V$  conductances without altering  $\text{K}^+$  conductances (Fig. 2; Nakajima et al., 1962; Narahashi et al., 1964; Nakamura et al., 1965; Takata et al., 1966). The originally parsimonious hypothesis that a single channel conducts both ions became untenable in the face of these results. Tetrodotoxin studies provided key data to reach the conclusion that the conductance changes underlying action potentials were from independent  $\text{Na}^+$  and  $\text{K}^+$  channels (Hille, 1970). This work

and the selectivity of tetrodotoxin allowed the field to move forward with general acceptance that  $\text{Na}^+$  and  $\text{K}^+$  currents were carried by molecularly distinct pores.

$\text{Na}_V$  channel openers allowed conduction to be studied without confounding inactivation.  $\text{Na}_V$  channels open very transiently in response to stimuli before they inactivate, making it difficult to study their properties at the single-channel level. The modification of  $\text{Na}_V$  channel gating by toxins including veratridine and batrachotoxin removes inactivation; consequently, channels remain open much longer. This method of studying  $\text{Na}_V$  channels led to a host of findings including identification of structural elements that limit ionic flux (Huang et al., 1979; Green et al., 1987; Wang et al., 1991), relative localization of blocker sites (Huang and Ehrenstein, 1981; Wang, 1990), and understanding of the state dependence of drug binding (Moczydlowski et al., 1984; Wang and Wang, 1994).

Scorpion toxins probed the external mouth of the  $\text{K}^+$  channel selectivity filter. Studies of charybdotoxin interactions with  $\text{K}^+$  channels seeded a new field of very fruitful ion channel probing techniques. A set of JGP papers on charybdotoxin mechanism were fundamental to understanding the toxin's precise action (Anderson et al., 1988; MacKinnon and Miller, 1988). These studies concluded that charybdotoxin inhibits channels by plugging their externally facing mouth. This fundamental mechanistic understanding of toxin action motivated the deployment of pore-blocking toxins in creative ways. Using pore-blocking toxins as electrophysiological probes, researchers established the functional stoichiometry of  $\text{K}^+$  channels (MacKinnon, 1991; Morin and Kobertz, 2007) and determined the molecular architecture of regions surrounding the channel pore (Gross and MacKinnon, 1996; Ranganathan et al., 1996).

Tarantula toxins exposed limits of voltage sensor movement. Peptide spider toxins that target  $\text{K}^+$  channels have been instrumental for determining how voltage sensors move. One particularly thorough JGP study definitively localized of the binding site of the tarantula venom peptide hanatoxin on a voltage-gated  $\text{K}^+$  channel laid the foundation for interpretation of voltage sensor toxin action (Li-Smerin and Swartz, 2000). This work identified the binding site as the outer half of the voltage sensor's third transmembrane segment. Comparative work has determined that many, if not all, voltage sensor toxins bind to a similar site in  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Ca}^{2+}$  channels, (Winterfield and Swartz, 2000; Li-Smerin and Swartz, 2001; Milescu et al., 2013). The clearly identified binding site on voltage sensors has enabled conclusions to be drawn about the conformational changes of the site itself. For example, the tox-

in's binding site does not fully traverse the membrane during voltage gating (Phillips et al., 2005).

A snail mucus toxin revealed that voltage sensors can cooperate without channel opening. The gating of channels modulated by a toxin from a marine snail's defensive secretion, 6-bromo-2-mercaptotryptamine, revealed fine details of voltage sensor conformational interplay. This toxin selectively modulates an early voltage sensor conformational change (Sack et al., 2004). Although these early voltage sensor movements are normally independent between subunits of the Shaker K<sup>+</sup> channel, 6-bromo-2-mercaptotryptamine induces subunit cooperativity (Sack and Aldrich, 2006). Thus, movement of early voltage sensors can become coupled before channel opening. These studies indicated that spatially separate voltage sensors have another mechanism (besides opening the channel pore) by which they can couple to each other.

Scorpion toxins indicated that Na<sup>+</sup> channel activation and inactivation are modulated by different voltage sensors. Toxins have enabled microdissections of the complicated conformational changes that comprise Na<sup>+</sup> channel gating (see Ahern et al., 2016). Scorpion venom peptides have helped elucidate how the four distinct voltage-sensing domains of Na<sup>+</sup> channels contribute to gating.  $\alpha$ -Scorpion toxins slow Na<sup>+</sup> channel inactivation by binding to the fourth voltage sensor domain and holding its gating charges on the intracellular side of the membrane (Catterall, 1979; Hanck and Sheets, 1995; Sheets and Hanck, 1995; Rogers et al., 1996; Bosmans et al., 2008; Campos et al., 2008; see Fig. 4). In contrast,  $\beta$ -scorpion toxins slow transitions of the second voltage sensor domain, revealing that this domain is particularly important for the voltage-dependent opening and closing (Cestèle et al., 2001; Campos et al., 2007; Leipold et al., 2012; see Fig. 5). Our understanding of Na<sup>+</sup> channel gating would not be so rich in thermodynamics and structural detail were it not for scorpions and the precise studies of their toxins' mechanisms of actions.

**Toxins have taught us much.** At every phase of ion channel research over the last six decades, toxins have provided unique insights into ion channel mechanism. The toxin-driven discoveries described above are but a few examples of how toxins have been integral in identifying and characterizing channels that underlie electrical impulses. Toxin studies have been instrumental in understanding both permeation and gating of many channels. In the past decade, most of JGP's articles on toxins targeting ion channels have investigated mechanisms of gating modulation. This trend suggests that gating modifier toxins will play important roles in the study of ion channels in the years to come.

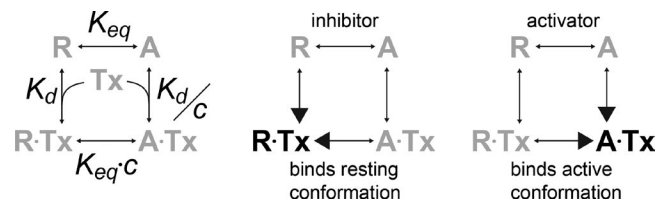


Figure 6. Schemes of relations between toxin (Tx) binding and stabilizing resting (R) or active (A) conformations of a protein.

#### What can toxins teach us now?

Ion channels are fascinating because they are dynamic, conformation-changing proteins. Decades of extensive studies, many of them in JGP, have determined what kinds of conformations channels enter. These conformations have been given functional names such as resting, active, open, inactive, and more. Determining structurally what these conformations are is a challenge of modern molecular physiology. Toxins can be especially valuable tools in this pursuit, because toxins have mastered the art of invoking conformational change. Many extremely potent toxins are allosteric modulators that stabilize certain conformations at the expense of others. The flip side of this conformational stabilization is binding preference. Toxins that stabilize a conformation also bind that conformation with better affinity (Fig. 6). This binding preference is mandated by the thermodynamic principle of microscopic reversibility (Wegscheider, 1901; Lewis, 1925; see Sack and Eum [2015] for further discussion). For an efficacious toxin, this strong preference means a toxin will essentially only bind a conformation it stabilizes. Thus, for their creator's own nefarious needs, venomous organisms have been evolving toxins that potently bind particular conformations of channels. What wonderful gifts from some creepy critters! The conformation-selective binding of toxins makes them powerful tools to study protein conformational change. Conformation-selective toxins are valuable in many realms of modern physiology, including structural characterization and imaging protein conformational change in cells.

**Toxins help channels pose for pictures.** Squirmy children are unlikely to smile naturally for a photograph, and ion channels are similarly troublesome. The dynamism that makes channels so interesting also makes determining their structures a special challenge. First, channels adopt many conformations, complicating the capture of a coherent snapshot of any single one. Second, structural data gathered from channels in a non-physiological environment do not necessarily represent a physiologically relevant conformation. Toxins that stabilize a conformation can make a population of channels more structurally homogeneous. Conformational homogeneity can make channels amenable to crystalli-

zation or electron microscopy reconstructions. Furthermore, a toxin's impact on function can help determine physiological relevance of a toxin-bound conformation. For both of these reasons, toxins have been valuable tools for obtaining structures and assigning them to a physiological context. Good examples of how toxins can aid in structural biology include toxin-bound structures of ASICs and transient receptor channels (TRPs).

The heat-, acid-, and capsaicin-sensing TRPV1 channel is opened painfully well by the "double knot" peptide from a Chinese bird spider's venom, and also by resiniferatoxin from cactus-like *Euphorbia* succulents. These state-selective toxins were the enabling factors for reconstruction of TRPV1 in an open state (Cao et al., 2013; Liao et al., 2013; Bae et al., 2016; Gao et al., 2016). Likewise, cocrystals of ASICs with peptide toxin gating modifiers, psalmotoxin-1 from the Trinidad chevron tarantula, or MitTx from the Texas coral snake have allowed glimpses of what these channels may look like in open or desensitized conformations (Baconguis and Gouaux, 2012; Dawson et al., 2012; Baconguis et al., 2014). Notably, psalmotoxin was originally reported to be an ASIC inhibitor, but careful characterization of its mechanism in a set of JGP papers revealed that psalmotoxin actually acts by sensitizing channels to acid (Chen et al., 2005, 2006). This mechanistic distinction was critical for interpretation of the structural work. Structural studies with TRPs and ASICs highlight both the difficulty in assigning structures to physiologically relevant conformations and how mechanistically characterized toxins can help. The conglomeration of gating modifier toxins that modulate ASICs and TRPs have been key for understanding what conformational transitions occur during channel gating. As you stare into the intensifying barrage of ion channel structures, watch for gating modifier toxins clinging to their sides.

**Toxins can show us where proteins are changing conformation.** A major question about protein conformational change is: What conformations do proteins adopt during physiological signaling? We have very advanced models of how ion channels react to stimuli. However, these models are mostly developed in isolated cells. Determining whether models accurately represent conformational changes during physiological signaling is a challenge in its own right. Cellular microenvironments modulate the gating of channels. Cells posttranslationally modify channels, as well as vary their accessory subunits and surrounding lipids. Such variation makes the behavior of endogenous channels difficult to predict, and even tougher to experimentally verify. We have limited means of measuring the actual conformational changes of distinct proteins in a living system. Imaging probes that identify when and where ion channels change conformation in tissue could help bring light to many unknown functions of ion channels.

Toxins with state-dependent affinities probe channel conformation. For example, we have found that fluorescently labeled variants of the guangxitoxin tarantula peptide label live cells where Kv2 ion channel subtypes have their voltage sensors in a resting conformation (Tilley et al., 2014). This fluorescent guangxitoxin acts as a probe of channel conformation. It dissociates from channels when they are voltage activated, thereby indicating where channel gating is modulated in tissue. This technique can enable imaging of endogenous channel gating throughout large regions of intact tissue. Furthermore, toxinologists around the world are cataloguing proteins targeted by a vast and growing collection of conformation-selective peptides (Kalia et al., 2015). This toxin library portends an expanding role for these peptides in discovering how proteins change conformation in live animals. How fulfilling it would be to see a channel symphony playing while the heart beats or the brain thinks!

**Toxin reign is forecast to continue.** The deployment of gating modifier toxins in structural biology and physiological imaging research highlights just two of their current uses. Until human engineering outflanks Mother Nature's ability to design protein modulators, toxins will continue to be important research tools. As the unrivalled masters of forcing conformational changes, toxins are expected to supply physiologists with valuable protein ligands for a long time yet. The best part is, the toxins for our future needs have already been made, evolving to deadly potency over the eons. All we have to do is find them.

#### Online supplemental material

The supplement lists the search terms used to identify toxin articles in the JGP archive. It then lists the title and year of publication of articles that were deemed to research mechanisms of toxin action; toxin articles are sorted under subheadings indicating the mechanism of toxin action.

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