ON THE VOLTAGE-DEPENDENT ACTION OF TETRODOTOXIN

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ABSTRACT The use of the maximum rate-of-rise of the action potential ($\dot{V}_{max}$) as a measure of the sodium conductance in excitable membranes is invalid. In the case of membrane action potentials, $\dot{V}_{max}$ depends on the total ionic current across the membrane; drugs or conditions that alter the potassium or leak conductances will also affect $\dot{V}_{max}$. Likewise, long-term depolarization of the membrane lessens the fraction of total ionic current that passes through the sodium channels by increasing potassium conductance and inactivating the sodium conductance, and thereby reduces the effect of $\dot{V}_{max}$ of drugs that specifically block sodium channels. The resultant artifact, an apparent voltage-dependent potency of such drugs, is theoretically simulated for the effects of tetrodotoxin on the Hodgkin-Huxley squid axon.

One approach to understanding the molecular basis of excitability is to study the pharmacological properties of ionic channels. Sodium channels, for example, are affected in characteristic, well-defined ways by protons and Ca^{2+} (1–4), local anesthetics (5–6), and a variety of alkaloids (7–8) and neurotoxins (9–12). The very similar pharmacological properties found in different excitable tissues from a variety of vertebrate and invertebrate species have strongly supported the notion that the specific ionic channels in all these membranes are nearly identical in structure (13). This observation is most valid for the effects of tetrodotoxin (TTX), a low-molecular-weight (319 daltons) poison that blocks action potentials at concentrations of 10^{-9}–10^{-8}M in many nerves and muscles (12). Studies of the binding of tetrodotoxin and saxitoxin (STX), an analogous poison, support the hypothesis that one toxin molecule binds in the external opening of one sodium channel, thus preventing ion passage (14). Because it has such a high affinity for this binding site, K_D = 1–10nM, a small molecule like TTX must fit quite intimately into the channel opening; slight modifications of TTX structure do render the toxin almost totally ineffective (15). Some investigators have even published speculations on the molecular dimensions and the chemical attributes of the sodium channel based somewhat on the structure of tetrodotoxin and saxitoxin (16).

Nevertheless, there are numerous reports of excitable cells whose activity is partially or totally insensitive to TTX or STX. Some of these cells rely on Ca^{2+} as the carrier for inward currents to produce regenerative electrical responses (17), but others are clearly Na^+-selective in this requirement. In particular, the neurons from animals that synthesize or sequester the toxins are refractory to their effects (18–20), as are the plasma membranes of neonatal (21) and denervated mammalian muscle (22) and the

membranes of mammalian cardiac ventricle (23). The last of these has recently been reported to have a TTX sensitivity that depends on the resting membrane potential of the ventricle (23), an observation that seems to contradict the direct results of studies on TTX and STX binding to nerve (14, 24, 25). As a result, the authors claim that ventricular muscle membrane has either tetrodotoxin receptors or sodium channels different from those in nerve. However, there is an alternative explanation of these experimental results.

In this paper we show that the apparent voltage-dependence of TTX effectiveness can arise as an artifact when the parameter $\dot{V}_{max}$ is used as a measure of the Na^+ conductance. The basis for our argument is that a membrane action potential (with zero applied current and zero axial current flow) has a maximum rate of rise, $\dot{V}_{max}$, which is proportional to the maximum total inward current crossing the membrane (i_m). This membrane current is the difference between inward sodium current (i_{Na}) and outward potassium (i_K) and "leak" currents (i_l). For a relatively "leaky" membrane, i_{Na} might be twice $i_K + i_l$ at $\dot{V}_{max}$, whereas for an "unleaky" membrane, the same i_{Na} would be 10 times $i_K + i_l$. Of course, the net inward i_m will be smaller for the leaky membrane and $\dot{V}_{max}$ will be lower. More important, if the sodium conductance is reduced to half in both membranes (for example, by applying the same concentration of TTX) then the leaky membrane will have zero net inward current and no action potential while the unleaky membrane will show a $\dot{V}_{max}$ that is 44% of the $\dot{V}_{max}$ without TTX. The nonlinearity between sodium conductance and $\dot{V}_{max}$ has already been amply illustrated in frog node of Ranvier by Ulbricht and Wagner (26). Clearly the use of $\dot{V}_{max}$ to measure the effectiveness of TTX in blocking sodium channels depends on a knowledge of the relative Na^+, K^+, and leak currents across the membrane.

By the same logic we can simulate a "voltage-dependent" action of TTX. Consider the same excitable membrane, held at two different resting potentials, −90 and −60 mV. Because of sodium inactivation, the available sodium conductance at −60 mV will only be 0.6 of that at −90 mV (27), and both $\dot{V}_{max}$ and net inward current will be less for action potentials in this situation. Thus, the same TTX concentration will reduce the net inward current by a larger percentage in the membrane held at −60 mV than in the one held at −90 mV, and will have a greater effect on $\dot{V}_{max}$, even though the binding of the toxin to sodium channels was identical at both resting potentials.

These artifactual variations in TTX effectiveness will appear most dramatically in cells that have relatively large leak and potassium currents compared to sodium currents. Nevertheless, we have simulated these effects on the Hodgkin-Huxley squid axon (27), a system with a leak conductance only 2.5×10^{-3} times the maximum Na conductance, and in which the height of the normal action potential reflects the value of the sodium equilibrium potential.

The Hodgkin-Huxley equations for a membrane action potential were programmed into a Hewlett-Packard 9810A calculator (Hewlett-Packard Co., Palo Alto, Calif.). The rate constants were chosen from the sample data given by Hodgkin and Huxley (2) for a squid axon at 6°C. The Runge-Kutta method for numerical integration of the dif-

ferential equations was chosen with a step width of 0.01 ms if $\dot{V}_m$ was greater than -50 V/s, and 0.05 ms if $\dot{V}_m$ was less than this magnitude.

The peak sodium conductance, $\bar{g}_{Na}$, was 120 mmho/cm^2 in the control and was reduced progressively to 60, 40, and 30 mmho/cm^2 at each of the holding potentials (-96, -78, -60, and -55 mV), to simulate a titration of sodium channels by TTX. Membrane action potentials were then initiated by instantaneous depolarizations of +26 mV.

This initial depolarization was chosen because it was found that at each $\bar{g}_{Na}$, $\dot{V}_{max}$ varied with the initial depolarization and this particular value of initial depolarization was found to produce the greatest $\dot{V}_{max}$ (within 10%) for nearly all experimental cases programmed.

Fig. 1 shows the rising phase of the simulated action potentials for different values of the holding potential and $\bar{g}_{Na}$. From the figure it is apparent that at more depolarized holding potentials the potentials at which ($\dot{V}_{max}$) is reached is more negative for equal values of $\bar{g}_{Na}$. The slopes of the traces show an obvious dependence on $\bar{g}_{Na}$. The less obvious modification of this dependence by the holding potential becomes apparent in Table I.

Table I lists the holding potential from which the membrane action potential was initiated, the TTX concentration, the fractions $\bar{g}_{Na}$ (TTX)/$\bar{g}_{Na}$ control (resulting from constant, voltage-independent TTX binding), $\dot{V}_{max}$ (TTX)/$\dot{V}_{max}$ control, and the apparent "K_D" for TTX effectiveness with $\dot{V}_{max}$ (TTX)/$\dot{V}_{max}$ control as a linear measure of $\bar{g}_{Na}$ (TTX)/$\bar{g}_{Na}$ (control). A reduction in holding potential from -78 to -60 to -55 mV results in a progressively increasing effect of reductions in $\bar{g}_{Na}$ (binding of TTX) on $\dot{V}_{max}$, thus producing an apparent increase in TTX affinity. Hyperpolarization to -96 mV seems to have little effect; it actually lowers $\dot{V}_{max}$ relative to its value at

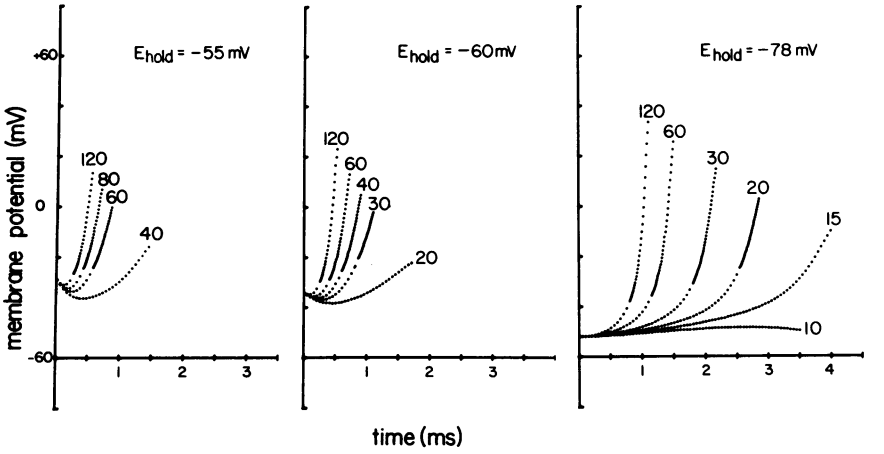

FIGURE 1 Rising phases of action potentials at three different holding potentials and various $\bar{g}_{Na}$'s labeled at the end of each trace. Each simulated upstroke contains one point beyond the maximal $\dot{V}$, so the end of each trace closely approximates the position of $\dot{V}_{max}$ in time and membrane potential.

TABLE I

Holding potential	[TTX]	$\frac{\bar{g}_{Na}\,(TTX)}{\bar{g}_{Na}\,(control)}$	$\frac{\dot{V}_{max}\,(TTX)}{\dot{V}_{max}\,(control)}$	Apparent K_D‡
−55	0	1.00	1.00	—
	2	0.66	0.68	4.33
	4	0.50	0.47	3.55
	8	0.33	0.16	1.48
−60	0	1.00	1.00	—
	4	0.50	0.61	6.26
	8	0.33	0.40	5.33
	11	0.25	0.26	4.22
	21	0.16	0.06	1.34
−78	0	1.00	1.00	—
	4	0.50	0.68	8.50
	12	0.25	0.40	8.04
	21	0.16	0.24	6.63
−96	0	1.00	1.00	—
	4	0.50	0.65	7.43
	12	0.25	0.30	5.14

*K_D = 4 nM, assumed for Langmuir binding of TTX to Na channels in squid at 6°C.
‡Apparent K_D calculated from the equation $K_D = [TTX](A/1 - A)$, where

$$A = [\dot{V}_{max}\,(TTX)]/[\dot{V}_{max}\,(control)]$$

−78 mV holding potential, due to the long lag period between the stimulation and the foot of the action potential (>3 ms) during which the sodium conductance can partially inactivate and the potassium current can develop.

These results represent an alternative explanation for a voltage-dependent action of TTX on $\dot{V}_{max}$. We emphasize that this treatment assumes only Langmuir binding of TTX with no requirement for any voltage-dependent behavior. Since the example we have chosen, the Hodgkin-Huxley squid axon, has a relatively small leak conductance, the apparent voltage-dependence of TTX action would be least impressive in this system, but would be more pronounced in membranes with relatively large leak currents. The analysis here has been restricted to membrane action potentials, where axial current flow is zero. To evaluate the relationship between $\dot{V}_{max}$ and $\bar{g}_{Na}$ at different holding potentials for propagated action potentials, a more elaborate simulation is necessary.

This work was supported by U.S. Public Health Service Grants 1-R01 NS12828-01 and 5S07RR0573604.

Received for publication 12 November 1976 and in revised form 3 January 1977.

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