Saxitoxin and Tetrodotoxin: Comparison of Nerve Blocking Mechanism

Abstract. *Saxitoxin at concentrations of 3 × 10–8 to 3 × 10–7 mole per liter blocks the conduction of lobster giant axon with no change in resting potential. Recovery of washed axons is faster in those that had been treated with saxitoxin than it is in those that were treated with tetrodotoxin. Peak transient increase in nerve membrane conductance is selectively blocked by saxitoxin with no change in late steady-state increase in conductance. The major mechanism of saxitoxin blockage is the same that of tetrodotoxin blockage.*

Saxitoxin (STX) is the active ingredient of the poison isolated from the toxic Alaska butter clams, *Saxidomas giganteus*. It is suggested that STX originally derives from the dinoflagellate *Gonyaulax catanella* (*1, 2*). Although the chemical structure of STX is not completely identified yet, there is evidence that its molecular formula is $C_{10}H_{17}N_7O_4 \cdot 2HCl$ (*1, 2*) and is somewhat different from tetrodotoxin (TTX), for which the chemical structure has been established as $C_{11}H_{17}N_3O_8$ (*3*). Since TTX blocks nerve excitation by specifically inhibiting the increase in voltage-dependent transient (sodium) conductance of the nerve membrane (*4–6*), it is of great interest to see whether STX blocks the nerve excitation in the same way TTX does.

Available data indicate that STX and clam poison block frog nerve fibers, frog sartorius muscle fibers, and electroplaques with no change in resting potential and delayed rectification (*7, 8*). These findings suggest a blocking mechanism similar to that of TTX; and in electroplaques (*9*) and in nodes of Ranvier (*10*), the peak transient current is indeed selectively blocked by STX. We have analyzed the blocking mechanism of STX in lobster giant axons, in which the TTX blocking mechanism has been studied in detail (*4*).

Resting potential and propagated action potentials were recorded at room temperature (22°C) from the partially isolated giant axons in the circumoesophageal connectives of the lobster *Homarus americanus* by means of capillary microelectrodes filled with 3*M* KCl. The method of sucrose-gap voltage-clamp with the completely isolated giant axons was essentially the same as that described previously for squid axons (*6*), except that the chamber was slightly modified to adapt to lobster axons. The voltage-clamp experiments were done at 7° to 10°C. Artificial seawater containing 468 mmole of Na^+, 10 mmole of K^+, 25 mmole of Ca^{2+}, 8 mmole of Mg^{2+}, 533 mmole of Cl^-, 4 mmole of SO_4^{2-}, 2.5 mmole of HCO_3^- (*p*H 7.9), was used as the bathing medium.

The time course of the change in resting potential, action potential, and the maximum rate of rise of action potential under the influence of STX and TTX is illustrated by Fig. 1. Both STX and TTX blocked the action potential, with no change in resting potential. That recovery following the first application of $1 \times 10^{-7}M$ TTX was slow confirms previous observations (*11*). The time constant of recovery was estimated as 18.5 minutes from the measurements of the maximum rate of rise. The recovery following the application of $1 \times 10^{-7}M$ STX was much faster, the time constant being 11.5 minutes, despite the fact that the STX was applied before the action potential completely recovered from the previously applied TTX. However, the second application of $1 \times 10^{-7}M$ TTX after the recovery from the STX resulted in a rather fast recovery, the time constant being 12 minutes, which was comparable to that after STX.

The results of five such experiments are given in Table 1. The time constants of recovery after treatments with different concentrations of STX cannot directly be compared with each other because the period of time during which the axon was soaked in each concentration was different; the test solution was washed out soon after the conduction was completely blocked. However, the period for which the axons were kept in $1 \times 10^{-7}M$ STX and in 1×10^{-7} TTX was kept constant in each experiment to enable direct comparison. The average time constant of recovery is nearly twice as long after treatment with $1 \times 10^{-7}M$ TTX as after that with $1 \times 10^{-7}M$ STX (Table 1). The acceleration of TTX recovery following exposure to STX was noted in two experiments (4-10-67A and B). If this effect had been exerted in the other three experiments in which TTX was applied after STX, the difference in the time constant of recovery between STX and TTX would have become even greater.

Voltage-clamp experiments revealed that the peak transient currents flowing both inward and outward are blocked by $3 \times 10^{-7}M$ STX, while the late steady-state currents remain unchanged.

The relations between current and

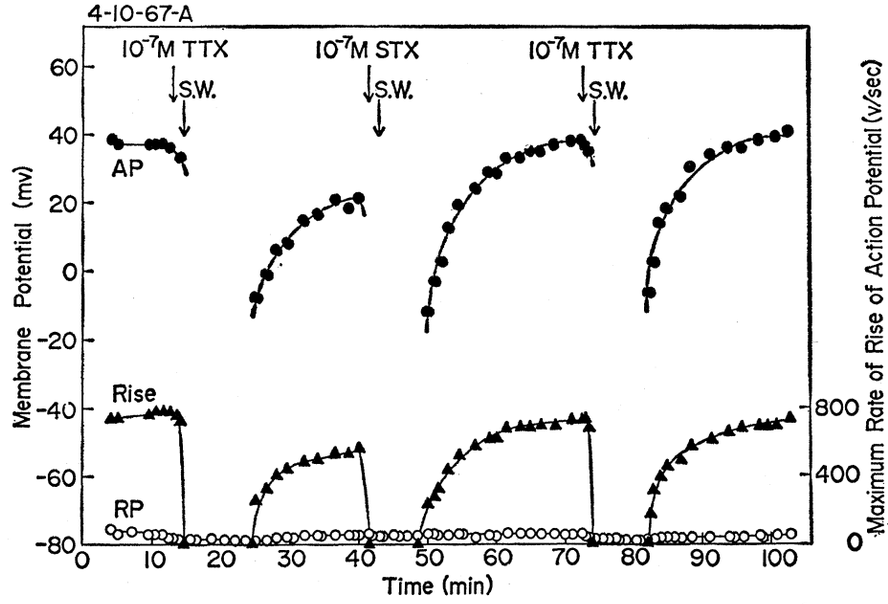

Fig. 1. Time course of the changes in resting potential and in action potential and its maximum rate of rise in a lobster axon during exposure to tetrodotoxin and saxitoxin, and after being washed with normal seawater.

Table 1. Time constant (minutes) for the recovery of the maximum rate of rise of action potentials from axons in normal seawater after exposure to tetrodotoxin and saxitoxin. The numbers in parentheses indicate the order of application.

Preparation	Tetrodotoxin ($1\times 10^{-7}M$)	Saxitoxin		
		$3\times 10^{-8}M$	$1\times 10^{-7}M$	$3\times 10^{-7}M$
4-3-67A	2.5(4)	3(1)	2(2)	3 (3)
4-3-67B	6(4)	1(1)	2(2)	4.5(3)
4-4-67B	6(3)	*(1)	2.2(2)	
4-10-67A	18.5(1)		11.5(2)	
	12(3)			
4-10-67B	7.2(1)		4.3(2)	
	4.6(3)			
		Mean		
	8.1	2	4.4	3.8

* Incomplete block.

voltage, after correction for leakage current, are plotted in Fig. 2, in which the selective blockage of the peak transient current is clearly shown. The membrane conductance (g_p) was calculated at the peak transient current (I_p) according to the equation $g_p = I_p/(E - E_p)$, where E is the membrane potential and E_p is the membrane potential where I_p reverses its polarity. The slope membrane conductance (g_{ss}) during the steady-state current (I_{ss}) was calculated by the equation, $g_{ss} = dI_{ss}/dE$, because of difficulty in estimating the equilibrium potential. The mean (± standard error) ratio of values during treatment to those before treatment with $3 \times 10^{-7}M$ TTX is calculated as 0.19 ± 0.025 (11 measurements)

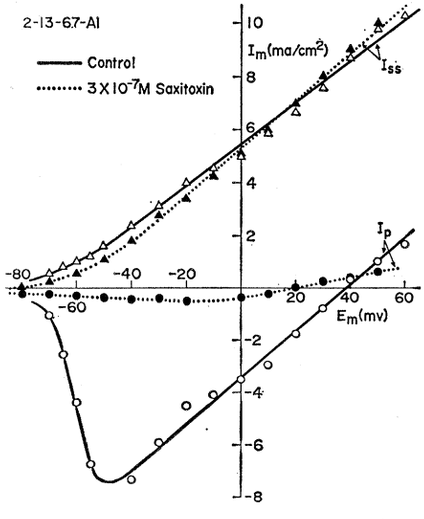

Fig. 2. Relations of current and voltage for the peak transient current (I_p) and for the steady-state current (I_{ss}) before and during application of $3 \times 10^{-7}M$ saxitoxin in a voltage-clamped lobster axon.

for g_p; that for g_{ss} is 0.97 ± 0.15 (11 measurements).

The time for peak transient current to be reached underwent little or no change during the course of STX blockage. The measurements in STX were made when the peak transient current was reduced to about one-fourth of the control value. Seven measurements of the ratio of values during treatment to those before treatment with $3 \times 10^{-7}M$ STX resulted in a mean (± standard error) value of 1.03 ± 0.009.

Our study shows that, like TTX, STX blocks the nerve conduction by inhibiting the peak transient conductance increase which is responsible for the rising phase of the action potential under normal conditions. Thus, STX resembles TTX (*4–6, 11*) in the following respects: (i) The blockage of the action potential is not accompanied by a change in the resting potential. (ii) The recovery after washing is slow compared to that of axons treated with local anesthetics such as procaine. (iii) The effective concentrations are very low. (iv) The peak transient conductance change is selectively blocked with no change in the steady-state conductance change. (v) The time to peak transient current undergoes little or no change. These similarities imply that both toxins behave in the same way as far as the mechanism of nerve blockage is concerned.

However, there are some known differences between STX and TTX: (i) Hypotension does not accompany muscular paralysis in the systemic action of STX, whereas it does in TTX (*7*). (ii) The nerves from the puffer fish *Spheroides maculatus* and from the newt *Taricha torosa* are highly resistant to TTX, whereas they are sensitive to STX (*1, 12*). (iii) When low concentrations of STX are applied to frog nerve fibers, there is often a transient increase in the spike amplitude which is not seen with similar treatment with TTX (*7, 8*). (iv) The block by STX is more readily reversible than that by TTX (*1, 10*).

Despite these differences between STX and TTX, the similarity in their nerve blocking mechanism is worth considering in view of the recent finding on the relationship of activity to structure in TTX derivatives (*11*); a minor change at carbon No. 4 of the TTX molecule could drastically reduce the ability to block the peak transient conductance as shown with deoxytetrodotoxin. Deoxytetrodotoxin, STX, and TTX contain a guanidinium group in their molecules. Thus, in contrast to deoxytetrodotoxin, the STX molecule can exert the same cellular effect as TTX despite its different chemical structure. This supports the notion that the guanidinium group is important and that it is this group of TTX which plugs the gate of the peak transient channel at the external surface of the nerve membrane, thereby preventing the flow of sodium ions normally occurring upon stimulation (*7, 11, 13*).

The faster recovery after treatment with STX than after that with TTX is also worthwhile to note because the rate of recovery after washing is one of the important factors that must be taken into account in determining the mechanism of the drug-receptor binding or interaction. Because of faster recovery after washing, STX may become a more useful and convenient tool than TTX in neurophysiology. When the chemical structure of STX is completely identified, derivatives useful clinically may be produced.

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