BRIEF COMMUNICATION

SODIUM INACTIVATION MECHANISM MODULATES QX-314 BLOCK OF SODIUM CHANNELS IN SQUID AXONS

JAY Z. YEH, *Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611 U.S.A.*

ABSTRACT Blocking action of Na channels by QX-314, a quaternary derivative of lidocaine, was studied in internally perfused and voltage-clamped axons of squid. In axons with intact Na inactivation, QX-314 exhibited both a frequency- and a voltage-dependent block of Na channels. Repetitive pulsing to more positive potentials enhanced the degree of block. Both frequency- and voltage-dependent blocks disappeared in axons in which Na inactivation had been destroyed by either pronase or *N*-bromoacetamide treatment. These results support the notion that Na inactivation not only modulates the frequency-dependent block but also involves the voltage-dependent binding reaction between QX-314 and Na channels.

Local anesthetics suppress the excitability of nerve by blocking Na channels (Taylor, 1959). Normal Na inactivation (Hodgkin and Huxley, 1952) has been proposed to play an important role in modulating the block of Na channels by some local anesthetics, especially the frequency- (use) and voltage-dependent block (Strichartz, 1973; Courtney, 1975; Hille, 1977). In voltage-clamped nodes of Ranvier of frogs, Strichartz (1973) observed that QX-314, a quaternary derivative of the local anesthetic lidocaine, blocked Na channels in a manner dependent upon the voltage and frequency of depolarizing pulses. He postulated that the voltage-dependent block arose from the drug-Na channel binding reaction being voltage dependent. Courtney (1975) extended this hypothesis to include that the h-gate of drug-bound channels may be affected by some tertiary amine local anesthetics. Namely, in drug-bound channels the voltage dependence of Na inactivation is shifted in the hyperpolarizing direction. In this report we present direct evidence showing that the Na inactivation mechanism is involved in QX-314 block of Na channels in squid axon membranes. It was found that pronase treatment, which destroys the normal Na inactivation mechanism, also removes both the frequency- and voltage-dependent block of Na channels by QX-314. Similar results were reported by Almers and Cahalan (1977).

Experiments were performed in giant axons of the squid *Loligo pealei* available at the Marine Biological Laboratory, Woods Hole, Mass. Axons were internally per-

fused using the roller technique and voltage clamped utilizing double axial electrodes (Wu and Narahashi, 1973). The external solution contained 150 mM NaCl, 50 mM $CaCl_2$, 300 mM tetramethylammonium chloride, and was buffered at pH 8.0 with Hepes buffer. The internal solution consisted of 50 mM NaF, 275 mM CsF, 400 mM sucrose, and was buffered at pH 7.3 with phosphate buffer. All experiments were carried out at 8±0.5°C.

Fig. 1 illustrates the effect of QX-314 on Na currents of a normal axon and an axon pretreated with pronase to destroy inactivation. In the normal axon (Fig. 1 A and B), QX-314 blocked Na currents and the blockade consisted of two phases, the tonic phase and the voltage-dependent phase. The former (Fig. 1 A) was obtained after a 2-min rest at holding potential of −80 mV, and the latter (Fig. 1 B) by frequent pulsing to the potentials which favored the opening of Na channels. Therefore, in almost every aspect, QX-314 block of Na channels observed in nodes of Ranvier (Strichartz, 1973) was confirmed here in squid axons.

Fig. 1 A also shows that Na inactivation is incomplete, especially so for large depolarizations (see the upper trace-labeled control). In the presence of 0.4 mM QX-314, Na current was suppressed more at the end of an 8-ms pulse than during the peak; numerical values for inhibition were 60.0 ± 11.6 and 25.0 ± 6.20% (both mean ±

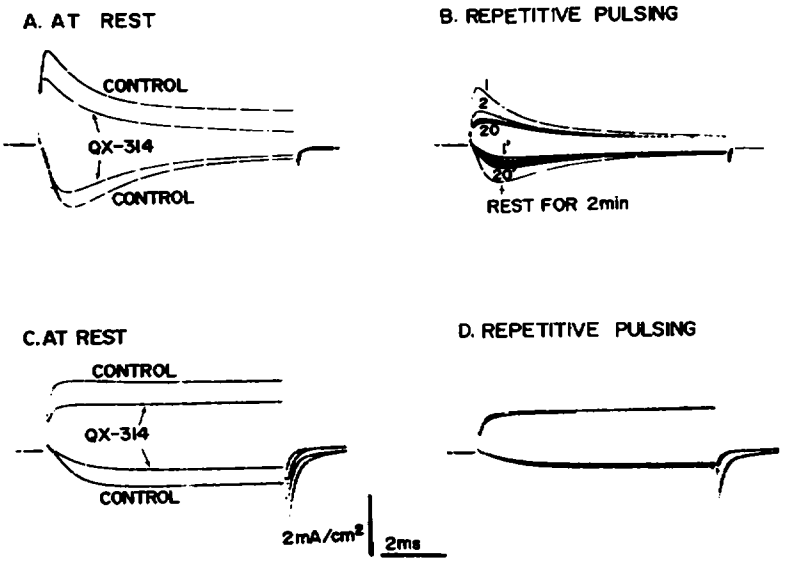

FIGURE 1 QX-314 blocks Na channel in Na inactivation (h-gate) intact axons (A and B) and in axons previously pretreated with pronase to remove h-gate (C and D). In all cases, the upper outward Na currents are associated with step depolarizations to +80 mV and the lower inward ones with step depolarizations to −20 mV from holding potential of −80 mV. In A and C, step depolarizations are applied at very low frequency (1/min) to obtain resting block of QX-314. In B, frequency-dependent block of QX-314 is evaluated at 1 Hz and a train of 20 step depolarization to +80 mV is applied first (as indicated by the number), then followed by trains of 20 step depolarization to −20 mV. The lower trace is obtained after 2 min of rest. The same scheme is applied in D.

SD, $n = 4$), respectively. This result shows that there is a time-dependent block present in the axons with intact h-gate, however, no time-dependent block is apparent in pronase-treated axons (see Fig. 1 C). Similar results were observed with alcohols (Swenson et al., 1978).

The upper traces in Fig. 1 B and D show outward Na currents associated with a train of twenty 8-ms step depolarization to +80 mV, pulsed at 1 Hz from a holding potential of −80 mV. In the presence of 0.4 mM QX-314 the amplitude of Na currents in a normal axon was progressively decreased from the trace labeled 1 until it reached a steady-state level with the trace-labeled 20 (see Fig. 1 B). The onset of block and the final steady-state level of block depend upon the frequency of pulsing, and therefore the block is referred to as frequency-dependent block.

The steady-state block obtained at +80 mV was partially relieved by pulsing to lower potentials. This is illustrated in the lower traces of Fig. 1 B, which represent the Na currents associated with step depolarizations to −20 mV. The amplitude of Na currents recovered from the most inhibited condition (the trace-labeled 1′) to the trace-labeled 20′ after a train of depolarization pulse to −20 mV at 1 Hz. Thus the steady-state level of QX-314 block of Na currents in a normal axon depended upon the magnitude of conditioning potentials. This voltage dependence was evaluated in a more detailed manner in Figs. 2 and 3.

In the pronase-treated axon, no time-dependent block is apparent and the frequency-dependent block almost disappeared as illustrated in Fig. 1 C and D. The pulse wave form used to evaluate the frequency and voltage dependence of QX-314 block was adopted from that used by Hille (1977) as shown in Fig. 2 A, and the pulse sequence is indicated at the top on Fig. 2 B with numbers. In wave form 2, which consisted of a 2-ms pulse to −20 mV and a 6-ms pulse to +80 mV, the former served to

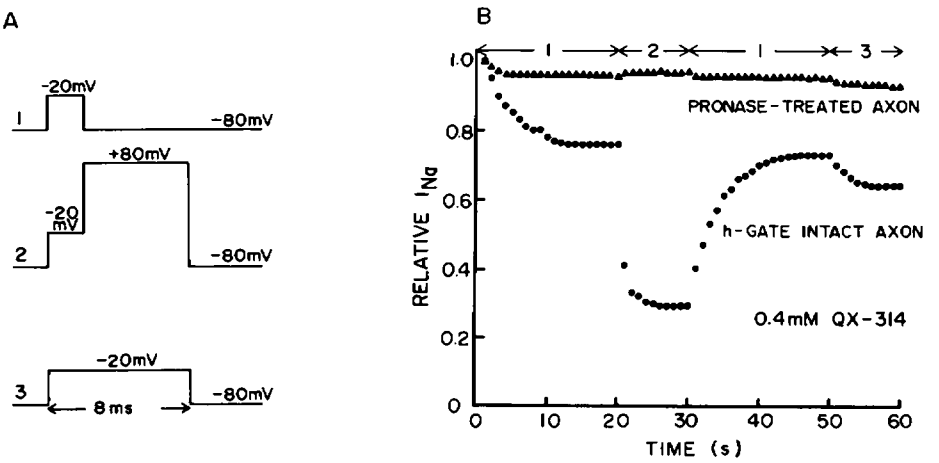

FIGURE 2 Frequency- and voltage-dependent block of Na current by 4×10^{-4} M QX-314 is removed by pronase treatment. The pulse sequence used to evaluate the frequency- and voltage-dependent QX-314 block of Na current is illustrated in A and labeled in B.

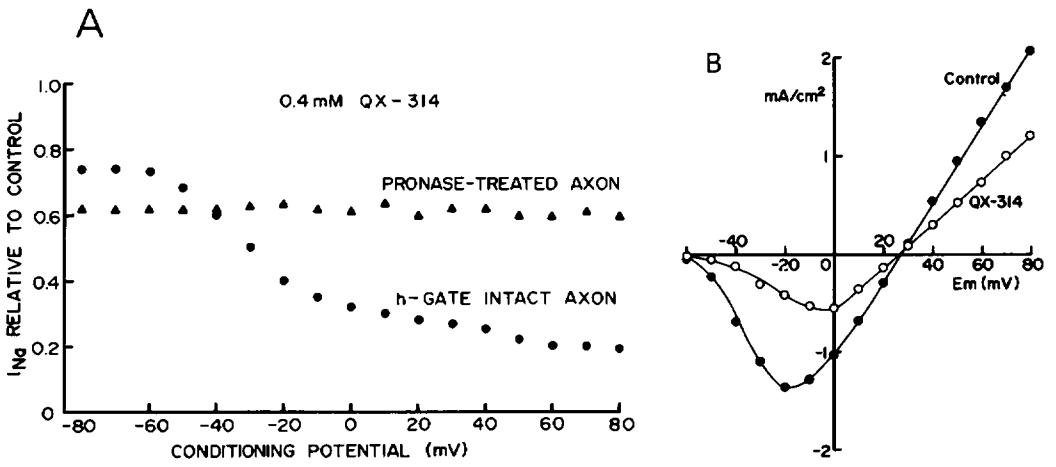

FIGURE 3 The steady-state voltage-dependent block of Na current by QX-314 is removed by pronase treatment. (A) Multiple method as shown in Fig. 2. (B) Single-pulse method. The membrane was depolarized to various magnitudes for a period of 8 ms from the holding potential of −80 mV, and the Na currents were measured at the end of each pulse.

open Na channels and also as a test pulse; the latter served as a conditioning pulse. The peak Na currents associated with the test pulse were measured and then normalized to the currents after 2 min resting. The normalized value is plotted as a function of time in seconds (or in numbers of pulse) as shown in Fig. 2 B. In a normal axon, even the short test pulse induced a gradual decrease in Na current as the pulse was given repetitively (wave form 1). Pulsing to the same potential but with longer duration (wave form 3) was slightly more effective than wave form 1. Pulsing to +80 mV in addition to −20 mV (wave form 2) further enhanced the block. The increase in block with wave form 2 over wave form 3 reflected the voltage-dependent effect. When the Na inactivation mechanism (h-gate) was destroyed by pronase treatment, most of the frequency- and voltage-dependent block disappeared, as clearly shown in Fig. 2 B (upper trace).

The voltage dependence was estimated over a wide range of conditioning potentials by varying the amplitude of second pulse in wave form 2. The steady-state level of Na current was measured, and the degree of block, expressed in terms of fraction of the control, is plotted as a function of membrane potential in Fig. 3 A. The steady-state block of Na currents in normal axons is clearly dependent upon the conditioning potential being more pronounced at more positive potentials. In contrast, Fig. 3 A shows that the block became independent of the conditioning potentials after inactivation had been destroyed by pronase pretreatment. However, upon removal of h-gate, the multiple pulse experiment may not reveal the true voltage dependence of QX-314 block because the drug comes off quickly between pulses. To measure equilibrium block at each potential, Na currents were measured at the end of an 8-ms pulse and plotted as a function of membrane potential as shown in Fig. 3 B. The current-voltage relation shows that the decrease in Na current in the presence of QX-314 is

the same at all membrane potentials more positive than 0 mV. However, the *I-V* curves for QX-314-treated axon is very different from the control one in the region of activation (−60 to 0 mV), being shifted in the depolarized direction.

The effectiveness of QX-314 in blocking Na channels decreased drastically upon removal of h-gate. The steady-state blocks achieved at +80 mV were 76.4 ± 3.38% (mean ± SD, n = 7) and 38.5 ± 4.00% (n = 5) for normal axons and pronase-treated axons, respectively.

The result of the present studies indicates that the effectiveness of QX-314 in blocking Na channels depends upon the state of gating. With the Na inactivation mechanism intact, the effectiveness is enhanced by repetitive pulsing at higher frequencies and more positive potentials. These results support Hille's modulated receptor model which assumes that the affinity of Na channels to drug molecules changes as the channel changes its gating states (Hille, 1977).

It has been shown that pronase treatment destroys the normal Na inactivation mechanism without affecting the activation mechanism (Armstrong et al., 1973; Rojas and Rudy, 1976; Oxford and Yeh, 1977). However, pronase treatment removes both frequency and voltage dependence of QX-314 blockade of Na channels. Similar results were observed by Cahalan and Shapiro with strychnine (1976) and by Almers and Cahalan (1977) with QX-314. The voltage dependence of QX-314 block is likely due to the actual binding reaction between QX-314 and sodium channels being voltage dependent (Strichartz, 1973). The loss of voltage dependence after pronase treatment to remove Na inactivation suggests that Na inactivation may be either directly involved in or indirectly catalyze this voltage-dependent binding reaction. The observations that a time-dependent block of Na channels by long-chain alcohols and QX-314 were seen only in normal axons further support this notion.

Alternatively, it is possible that pronase treatment removes or modifies some of the membrane constituents that are essential for QX-314 binding apart from the one responsible for Na inactivation. Two observations seem to argue against this possibility. First, similar results have been obtained by using other means to remove Na inactivation (Yeh and Wu, 1978). This includes using the protein reagent N-bromoacetamide (Oxford et al., 1976) and the detergent deoxycholate (Wu et al., 1977). Considering the different nature of reagents applied, it is rather unlikely that pronase, N-bromacetamide, and deoxycholate all destroy the same constituent required for voltage-dependent drug binding. Second, the voltage dependence of 9-aminoacridine block of Na channels in squid axon membranes was not affected although its frequency dependence was abolished by removal of Na inactivation (Yeh and Narahashi, 1976). These results support the notion that, in the case of QX-314, the membrane constituent which has been modified by these agents is responsible for normal Na inactivation, and the intactness of Na inactivation mechanism is essential in modulating the frequency as well as voltage-dependent block. In the case of 9-aminoacridine, the macromolecule responsible for Na inactivation plays an important role in modulating the frequency-dependent block only but does not seem to be required for voltage-dependent binding reaction to occur.

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