NA CHANNEL ACTIVATION GATE MODULATES SLOW RECOVERY FROM USE-DEPENDENT BLOCK BY LOCAL ANESTHETICS IN SQUID GIANT AXONS

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ABSTRACT The time course of recovery from use-dependent block of sodium channels caused by local anesthetics was studied in squid axons. In the presence of lidocaine or its quaternary derivatives, QX-222 and QX-314, or 9-aminoacridine (9-AA), recovery from use-dependent block occurred in two phases: a fast phase and a slow phase. Only the fast phase was observed in the presence of benzocaine. The fast phase had a time constant of several milliseconds and resembled recovery from the fast Na inactivation in the absence of drug. Depending on the drug present, the magnitude of the time constant of the slow phase varied (for example at −80 mV): lidocaine, 270 ms; QX-222, 4.4 s; QX-314, 17 s; and 9-AA, 14 s. The two phases differed in the voltage dependence of recovery time constants. When the membrane was hyperpolarized, the recovery time constant for the fast phase was decreased, whereas that for the slow phase was increased for QX-compounds and 9-AA or unchanged for lidocaine. The fast phase is interpreted as representing the unblocked channels recovering from the fast Na inactivation, and the slow phase as representing the bound and blocked channels recovering from the use-dependent block accumulated by repetitive depolarizing pulse. The voltage dependence of time constants for the slow recovery is consistent with the m-gate trapping hypothesis. According to this hypothesis, the drug molecule is trapped by the activation gate (the m-gate) of the channel. The cationic form of drug molecule leaves the channel through the hydrophilic pathway, when the channel is open. However, lidocaine, after losing its proton, may leave the closed channel rapidly through the hydrophobic pathway.

INTRODUCTION

Most local anesthetic agents block sodium current in a manner dependent on the frequency of stimulation, resulting in two phases of block: a resting block and a phasic block. The resting block is obtained when an axon is at rest and the phasic block is obtained when an axon has been stimulated by a train of repetitive depolarizing pulses. The phasic block is often referred to as use-, rate-, or frequency-dependent block. The use-dependent block can be viewed as being composed of two opposing events: the first, which occurs during the depolarizing phase, leads to a net blocking action; the second, which occurs during the interpulse interval (at the holding potential), leads to a net unblocking action. During the depolarizing phase, the Na inactivation is essential for producing the use-dependent block of Na currents (Strichartz, 1973; Hille, 1977; Cahalan, 1978; Yeh, 1978, 1979, 1982; Courtney, 1975). During the interpulse interval, Na currents recover from the use-dependent block slowly with a time constant on the order of hundreds of milliseconds to tens of seconds, depending on the local anesthetic agents present. The nature of this slow recovery from the use-dependent block is not well understood and will be the main focus of the present investigation.

There are two general mechanisms by which the slow recovery from use-dependent block could occur. One assumes that the drug dissociation step is the rate-limiting step for blocked channels to recover (Courtney, 1981). This mechanism implies that the recovery is slow because the rate with which the bound drug could dissociate from the channel is slow. The second mechanism makes the assumption that the channel gating step, rather than the drug dissociation step, is rate limiting: the channel could not unbind drug molecule until the channel opens. According to this mechanism, two different hypotheses can be considered: the inactivation hypothesis and the m-gate trapping hypothesis.

In the inactivation hypothesis, the drug molecule is trapped by inactivation gates. Since recovery from the fast Na inactivation occurs with a time constant of few milliseconds, the fast inactivation gate (Hodgkin and Huxley, 1952) could not serve as a trap of the drug molecule. In addition to the fast Na inactivation, Na channels can enter inactivated states from which recovery is very slow with time constants ranging from hundreds of milliseconds to

several minutes, depending on the duration of depolarizing pulse (Narahashi, 1964; Adelman and Palti, 1969; Chandler and Meves, 1970; Rudy, 1981*a*, *b*). These various types of slow inactivation may be involved in slowing recovery from the use-dependent block (see Khodorov et al., 1976; Hille, 1977; Matsuki et al., 1984). This will be referred to as the slow inactivation hypothesis.

In the m-gate trapping hypothesis, it is suggested that the drug molecule is trapped within the channel by the activation (m) gate, and recovery from the block is determined by the rate with which the drug molecule escapes from the channel. This rate will be determined by the channel opening rate, which is presumably quite slow at the holding potential. This gate-trapping hypothesis was originally suggested by Yeh (1979) to explain the slow recovery of Na channels from 9-aminoacridine (9-AA) block in squid axons and later elaborated by Starmer et al. (1984) to account for the use-dependent block by local anesthetics.

Thus, the two hypotheses make the common prediction that the recovery time course will be slow. However, in the m-gate trapping hypothesis, the recovery time course would be slowed as the membrane is hyperpolarized, because the probability of channel opening is decreased. The slow inactivation hypothesis makes the opposite prediction, because recovery from the slow inactivated state is accelerated by hyperpolarizing the membrane (Rudy, 1981*b*).

This investigation was undertaken in an attempt to differentiate between these two hypotheses by measuring the potential dependency of the time constant for recovery from use-dependent block caused by the presence of quaternary derivatives of lidocaine, QX-222 and QX-314, and another tertiary amine, 9-aminoacridine, which has been shown to produce use-dependent block similar to that produced by QX-314. For the purpose of comparison, benzocaine was also included in this study even though it did not produce a use-dependent block. We found that hyperpolarization slowed the time course of the slow phase of recovery for QX-222, QX-314, and 9-AA. This result is consistent with the m-gate trapping hypothesis, rather than the slow inactivation hypothesis.

METHODS

Experiments were performed on giant axons isolated from squid, *Loligo pealei*, obtained at the Marine Biological Laboratory (Woods Hole, MA). Axons were internally perfused with the roller method (Baker et al., 1961) and voltage clamped with an axial wire electrode assembly. We used two sets of guard electrodes on either side of the axon and air-gaps on either end to improve the space clamp as described by Oxford (1981). The rise time of the clamp was 3 μs (10–90% of step command pulse). Feedback compensation was used in all experiments to compensate for errors arising from approximately two-thirds of the measured 3–4 Ω cm^2 of series resistance. The axons were perfused externally with artificial sea water containing ions in the following concentrations (in millimoles per liter): Na^+, 450; Ca^{2+}, 50; HEPES buffer, 10; Cl^-, 550. The pH of the external solution was adjusted to 8.0 and its osmolarity to 1,000 mOsMol. The internal solution was composed as follows (in millimoles per liter): Na^+, 50; Cs^+, 275; glutamate$^-$, 275; F^-, 50; sucrose, 400; phosphate buffer, 15. It was adjusted to a pH of 7.3 and to an osmolarity of 1,040 mOsMol. Drugs were internally applied to internally perfused axons. Lidocaine, QX-222 and QX-314, were generously supplied by Dr. B. Takmann of Astra Pharmaceutical Products (Framingham, MA). Benzocaine and 9-AA hydrochloride were purchased from K and K Laboratories Inc. (Plainview, NY). All experiments were performed at a temperature of 8.5 ± 0.5°C.

The time course of recovery of Na currents from the use-dependent block was assessed as follows. The computer generated a pulse sequence consisting of a series of conditioning pulses, followed by a recovery pulse and a test pulse. Both the series of conditioning pulses and the test pulse were kept constant: the conditioning pulses used to produce use-dependent block consisted of a train of 15 pulses to +100 mV, each of 8 ms in duration, applied at 4 Hz from a holding potential of −80 mV; a 4-ms test pulse to 0 mV was used to monitor the Na current. To study the voltage dependence of the recovery time constant, the membrane potential between the train of conditioning pulses and the test pulse was varied from −120 to −70 mV. For example, to assess the recovery time course at −100 mV for the time of 1 ms, the computer would generate the first pulse episode consisting of a train of 15 conditioning pulses from −80 to +100 mV applied at 4 Hz, followed by a pulse back to the recovery potential of −100 mV for 1 ms, and followed by a 4-ms test pulse to 0 mV. This whole episode was repeated every 15 s with the recovery time being increased each time until full recovery of the Na current was observed. The advantage of this protocol was that a full recovery from the previous use-dependent block was not required before beginniing the next episode because a maximal degree of block was always achieved by the train of conditioning pulses. Depending on the recovery potential to be studied, the time required for full recovery ranged from 10 to 300 s. The peak Na current for a given recovery time was normalized to the fully recovered peak current, and the normalized values were then plotted as a function of the recovery time.

RESULTS

When a single depolarizing pulse was applied at low frequency (1 per 2 min), benzocaine (1 mM), lidocaine (1 mM), QX-222 (1 mM), QX-314 (0.4 mM), and 9-AA (0.1 mM) all produced a similar degree of block, which amounted to a reduction in the peak Na current of 19–25% (Table I). This was the resting block. When a train of depolarizing pulses was applied, an additional block was observed. The block, which is above and beyond the resting block, is called use-dependent block. These compounds differed markedly in their amount of use-dependent block.

TABLE I
COMPARISON OF RESTING AND USE-DEPENDENT BLOCK CAUSED BY NEUTRAL, TERTIARY, AND QUATERNARY COMPOUNDS

Drugs	Concentration	Resting block	Use-dependent block	*N*
	mM	%	%	
Benzocaine	1.0	21.5 ± 8.5	0	4
Lidocaine	1.0	19.0 ± 3.5	5.6 ± 3.2	5
QX-222	1.0	21.7 ± 2.3	29.0 ± 3.6	3
QX-314	0.4	25.0 ± 6.2	55.0 ± 5.0	4
9-AA	0.1	21.1 ± 3.2	60.6 ± 5.2	5

Use-dependent block was measured following a train of conditioning pulses to +80 mV applied at 1 Hz from a holding potential of −80 mV.

Table I summarized the degree of the use-dependent block following a train of depolarizing pulses to +80 mV applied at the frequency of 1 Hz: 60% for QX-314 and 9-AA, 30% for QX-222, 6% for lidocaine. The use-dependent block could be increased by increasing the frequency of pulsing. For lidocaine, as an example, when the frequency of conditioning pulses was increased to 4 Hz, the degree of use-dependent block was increased to 30%. In the case of benzocaine, no use-dependent block was observed at frequencies up to 30 Hz.

Time Course of Recovery from Use-dependent Block Caused by QX-222

Fig. 1 shows the inward Na currents associated with a test pulse to 0 mV at various recovery times following a train of conditioning pulses (see the protocol at the bottom of Fig. 1). In the absence of drug, Na current increased in amplitude rapidly as the recovery time (the interval between the last conditioning pulse and the test pulse) was lengthened (Fig. 1 *A*). After a 16-ms recovery time at −80 mV, the Na current had returned to the original value seen before applying the conditioning protocol. In the presence of QX-222 (Fig. 1 *B*), the recovery time course exhibited two distinct phases: a fast phase and a slow phase. The fast phase had a time course similar to that observed in the control case, and saturated at the recovery time of 16 ms. The slow phase had a time course on the order of several seconds.

Time Constant of Recovery from the Fast Na Inactivation

The time constant for recovery from the fast Na inactivation was graphically determined as shown in Fig. 2 *A*. A semilogarithmic plot of $(1 - I_t/I_\infty)$, as a function of recovery time followed a straight line, indicating that the recovery from Na inactivation is a single exponential process. The time constants were obtained from the best-fitting line. The values are 3.35, 2.15, and 0.85 ms for the membrane potentials of −80, −100, and −110 mV, respectively. These values correspond to the time constants for recovery from the fast Na inactivation induced by a single depolarizing pulse (Hodgkin and Huxley, 1952). Thus, in the control, the conditioning protocol used in this experiment produces only the fast Na inactivation. Fig. 2 *B* shows that the time constant became smaller as the membrane was hyperpolarized. A 25-mV hyperpolarization produced an *e*-fold decrease in the time constant for recovery from the fast Na inactivation. Thus hyperpolarization of the membrane speeds up recovery from fast Na inactivation.

Time Constants of Recovery from Use-dependent Block

The time constants associated with the two phases of recovery from use-dependent block could be determined by the graphic method, when these two phases differed mark-

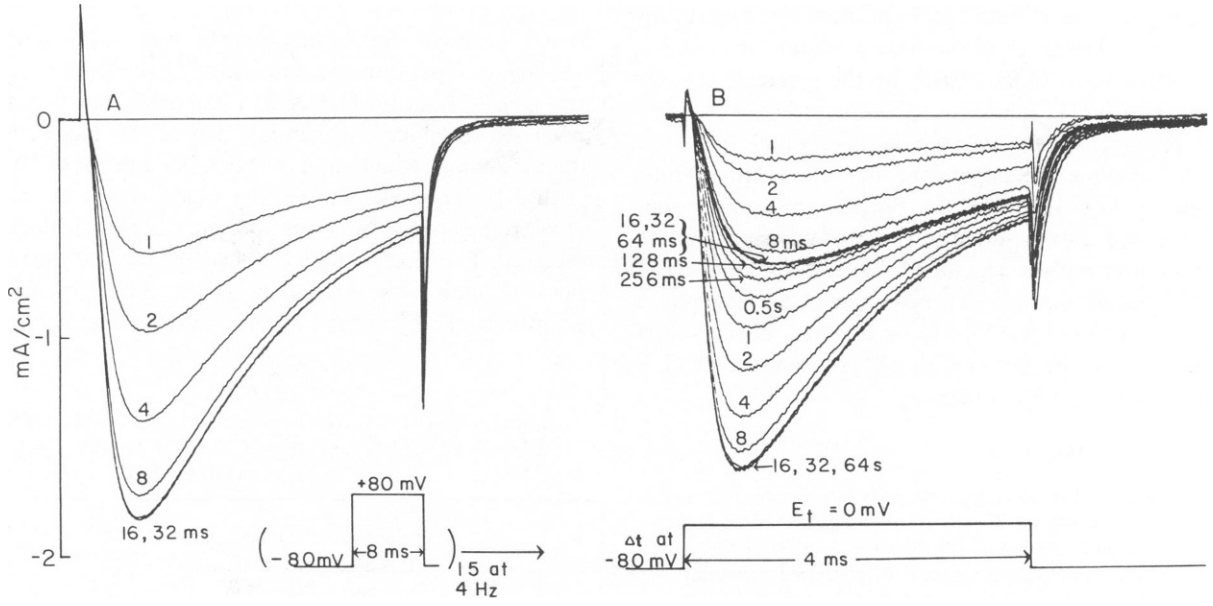

FIGURE 1 Recovery of Na currents from the depolarization-induced Na inactivation in the control (*A*) and from the use-dependent block in the presence of 1 mM QX-222 (*B*). In both cases, the inward currents are associated with a test pulse to 0 mV and the recovery times (at −80 mV) are labeled near each current trace. Inactivation of Na currents was produced by conditioning protocol shown at the bottom of this figure. In the absence of drug, recovery from Na inactivation occurred rapidly, reaching the steady state value at 16 ms (note that the traces for 16 and 32 ms are superimposed on each other; for the clarity of presentation, the traces for longer recovery times were not shown). In the presence of QX-222 internally applied at 1 mM, the recovery from the use-dependent block consisted of two phases. The fast phase, like the control, occurred rapidly and saturated at the recovery time of 16 ms (note that traces for 16, 32, and 64 ms are superimposed on one another). The slow phase, not seen in the control, occurred slowly and saturated at 16 s.

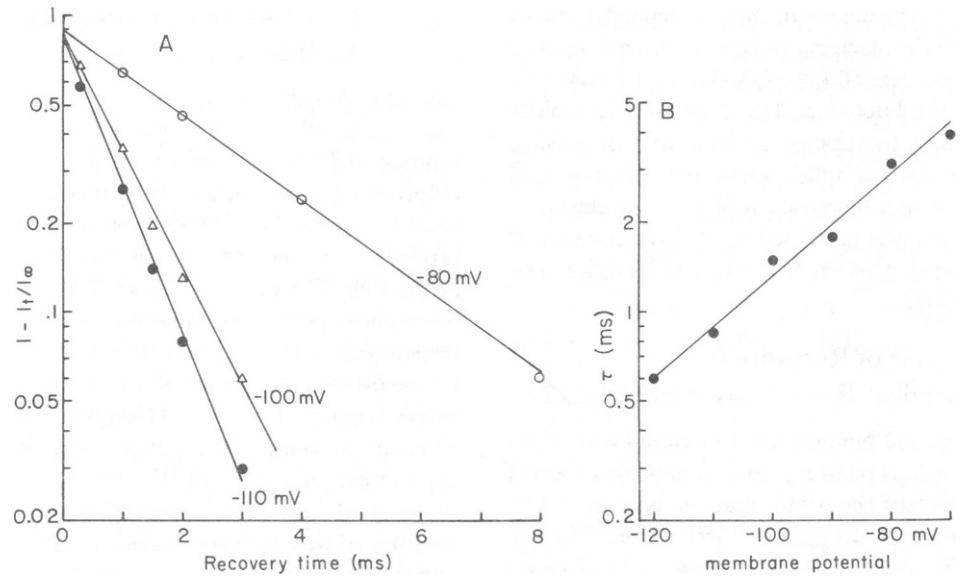

FIGURE 2 Voltage dependence of time constants for recovery from Na inactivation in the absence of drug. Fig. 2 *A* shows the plot of the semilogarithmic value of $(1 - I_t/I\infty)$ as a function of recovery time at −80, −100, and −110 mV. A linear relation suggests that recovery process is a single exponential function. The time constant (τ) was obtained from this linear plot. Fig. 2 *B* shows the voltage dependence of τ. The time constant became smaller as the membrane was hyperpolarized.

edly in their time course. This is illustrated in Fig. 3 for QX-222 at membrane potentials of −80, −100, and −120 mV. For the fast phase (Fig. 3 *A*), the steady state value was chosen at the recovery time of 32 ms and this value was used to normalize the current measured at any given recovery time up to 32 ms. The value, $1 - I_t/I_{32\,ms}$, plotted semilogarithmically as a function of the recovery time gave a straight line, indicating that the fast phase of recovery follows a single exponential time course. The values obtained from this relation are 4.2, 2.0, and 1.55 ms for

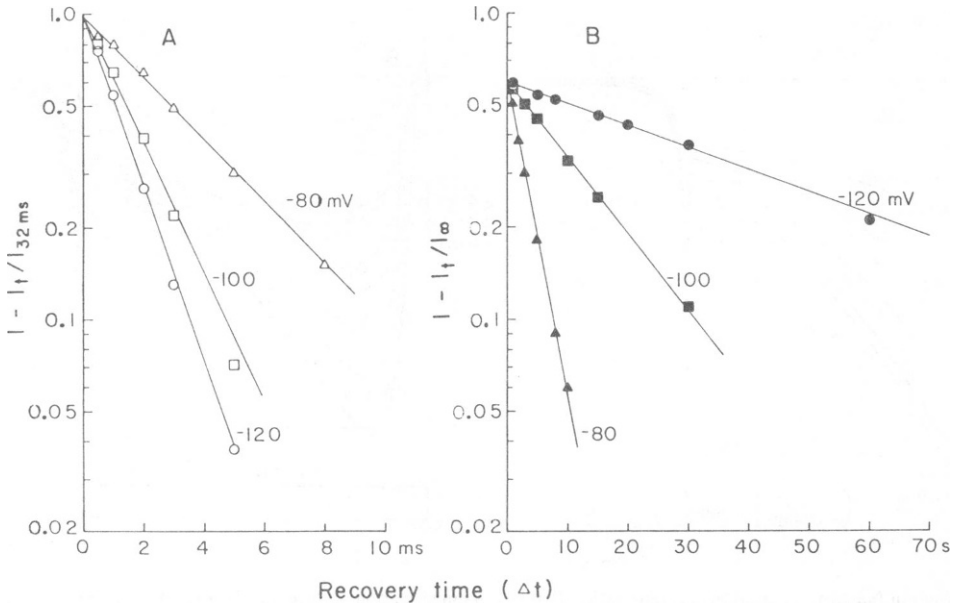

FIGURE 3 Two phases of recovery of Na currents from the use-dependent block in the presence of 1 mM QX-222. For the fast phase (*A*) of recovery, the peak of Na currents (I_t) was normalized to the one following 32 ms recovery period (I_{32}). The value $(1 - I_t/I_{32})$ plotted semilogarithmically as a function of recovery time followed a linear relation, suggesting that the fast phase follows a single exponential time course. The time constants were 4.2, 2.0, and 1.55 ms for recovery obtained at −80, −100, and −120 mV. For the slow phase (*B*), the peak of Na currents (I_t) was normalized to the one following a long period of resting (I_∞). The values, $1 - I_t/I_\infty$, plotted semilogarithmically as a function of recovery time followed a linear relation. The time constants obtained from this relation for membrane potentials of −80, −100, −120 mV were 4.2, 17.5, and 60 s, respectively.

−80, −100, and −120 mV, respectively. Thus the time constants of the fast phase was on the same order of magnitude as that of the Na fast inactivation.

For the slow phase of recovery (Fig. 3 *B*), the value, $1 - I_t/I_\infty$, plotted semilogarithmically as a function of recovery time also gave a straight line, indicating that the slow phase of recovery follows a single exponential time course. The time constants obtained from the linear relation are 4.2, 17.5, and 60 s for −80, −100, and −120 mV, respectively. Thus, the time constant for the slow phase of recovery became larger as the membrane was hyperpolarized.

Differences in the Voltage Dependence of Recovery Time Constants

As illustrated in Fig. 4, the voltage dependence of the time constant for the fast phase differed from that of the slow phase in the presence of QX-222. The time constant for the fast phase decreased as the membrane was hyperpolarized, but saturated at −110 mV (Fig. 4 *A*). In contrast, as shown in Fig. 4 *B*, the time constant for the slow phase became larger as the membrane was hyperpolarized. A 14-mV hyperpolarization produced an *e*-fold increase in the time constant.

Effects of Drug Concentration on Time Course of Recovery

The effects of drug concentration on the time course of the two exponential phases of recovery are illustrated in Fig. 5. As the two time constants differed by three orders of magnitude, the time was expressed in logarithmic scale in the abcissa. The control-normalized data points were fitted using the equation

$$I_t/I_\infty = 1 - A\exp(-t/\tau), \quad (1)$$

where A is the coefficient and τ is the time constant, both of which were determined using linear least-square regression on logarithmically transformed data. In this example, the value of A is 0.88, which represents the inactivating component of the Na current, and τ is 2.13 ms, which corresponds to the time constant of recovery from the fast Na inactivation.

In the presence of QX-222, the data points were fitted to an exponential function of the form

$$I_t/I_\infty = 1 - A\exp(-t/\tau_f) - B\exp(-t/\tau_s), \quad (2)$$

where A and B represent the fraction of the fast and slow phases, respectively, and τ_f and τ_s are the time constants associated with the fast phase and the slow phase of recovery.

Fig. 5 shows the recovery time course in the presence of QX-222 at concentrations of 0.3, 1, 2, and 3 mM. The fractions associated with the two phases of recovery were drug-concentration dependent. As the concentration was increased from 0.3 to 1, to 2, and to 3 mM, the fraction associated with the slow phase was increased from 0.33, to 0.53, to 0.66, and to 0.70, and the fraction associated with the fast phase was decreased proportionally. The change in drug concentration, however, did not significantly affect the time constant for the slow phase of recovery. The time constants were 2.42, 2.0, 2.35, and 2.51 s, respectively, for the concentrations at 0.3, 1, 2, and 3 mM QX-222. On the other hand, QX-222 at concentrations higher than 0.3 mM

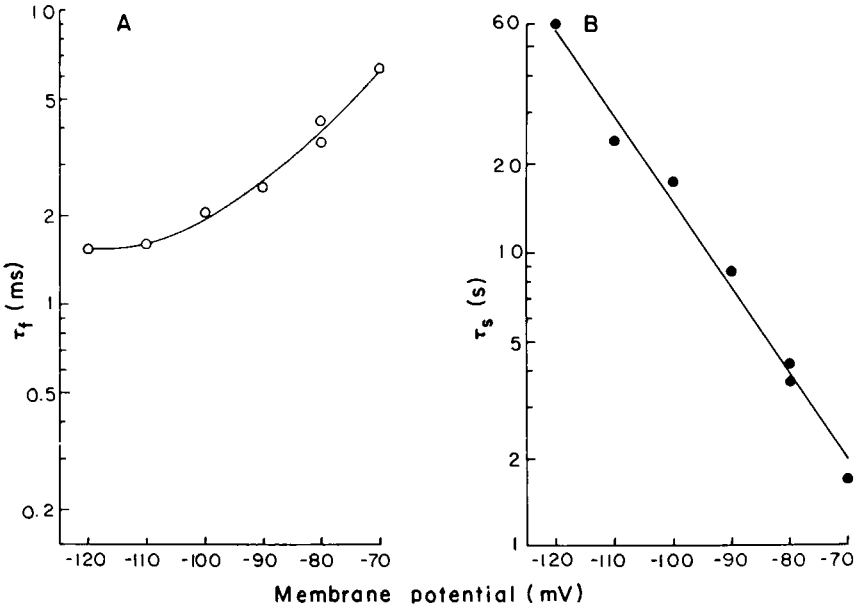

FIGURE 4 The voltage dependence of time constants for two phases of recovery in the presence of 1 mM QX-222. The time constants for the fast phase (τ_f) became smaller as the membrane was hyperpolarized (*A*). The time constants for the slow phase (τ_s) became larger as the membrane was hyperpolarized (*B*).

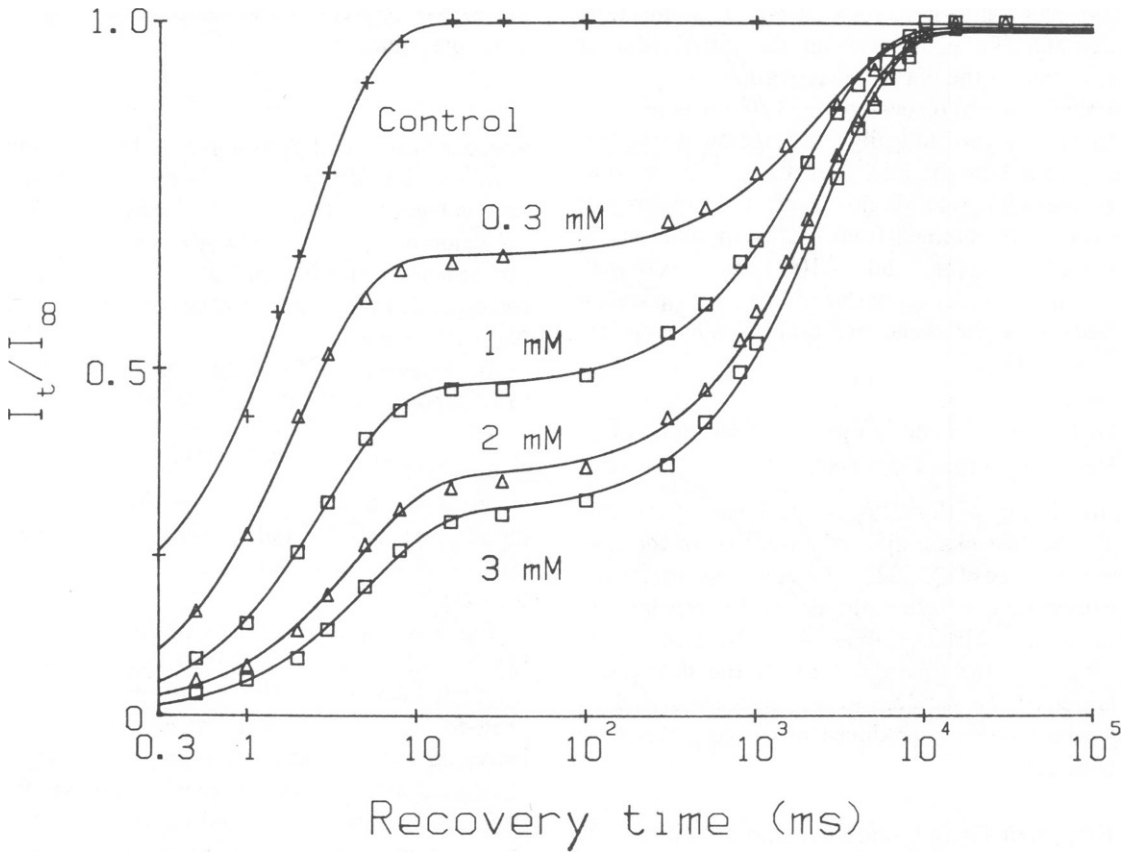

FIGURE 5 Effects of drug concentration on the two phases of recovery from use-dependent block in the presence of QX-222. The time course of recovery was measured at −80 mV in the presence of 0.3, 1, 2, and 3 mM QX-222. The two phases of recovery were fitted to the double exponential function (Eq. 2). Increasing drug concentration increases the fraction associated with the slow phase of recovery: 33, 53, 66, and 70% for the concentrations at 0.3, 1, 2, and 3 mM, respectively. The time constants calculated for the slow phase were not significantly affected by changes in drug concentration: 2.4, 2.0, 2.35, and 2.5 s at 0.3, 1, 2, and 3 mM. The time constants for the fast phase were increased at the drug concentrations higher than 0.3 mM: 2.13, 1.96, 2.88, 4.34, and 5.17 ms for 0, 0.3, 1, 2, and 3 mM QX-222.

increased the recovery time constant for the fast phase. The time constants were: 2.13, 1.96, 2.88, 4.34, and 5.17 ms for 0, 0.3, 1, 2, and 3 mM QX-222, respectively.

Comparison of Time Constants of Recovery among Local Anesthetics and 9-AA

Fig. 6 compares the time course of recovery from use-dependent block in the presence of benzocaine, lidocaine, QX-222, and QX-314. In the presence of benzocaine, only the fast phase of recovery was observed, and it still followed a single exponential time course, as seen in the control. As shown with QX-222, two phases of recovery from the use-dependent block of Na currents were observed in the presence of lidocaine, QX-314, or 9-AA (not shown in this figure).

Regardless of local anesthetics present, the time constant for the fast phase of recovery was similar, and was consistently larger than that for the control (see Table II). For example, at −80 mV, the time constant was 3.30 ± 1.10 ms (mean ±SD, n = 11) for the control, and 3.90 ± 0.60 ms (mean ±SD, n = 4) for that during the QX-222 application. In contrast to the fast phase, the slow phase differed among these four drugs in three respects. First, the fraction associated with the slow phase depended on the drug present: 0.45, 0.64, and 0.67 for 2 mM lidocaine, 2 mM QX-222, and 0.4 mM QX-314, respectively. Since the slow fraction is interpreted to represent the degree of use-dependent block, QX-314 is the most effective among these agents in producing a use-dependent block. Second, the time constant varied depending on the chemical structure of drugs (Table II). The time constants of the slow phase of recovery shown in Fig. 6 were estimated to be (at −80 mV): 0.14, 2.35, and 16.0 s for lidocaine, QX-222, and QX-314, respectively. Third, QX-222, QX-314, and 9-AA showd a similar voltage dependence of recovery time constant, whereas the tertiary amine lidocaine showed no voltage dependence (Fig. 7). As seen in the presence of QX-222, increasing drug concentrations led to an increase in the fraction associated with the slow phase, but had no effect on its time constant or its voltage dependence.

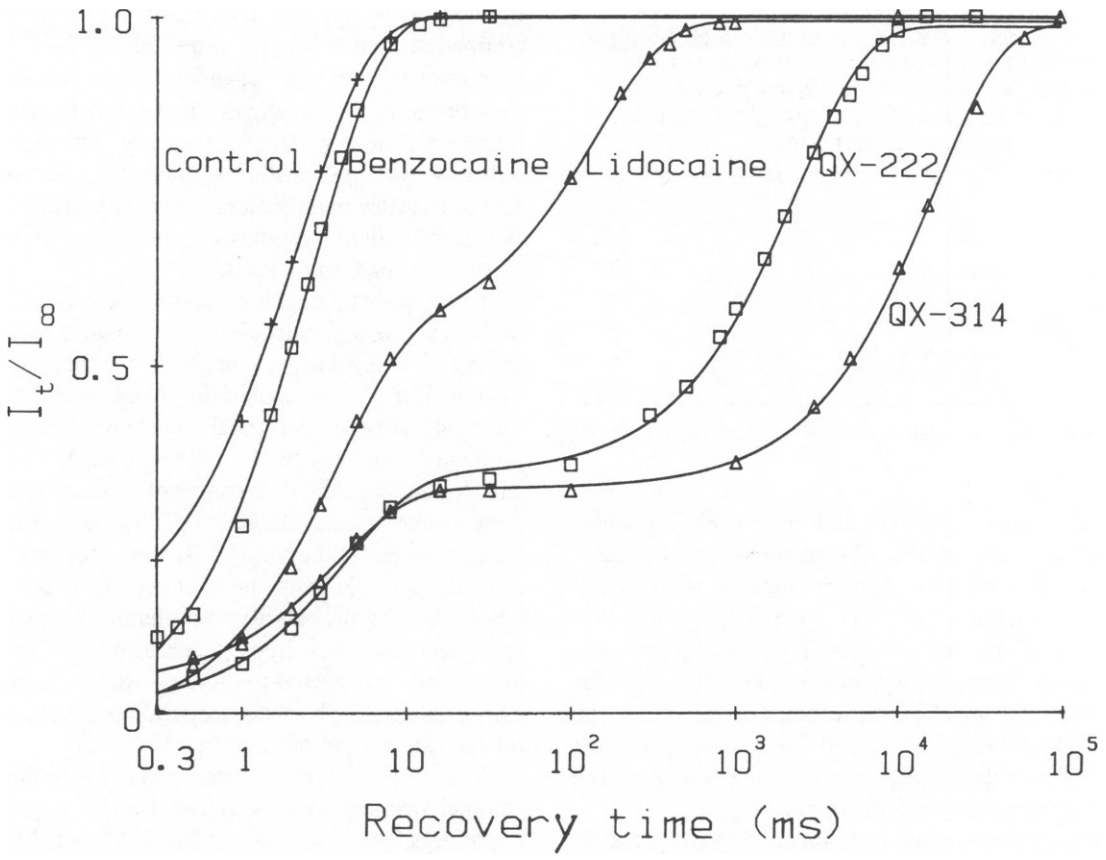

FIGURE 6 The time course of recovery from the inactivation of Na currents in the control or from use-dependent block in the presence of local anesthetics. The peaks of inward Na currents associated with a test pulse to 0 mV following various recovery times (I_t), as shown in Fig. 1, were normalized to the one following a long resting period (I_∞). The normalized values, I_t/I_∞, were plotted logarithmically as a function of recovery time, t, in log scale. The control data were the mean of eight experiments and were fitted to a single exponential function (Eq. 1) with a time constant of 2.17 ms and an A of 0.88. In the presence of 1 mM benzocaine, the recovery time course remained a single exponential with a time constant of 3.05 ms. In the presence of 2 mM lidocaine, 1 mM QX-222 or 0.4 mM QX-314, the data were fitted to two exponential functions (Eq. 2). The time constants for the fast phase were: 3.6 ms for lidocaine, 3.14 ms for QX-222, and 2.95 ms for QX-222. The time constants for the slow phase were: 0.14 s for lidocaine, 2.80 s for QX-222, and 15.80 s for QX-314. The fraction associated with the slow phase were: 42% for lidocaine, 58% for QX-222, and 67% for QX-314.

DISCUSSION

The present study demonstrated that the time course of recovery of Na channels from use-dependent block in the presence of charged local anesthetics exhibited two phases: a fast phase and a slow phase. The fast phase had a time constant on the order of several milliseconds, whereas the slow phase had a time constant two to three orders of magnitude larger than that of the fast phase, ranging from several hundreds of milliseconds to several tens of seconds, depending on the local anesthetic present. In addition, these two phases differed in the voltage dependence of recovery time constants. When the membrane was hyperpolarized, the time constant for the fast phase was decreased, whereas the time constant for the slow phase was increased. In the presence of neutral benzocaine, only the fast phase was observed.

Since the time course for the fast phase of recovery from the use-dependent block was similar to that observed in the control, we interpret the fast phase of recovery to represent unblocked channels recovering from the fast Na inactivation. However, the magnitude and voltage dependence of the fast recovery time constant differed from those for the fast Na inactivation. This suggests that local anesthetic agents might affect the fast Na inactivation of the unblocked Na channels. This action does not depend on the charge of the molecule, since it was observed in the presence of both the neutral molecule benzocaine and the charged molecules QX-222 and QX-314. The slow phase of recovery is interpreted to represent the recoverey of bound and blocked channels, during the interpulse interval, from the use-dependent block accumulated by repetitive pulsing. Two possible mechanisms by which this slow recovery could occur were examined here: one involves Na inactivation and the other involves the m-gate trapping mechanism.

The role of Na inactivation in producing use-dependent block was first suggested by Strichartz (1973) and later

TABLE II
TIME CONSTANTS OF RECOVERY FROM DEPOLARIZATION-INDUCED NA INACTIVATION IN THE ABSENCE OF DRUG OR FROM USE-DEPENDENT BLOCK IN PRESENCE OF LOCAL ANESTHETICS

Drugs	τ_f	τ_s	N
Control	3.30 ± 1.10	—	11
Benzocaine	3.38 ± 0.36	—	5
Lidocaine	3.40 ± 0.90	0.29 ± 0.09	4
QX-222	3.90 ± 0.60	3.70 ± 0.30	4
QX-314	3.80 ± 0.50	17.0 ± 5.0	3
9-AA	4.60 ± 1.04	13.8 ± 2.6	5

The time constants were estimated from time courses of recovery at −80 mV.

elaborated by Courtney (1975) and Hille (1977). Consistent with this view is the observation that the use-dependent block disappears upon removal of the fast Na inactivation (Cahalan, 1978; Yeh, 1978, 1979). However, the magnitude of the time constant for recovery from the fast Na inactivation is 1,000 times smaller than that for recovery from the use-dependent block observed in the presence of QX-314, QX-222, and 9-AA. Thus, the fast Na inactivation in its simplest form could not explain the slow recovery from the use-dependent block.

The Na inactivation could be influenced by the presence of a local anesthetic agent, which stabilizes the Na channel in an inactivated state from which recovery could be slow. In terms of the Hodgkin-Huxley equation for the Na inactivation, this slow recovery could be explained by a decrease of the rate constant α_h by the drug (Hille, 1977). The extent of slowing in the time constant of recovery would reflect the degree of decrease in α_h, which would depend on the local anesthetics present. In terms of the multicomponent process for slow Na inactivation (Khodorov et al., 1976), the slow inactivation is coupled to the fast one in such a way that only the inactivated Na channels can be converted to the slow inactive state. This model assumes that this transition to the slow inactivated state is a direct result of the drug binding to the fast inactivated state, and that the recovery from the slow inactivated state is slow. In this case, the extent of slowing in the time constant of the recovery process would reflect the degree of decrease in α_s, which varies depending on the local anesthetic present. Thus, these two modified inactivation hypotheses could explain vastly different time constants of the slow recovery from a use-dependent block.

However, the inactivation hypothesis, either in its simplest form or in modified forms, could not explain the voltage dependence of the time constants. Chandler and Meves (1970) and Rudy (1978; 1981*a*, *b*) found that the time constant of recovery from the slow Na inactivation is very potential dependent. Hyperpolarizing the membrane from −60 to −100 mV accelerates the time course of recovery. In other words, the time constants decrease as the membrane is hyperpolarized. In the presence of QX-compounds and 9-AA, the time constant of the slow phase of recovery from use-dependent block depended on the membrane potential. Fig. 7 shows that hyperpolarization increases time constants of recovery. This voltage dependence of the time constant is opposite to that of either the fast or the slow inactivation. This result argues against the Na inactivation hypothesis, but is consistent with the m-gate trapping hypothesis.

In the m-gate trapping hypothesis, it is assumed that a quaternary drug molecule that is bound to the channel during the depolarizing phase is trapped in the closed channel during the repolarizing phase. The trapped molecule could escape from the channel only when the channel is open (through the hydrophilic pathway). This hypothesis can explain the slow time course of recovery and its voltage dependence. Since channel opening is a rare event at holding potentials between −70 and −120 mV, the escape of drug molecule from the channel would be slow. Moreover, since the probability of a channel having its m-gate open decreases with hyperpolarization, the time constant of recovery is expected to increase with hyperpolarization. This prediction is borne out by the results shown in Fig. 7 for the QX compounds and 9-AA.

If the recovery process were strictly rate limited by the channel opening as is required by the m-gate trapping hypothesis, one would expect the following. First, all local anesthetics should exhibit the same voltage dependence of recovery. This voltage dependence should be identical to that of the activation process. Second, the recovery time constant should be the same for all local anesthetic agents. Consistent with the first expectation is the observation that QX-314, QX-222, and 9-AA all show a similar voltage dependence. Fig. 7 shows that a 14-mV hyperpolarization in the membrane potential produces an *e*-fold increase in the time constant. However, the voltage dependence of the activation process estimated from the conductance-voltage relationship gives an *e*-fold increase in Na conductance per 5–6 mV increase in membrane potentials between −60 and −40 mV (Hodgkin and Huxley, 1952; Oxford, 1981). The voltage dependence for the conductance change is much steeper than that for the time constant of recovery from use-dependent block. The disparities in the voltage dependence could arise in several ways. First, the measurement of the voltage dependence of the activation and that of the slow recovery process was performed in two different membrane potential ranges. Second, the drug-bound channel might differ from the normal channel in the voltage dependence of activation. That is, the activation of the drug-bound Na channel might be less sensitive to a voltage change. Third, whereas the drug-blocked channels may undergo transitions similar to those of the control, the trapped drug molecule can dissociate from the channel before channel opening. Finally, the rate constant governing the dissociation of a drug molecule from a site within the channel could contribute to a slight decrease in

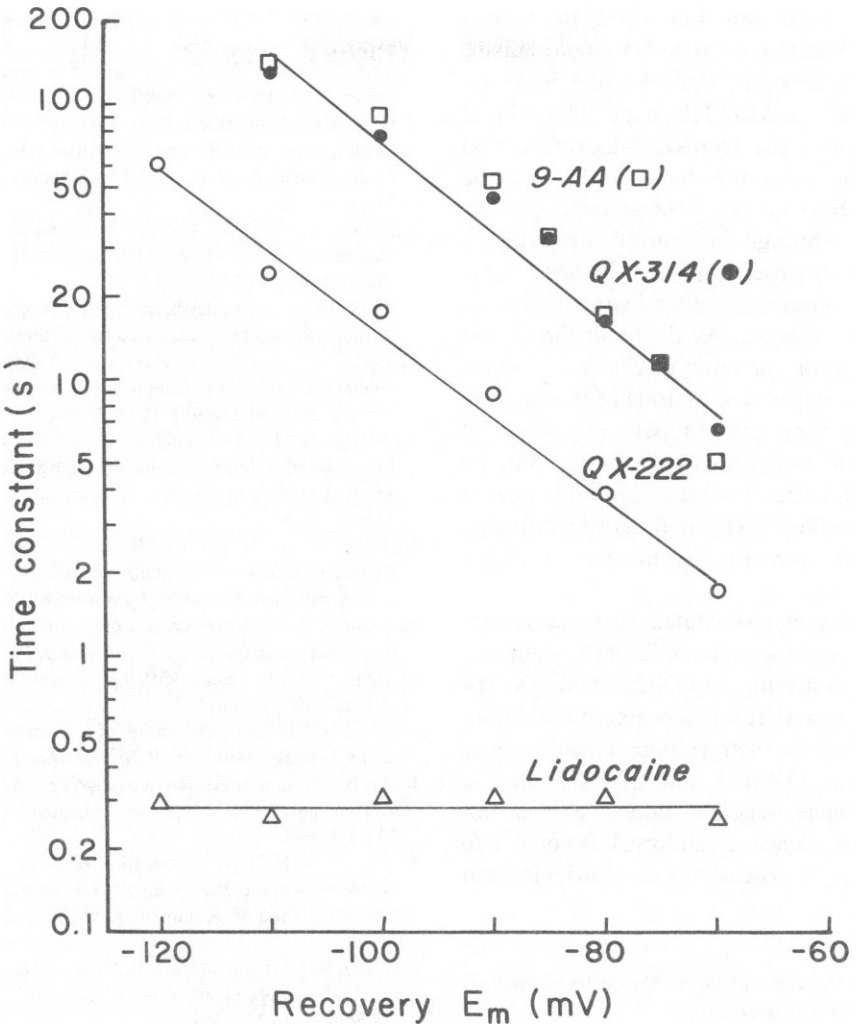

FIGURE 7 Time constant of the slow phase of recovery from use-dependent block depend on local anesthetics present and on membrane potential. All drugs were applied internally to the axon at the concentrations of 0.4 mM for QX-314, 1 mM for QX-222, 1 mM for lidocaine, and 0.1 mM for 9-AA. The voltage dependence of the slow recovery time constant for QX-314, QX-222, and 9-AA showed an e-fold increase per 14-mV hyperpolarization. No voltage dependence was observed for lidocaine.

voltage dependence of the recovery time constant, because the voltage dependence of drug unbinding is opposite to that of channel opening (Cahalan, 1978; Yamamoto and Yeh, 1984). Lidocaine did not exhibit any voltage dependence of its recovery time constant. This will be discussed in detail later.

As to the magnitude of the recovery time constant, note that lidocaine and its derivatives differ markedly in their recovery time constants. The recovery from the QX-314 block is 4–5 times slower than that block from QX-222, which, in turn, is 12 times slower than that from the lidocaine block (Fig. 7). This difference between the QX-314 and QX-222 recovery time constants may result in part from the difference in molecular weight among these compounds because the smaller molecule is thought to escape from the channel faster (Courtney, 1980).

For tertiary amine drugs, the m-gate trapping hypothesis has to be modified to include the alternative pathway for the drug molecule to leave the channel, that is to include the hydrophobic pathway by which the neutral molecule could escape from the channel. According to this view, the cationic form of a tertiary amine local anesthetic is trapped in the closed channel until the channel opens or it loses its proton. The overall rate at which the trapped molecule could escape from the channel would depend on the lifetime of the cationic species and on the partition coefficient of the neutral molecule.

The lifetime of a cationic molecule is determined by its deprotonation rate constant, which can be estimated from the pK_a and the protonation rate constant. For lidocaine, if one uses the protonation rate constant of $5 \times 10^8\ M^{-1}s^{-1}$ as used by Schwarz et al. (1978) and if one assumes that the pK_a value of the bound molecule is the same as that measured in the solution, then the lifetime of the cationic species would be 125 ms for a pK_a of 7.8 and 250 ms for a pK_a of 8.1. Similar calculation shows that the lifetime of

9-AA cation (with pK_a of 10) would be ~20 s. Because of this long lifetime of its cationic form, 9-AA would behave as a quaternary amine compound. For lidocaine, however, the cationic species is short lived and the neutral form has a large partition coefficient, the trapped lidocaine cation itself needs not escape from the channel through the hydrophilic pathway. Instead, the neutral molecule can escape from the channel through the hydrophobic pathway as soon as the cationic form loses its proton. Thus, the time constant of recovery becomes rate limited by the lifetime of the cationic form in the channel. As shown in Fig. 7, the recovery time constant is on the order of 270 ms, which is within the ranges of the calculated lifetime of the cationic species. Moreover, the time constant was not potential dependent. This may be explained by assuming that the protonation and deprotonation processes are only weakly voltage dependent (Woodhull, 1973) and that the diffusion of the neutral molecule through the membrane is not potential dependent.

The present study has demonstrated that quaternary derivatives in many respects behave differently from tertiary amines. The time constants for QX-222 and QX-314 are 10–40 times as large as that for lidocaine. In addition, we found that they differ in their voltage dependence of recovery time constants: QX-314 and QX-222 show a strong voltage dependence, whereas lidocaine does not show such a voltage dependence. Therefore, it is not safe to assume that a quaternary derivative is just a cationic form of a tertiary amine.

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