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MECHANISM OF FREQUENCY-DEPENDENT INHIBITION OF SODIUM CURRENTS IN FROG MYELINATED NERVE BY THE LIDOCAINE DERIVATIVE GEA 968[1]

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ABSTRACT

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A new lidocaine derivative (Astra, GEA 968) depresses excitability of myelinated frog nerve in a manner which depends upon the rate of use of the nerve. This phenomenon has been shown, under voltage clamp conditions, to involve "frequency-" or "use-dependent" inhibition of the transient inward sodium currents at the node of Ranvier. With 0.6 mM GEA 968 in the solution bathing the node, the inward sodium currents produced by 5-msec depolarizing pulses to −20 mV are reduced to 40% of control values *if* the node is rested for a few hundred seconds prior to the test pulse. Repetitive opening of the sodium channels by depolarizing pulses enhances this inhibition, for example, currents are eventually reduced to 10 to 20% of control with repetitive depolarization at 2 sec^{-1}. If the preparation is then allowed to rest, this use-dependent increment in inhibition gradually declines with a time constant of about 10 seconds. Repetitive opening of the sodium channels by depolarizing pulses preceded by large hyperpolarizing prepulses reverses the inhibition caused by application of depolarizing pulses alone. It is hypothesized that the GEA 968 molecule binds to open sodium channels and, in doing so, simultaneously blocks the channel and shifts the curve relating sodium inactivation to membrane potential by 20 to 40 mV in the hyperpolarizing direction. Several kinds of evidence supporting this molecular hypothesis are presented. Lidocaine, procaine, procaine amide and a quaternary lidocaine derivative QX-314 also cause use-dependent depression of sodium currents in this preparation. This common mode of action of tertiary and quaternary anesthetics implies that the cationic form of tertiary anesthetics is active.

This paper concerns the mechanism by which local anesthetics block excitability in myelinated nerve. This work extends the studies of Strichartz (1973) on the interaction of a quaternary lidocaine derivative, QX-314, with sodium channels in frog myelinated nerve. He showed, using voltage clamp techniques, that the influence of this drug is remarkably dependent upon the previous history of use of sodium channels. In particular, inhibition of sodium currents is enhanced by membrane depolarizations of suffi-

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cient magnitude to "open" sodium channels. This phenomenon will be referred to as "frequency-dependent" or "use-dependent" inhibition of the sodium conductance mechanism since the rate of use of the sodium conductance mechanism is a powerful determinant of the blocking action of this quaternarized local anesthetic. It is important to know if tertiary local anesthetics affect the sodium mechanism in a similar way to their quaternary analogs thus implying that quaternary and tertiary anesthetics share the same membrane receptor. Evidence presented in this paper shows that this is, in fact, the case.

More specifically this paper attempts to provide an adequate explanation of the stabilizing action of a new local anesthetic, GEA 968 (Astra Pharmaceutical Products, Inc., Worcester, Mass.). Dr. Helen Vassallo called our attention to this compound as an agent that blocked propagation of trains of action potentials far more effectively than it blocked propagation of single impulses. This tertiary drug is also found to give a use-dependent block of the sodium channels responsible for excitation in frog myelinated nerves. A specific molecular model for this use-dependent block of the sodium channel is suggested by evidence given in this paper. It is hypothesized that the GEA 968 molecule binds to open sodium channels and, in doing so, blocks the channel and shifts the curve relating sodium inactivation to membrane potential 20 to 40 mV in the hyperpolarizing direction. Since a similar sodium conductance mechanism may be involved in cardiac excitation (Trautwein, 1963), these studies may prove useful to the understanding of the antiarrhythmic effectiveness of local anesthetics in cardiac tissue as well.

Methods

Single myelinated nerve fibers were dissected from the sciatic nerve of the frog *Rana pipiens*. The voltage clamp technique used was that of Dodge and Frankenhaeuser (1958) with modifications described by Hille (1971a,b). Briefly, currents carried across the membrane by sodium ions can be measured as the membrane potential of one node of Ranvier is forced to follow a predetermined time course by a feedback amplifier. A depolarizing test pulse from rest to about −20 mV triggers a transient increase in sodium permeability which is monitored as a transient inward ionic current. Between test pulses, the node is held at a potential of approximately −80 mV. This potential is near the normal resting potential and is also the potential at which about 40% of the sodium conductance system is inactivated.

Drugs applied to the node were dissolved in stock Ringer's solution containing 103 mM NaCl, 1.8 mM $CaCl_2$, 2.2 mM KCl, 7.2 mM tetraethylammonium bromide, and 9.6 mM tris(hydroxymethyl)amino methane (Tris). The node illustrated in figure 4, an early experiment, was bathed in Ringer's solution buffered with 1 mM Tris at pH 6.8 rather than 9.6 mM Tris at pH 7.6 as used in all other reported experiments. The tetraethylammonium bromide ion blocks K^+ channels when applied to the outside of the node of Ranvier in frog myelinated nerve (Hille, 1967a,b). The 7-mM concentration of tetraethylammonium bromide used blocked 95% of the potassium currents (K_i = 0.4 mM), and leakage currents were electronically subtracted (Armstrong and Hille, 1972) so that the measured ionic currents consisted primarily of currents in sodium channels. After drugs were applied, approximately 3 to 5 minutes elapsed before new measurements were begun. Early experiments showed that the effects of GEA 968, at pH 7.6, develop within 50 to 100 seconds after application to outside the node of Ranvier.

A sample of GEA 968, a new experimental local anesthetic and potential antiarrhythmic agent, was obtained from Astra Pharmaceutical Products for studies of its effect on excitability mechanisms in nerve. This compound (fig. 1) differs from lidocaine only by the inclusion of one glycyl amino acid residue in peptide linkage between the carbonyl and anilino nitrogen of lidocaine. The pK_a is 7.7 compared with 7.9 for lidocaine, and the cod liver oil water partition coefficient is 1.3.

The computer simulations depicted in figure 8 were performed on a Raytheon 440 computer operating in a Digital-Analog Simulation mode.

Results

With GEA 968 in the solution bathing the voltage clamped node of Ranvier, inward sodium currents produced by 5-msec depolarizing pulses are depressed below control values. If care is taken to rest the node for a few hundred seconds prior to each test depolarization, then the concentration dependence of the depression can be determined. Under these conditions, peak sodium currents are reduced to 50% of control with a 0.4 mM GEA concentration. If depolarizations are applied at a more rapid rate, then the GEA block is enhanced as described below. A study of this rate-dependent enhancement of the sodium conductance block is the focus of this paper since an understanding of

this phenomenon may help explain the anti-arrhythmic effectiveness of local anesthetics.

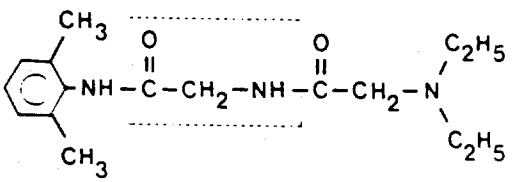
Use-dependent inhibition under voltage clamp. Strichartz (1973) found that repetitive use of sodium channels by short depolarizing voltage pulses progressively reduced the size of sodium currents in axons treated with quaternary lidocaine inside. A similar effect of use is found with tertiary GEA 968 applied outside and is shown in figure 2. Here sodium currents under voltage clamp are represented conventionally as downward going traces that reach a peak a fraction of a millisecond after the depolarizing step starts and then decay exponentially (inactivate) in the next several milliseconds. The peak value of the inward sodium current is a measure of the peak sodium conductance that is available on that test pulse. In this experiment the node was rested for several minutes and then depolarized at a rate of 2 sec^{-1}. Repetitive depolarization increased the inhibition of I_{Na} (sodium current), gradually decreasing peak I_{Na} until after 25 pulses only 24% of that observed after a rest remained. If peak current is plotted against time within the pulse train, then the block is observed to accumulate exponentially with time constant 0.50 sec^{-1} in this experiment. In the absence of drug, repetitive pulses at rates up to 10 sec^{-1} did not cause any progressive loss of sodium currents.

If the preparation is allowed to rest after receiving a train of depolarizations, the accumulated increment in inhibition gradually declines again. In three nodes exposed to 0.6 mM GEA, the time constant for this recovery at rest measured 12, 14 and 10 seconds; two experiments at 1.8 mM GEA gave recovery time constants between 5 and 6 seconds.

Currents that flow through the sodium channel during a depolarizing pulse (to E) can be related to a time varying conductance change g_{Na} and the reversal potential (E_{Na}) for ions flowing through the channel according to Ohm's law:

$$I_{Na} = g_{Na}(E-E_{Na}) \qquad (1)$$

In four experiments, the reversal potential for sodium currents changed from 30 ± 4 mV (mean ± S.E.) before drug treatment to 39 ± 8 mV during drug treatment. Since no significant reduction in the reversal potential was found



GEA 968

FIG. 1. Relationship of the new local anesthetic, GEA 968, to lidocaine is indicated by insertion of glycyl amino acid residue (dotted enclosure) into the lidocaine structure. GEA 968 was synthesized by Ulf Lindberg at Astra Pharmaceutical Products, Södertälje, Sweden.

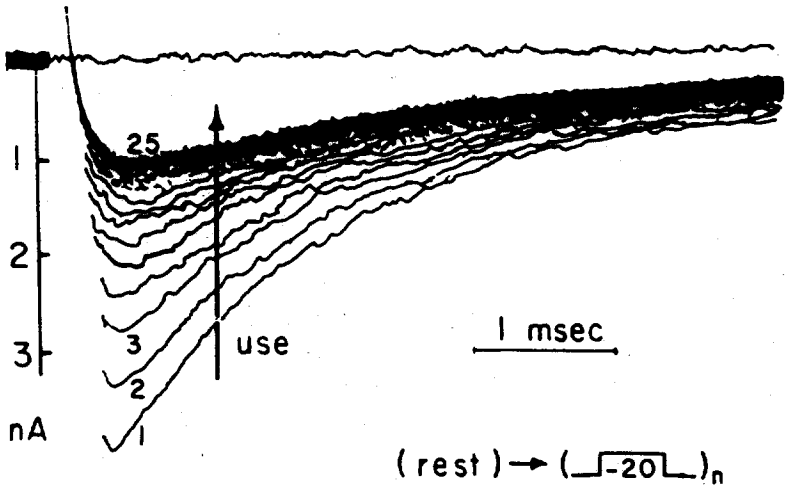


FIG. 2. Sodium currents are diminished by repetitive depolarizations. Node rested then depolarized for 5 msec at rate of 2 sec^{-1} causing gradual reductions in pictured sodium currents with use. GEA 968 applied to pool bathing node at a concentration of 0.5 mM, node 4/8.

with drug treatment, the large reductions in current can be attributed to decreases in sodium conductance.

Use-dependent removal of inhibition. When a voltage-clamped node of Ranvier from frog myelinated nerve is exposed to solutions containing GEA 968, inhibition of sodium conductance develops. A certain amount of inhibition is observed if a long interval elapses between test depolarizations; more inhibition develops if the sodium channels are repetitively activated. If 5-msec depolarizing pulses are applied repetitively until currents are strongly inhibited, and then a large hyperpolarizing prepulse of 50-msec duration is applied before each subsequent test pulse, the currents will grow again for several successive depolarizations (fig. 3). When the response with prepulse has stabilized after 10 to 15 prepulse/pulse sequences, the sodium conductance is generally greater than after a long period of rest, although it is still less than in the absence of the drug. This modulation of sodium conductance is still observed even when taking into account changes in conductance due to prepulse potential (normal sodium inactivation—see below). Thus, use of the sodium channels with depolarizing pulses alone reduces conductance to levels below that available at rest whereas use of the channels with large hyperpolarizing prepulses preceding the pulses increases conductance to levels above that available at rest.

Does GEA 968 alter sodium inactivation? Weidmann (1955), working with mammalian Purkinje fibers, observed a loss of sodium conductance with cocaine treatment that was restored by hyperpolarizing prepulses; he interpreted this as a cocaine-induced shift in the voltage dependence of the sodium inactivation process in the hyperpolarizing direction. Later voltage-clamp studies on giant squid axons failed to find such a shift of sodium inactivation with procaine treatment (Taylor, 1959). However, depending on measuring procedures, GEA 968 does or does not show an apparent inactivation shift. Figure 4 shows sodium inactivation measured in the conventional manner before drug treatment and inactivation curves measured in two ways on the same node during GEA 968 treatment. Because sodium currents depend on the history of use during drug treatment, the conditioning sequences of prepulse/pulse indicated in figure 4 are applied until the current levels are stable ($I_{p\,max}$) before each measurement for more depolarizing prepulse levels. During drug treatment, two very different inactivation curves are obtained by comparing peak current values on the first and last pulse at the new prepulse level to that on the last conditioning pulse. The curve labeled "first pulse" (●)

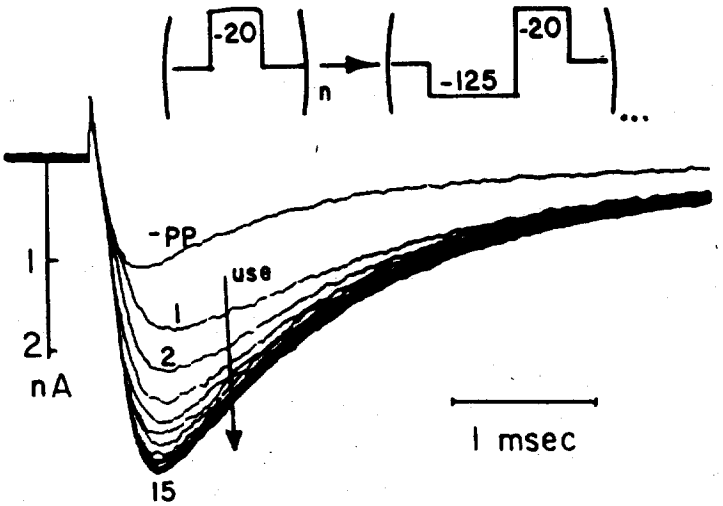


FIG. 3. Use-dependent removal of inhibition caused by adding large hyperpolarizing prepulses. Nodal sodium currents inhibited with about 20 pulses to −20 mV at 1 sec⁻¹, then 50-msec prepulse to −125 mV coupled with each depolarizing pulse causing use-dependent increases in sodium currents. The final trace without prepulse (−PP) is shown and currents for prepulse plus pulse are numbered in sequential order. Node 3/27B, 0.9 mM GEA.

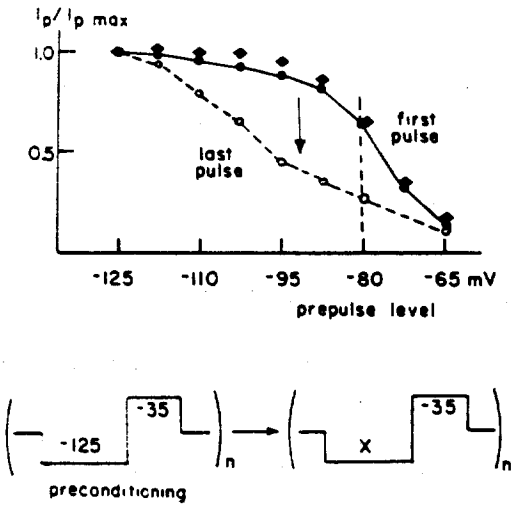


FIG. 4. Prepulse dependence of sodium currents before and during exposure to GEA 968. Predrug inactivation values (◆) measured by comparing peak sodium currents with varying prepulse to the maximum current obtained with prepulse to −125 mV and plotting $I_p/I_{p\ max}$. During drug exposure, a conditioning prepulse/pulse sequence is applied until currents are stable before stepping to new prepulse level. Peak current on first pulse after conditioning (●) and 20th to 25th pulse at new prepulse level after conditioning (○) generate the two indicated curves; $I_{p\ max}$ is last peak current value of conditioning period in both these cases. Node 12/1, exposed to 0.6 mM GEA at pH 6.8, 50-msec prepulses and 5-msec pulses to −20 mV applied at 1 sec^{-1}.

looks much like the normal inactivation curve (◆, data points). On this basis, one would say GEA 968 has little effect on sodium inactivation. However, repetitive use of the sodium channels with the new prepulse gradually reduces the amount of available conductance until a very different "inactivation" curve develops. By this criterion, which includes the contribution of use-dependent inhibition, sodium inactivation seems greatly changed.

In order to look for changes in sodium inactivation in a different way, the shape of inward sodium current traces have been compared before and during treatment with GEA. Although amplitudes of the sodium currents were reduced, the time courses were not altered to any appreciable degree. The time constant for sodium inactivation during depolarizing steps (time constant for the falling phase of the sodium current) generally increased only slightly with GEA treatment; τ_h values increased by an average of 10% in seven experiments.

Prepulse and pulse influences on the blocking and unblocking process. The experiments described next are reported since they will support the proposed model for GEA 968 action that is presented later in this paper. These experiments are complex but all have the following common protocol. 1) The node under study is first conditioned to a stable level of block with many repetitive pulses. 2) A change from the conditioning pulse format is introduced for two trials. 3) The conditioning pulse format is used again to test for the change (more or less block) caused by the two intervening pulse sequences. With this protocol, the effect of varying prepulse and pulse levels and durations on the initial rate of change of the GEA block can be studied.

In order to determine the prepulse influence on use-induced changes in GEA block, experiments were designed to measure the initial rates of change block after introduction of various prepulse levels. To study block removal, the node is first put into a relatively inhibited state with repetitive depolarizing pulses and then a prepulse is introduced for two trials. After this a final standard test depolarization is used to assay the amount of inhibition removed (see inset in fig. 5A). Removal of block was found to be strongly enhanced by large hyperpolarizing prepulses (fig. 5A). Measurements of this type on four different nodes always showed that the prepulse dependence curve for block removal is situated 20 to 40 mV to the left of the prepulse dependence curve that characterizes normal sodium inactivation (labeled h_∞). Prepulse durations as short as 20 msec were adequate to produce all the block removal reported for prepulses to −125 mV.

Another kind of prepulse dependence experiment is shown in figure 5B. In this case, the preparation was put into a relatively unblocked state by a train of large hyperpolarizing prepulses and depolarizing pulses until the currents were stable (see inset). After this conditioning procedure, two pulses with a new level of 50-msec prepulse were applied and finally the sodium conductance was tested with a standard prepulse and pulse. This kind of experiment assays the prepulse dependence of block onset and results from three nodes indicate that prepulses to about −95 mV are most effective in inducing more block.

Strichartz (1973) showed that channels

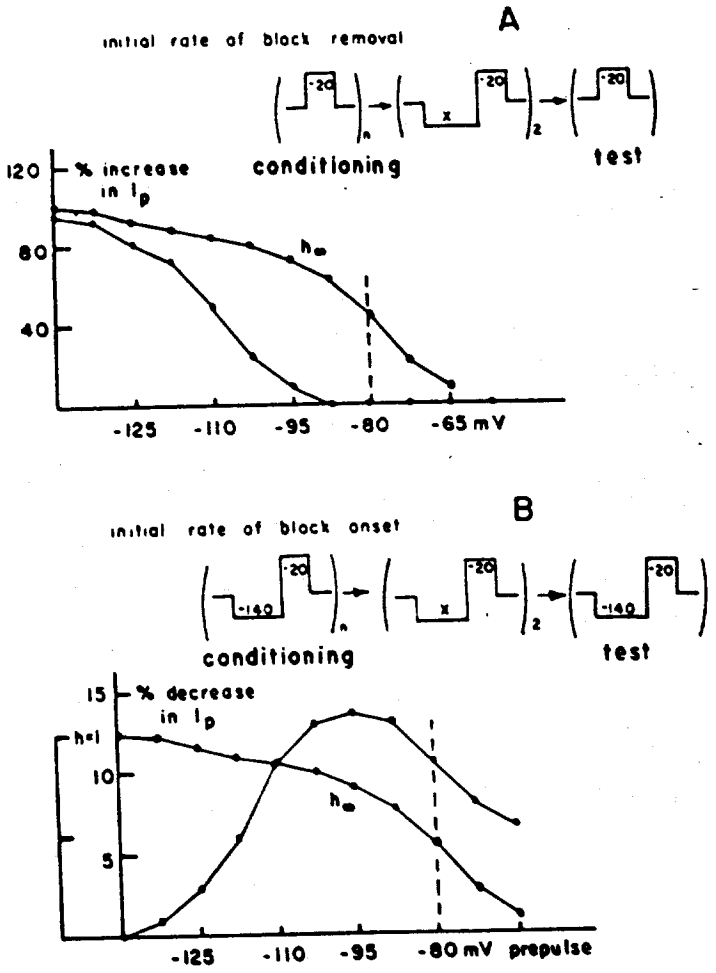


FIG. 5. Prepulse dependence of initial increases and decreases in sodium currents. A. Initial increase in peak sodium current (block removal) measured by conditioning to a high level of inhibition with about 20 5-msec pulses to −20 mV at 2 sec^{-1}, then inserting a 50-msec prepulse for two trials (see inset) before returning to test for the increased current. Unlabeled curve is a plot of test current increase over last conditioning current level. Inactivation curve (labeled h_∞) measured as in B with 100% ordinate corresponding to h_∞ = 1. B. Initial decrease in peak current (block onset) measured by conditioning to a low level of inhibition with −140 mV prepulse and pulse to −20 mV at 2 sec^{-1}, then interrupting with two clamp sequences at desired prepulse level before returning to test for the decreased current. Unlabeled curve plots decrease in current from last conditional level. Inactivation curve (labeled h_∞) measured by comparing peak current obtained on first x prepulse trial with that obtained on last conditioning trial. Dotted lines indicate normal holding potential for node 4/17, 0.5 mM GEA.

needed to be opened by a depolarization in order for inhibition to occur with quaternary lidocaine. I find an analogous requirement for the enhancement of block with tertiary GEA 968. Figure 6A shows an experiment similar to that just described but with various levels of the 5-msec pulses during the two intermediate pulses. This protocol investigates the pulse level dependence of the initial rate of onset of block. In this node, and the three other nodes tested, increases in the amount of the block did not occur until depolarizations sufficient to open sodium channels were used. However, additional inhibition developed for depolarizations beyond that where the channels are fully opened (about −20 mV). This implies that there is an additional voltage dependence of the blocking process as found by Strichartz (1973).

Strichartz also concluded that opening of sodium channels was required for the block to be removed. This may also be the case for GEA blocking of sodium channels. Figure 6B illus-

trates the results of an experiment designed to determine the pulse level dependence of block removal. Measurements on four different nodes gave very similar results: the curve for current increase (block removal) always peaked at about the same pulse level where sodium channels are fully opened. In both cases above (initial block onset and removal) pulses to −20 mV for only 5 msec were adequate to produce the full reported changes in peak sodium current.

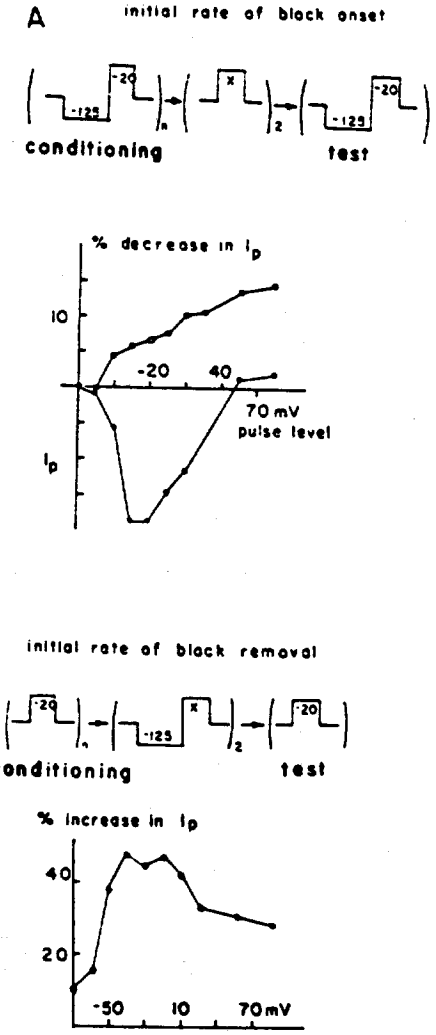


FIG. 6. Pulse level dependence of initial decreases and increases in sodium currents. A. Node was conditioned with hyperpolarizing prepulse/pulse sequence at 2 sec^{-1} until current levels were stable and then prepulse was removed for two trials to induce an increase in inhibition. Upper graph indicates percent decrease in I_p on test pulse (compared to conditioned level of current). Lower graph of part A indicates I_p measured on first trial at new pulse level after conditioning. B. Same node conditioned without prepulses to cause a stable high level of inhibition and then interrupted with two pulses at designated level with a prepulse to −125 mV preceding. This procedure removes some inhibition which can be monitored with next text pulse. Both graphs represent average measurements on two such trials at each intervening pulse level. Node 4/9A, 0.5 mM GEA.

Use dependence with other local anesthetics. Other local anesthetics show this use-dependent inhibition of the sodium mechanism (Courtney, 1974; Hille *et al.*, 1975). Depolarization frequencies of at least 2 sec^{-1} were required to observe this effect clearly for 0.5 mM lidocaine, however. The lack of the use-dependent property with lidocaine at lower frequencies may explain why previous voltage-clamp investigations have neglected this phenomenon. The other local anesthetics tested included procaine (0.74 mM) and procaine amide (3 mM) and gave similar effects. Therefore, several local anesthetics, quaternary (Strichartz, 1973) as well as tertiary, are capable of inducing use-dependent inhibition of sodium currents in myelinated nerve.

Discussion

The hypothesis. Hodgkin and Huxley (1952) first described a process whereby the membrane potential preceding a sudden depolarizing test pulse affects the sodium current that flows during that test pulse; hyperpolarizing prepulse potentials induce a greater "availability" of the sodium permeability mechanism so that larger sodium currents are observed upon depolarization from more hyperpolarized levels. Thus, depolarization of the membrane "inactivates" sodium channels and hyperpolarization removes this inactivation.

The observation that depolarizing pulses coupled with hyperpolarizing prepulses reverse the GEA inhibition caused by use of depolarizing pulses alone suggests a hypothesis for the mechanism of action of this drug. The hypothesis is (see fig. 7A): 1) the GEA 968 molecule can interact better with open sodium channels than with closed sodium channels; 2) when a channel-GEA complex is formed, the channel is blocked and, in addition, the voltage dependence of the sodium inactivation process in that channel is shifted in the hyperpolarizing direction thus increasing the degree of inactivation at any given membrane potential; 3) opening of blocked channels facilitates the unblocking

process; and 4) a slow reversal of the GEA block occurs in the absence of depolarizing pulses (*e.g.*, when the preparation is rested).

Strichartz (1973) has already concluded that "opening" of sodium channels by depolarizing pulses enhances the blocking and unblocking steps with a quaternary lidocaine derivative. The present hypothesis extends Strichartz's model by adding the idea of shifted voltage dependence of sodium inactivation for complexed channels. Weidmann (1955) had previously suggested a shift in sodium inactivation for the local anesthetic action of cocaine; the proposed hypothesis differs from Weidmann's in requiring only that the complexed or bound fraction of sodium channels shows shifted sodium inactivation. The unblocked channels are thought to be unaltered.

The loss of sodium conductance that occurs during depolarizing pulses and the recovery of sodium conductance that occurs during depolarizing pulses preceded by large hyperpolarizing prepulses are explained by the proposed hypothesis in the following way (see fig. 7, B and C). Since channels can most readily transfer back and forth between blocked and unblocked pools when they are open, depolarizing pulses open and move some channels into the blocked (*) pool. Because inactivation of the blocked channels is supposed to be almost complete, they end up in the pool labeled inactive.* Movement out of the blocked population at the resting potential is very difficult during subsequent pulses. Repeated pulses gradually shift channels from the unblocked to the blocked pool (fig. 7B) until an equilibrium is attained. When large hyperpolarizing prepulses are introduced before the test depolarizations, the inactivation of the blocked channels is removed so the channels can now open and shift back to the unblocked states. This pulse procedure shifts the equilibrium away from the blocked pool toward the unblocked pool of channels (fig. 7C); many pulses are again required to attain this new equilibrium.

Evidence supporting the hypothesis. A key feature of the hypothesis is that prepulses enhance block removal far more than they enhance block onset due to the shifted voltage dependence of inactivation for the blocked channels. The inactivation removal (**) reaction is proposed as the prepulse sensitive step. This interpretation is supported by direct measurement of the initial rate of block removal as a function of prepulse level (fig. 5A), where the prepulse dependence curve for block removal is seen to be many millivolts to the left of the prepulse dependence curve for normal steady state inactivation. These experiments therefore support point 2 of the hypothesis.

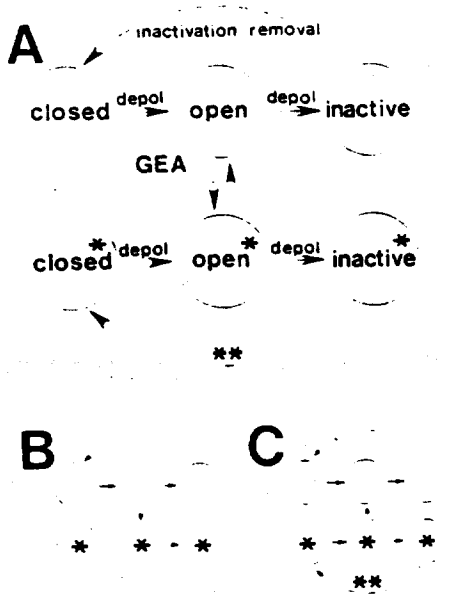


FIG. 7. Hypothesized scheme for pulse-mediated sodium conductance changes with GEA 968 treatment. A. Normal sodium channels above are opened by depolarizing pulses and can then interact with GEA 968 molecule. Dotted lines indicate inactivation removal that is determined by the prepulse potential level. GEA complexed channels (*) are blocked and voltage dependence of sodium inactivation process is shifted in the hyperpolarizing direction (**). B. Major state transformations (more block) that occur during application of depolarizing pulses alone. C. Major state transformations (less block, if previously conditioned to high level of block) that occur when a large hyperpolarization precedes each depolarizing pulse.

The hypothesis presented also requires that the major change in the GEA block occurs when channels are opened. The experiments of figure 6, A and B, showed that blocking and unblocking are not facilitated until depolarizations sufficient to open sodium channels are used. Furthermore prepulses applied by themselves are not nearly as effective in removing inhibition as prepulses followed by channel-opening depolarizing pulses. These findings support points 1 and 3 of the hypothesis.

Another test of the hypothesis can be made using a computer simulation of the Hodgkin-

Huxley equations. A simplified 10-state kinetic scheme is depicted at the top of figure 8: the upper five states, labeled unblocked, are an approximate representation of the opening and closing kinetics of normal channels while the lower five states represent the corresponding transitions for channels blocked by anesthetic. Four of the five states are "closed." The three left most transitions correspond to the opening of "m gates" (activation of the channels) and the rightmost transition to the closing of the "h gate" (inactivation during a depolarizing pulse). Rate constants for these transitions are taken from Hille (1971b) for 10°C. Since the lower five states represent blocked states, the rate constants governing their transitions could be different; in particular, the voltage dependence of h gating (β^*_h) is assumed to be shifted toward more hyperpolarizing voltages by 40 mV while the m gating process (α^*_m) is assumed to be

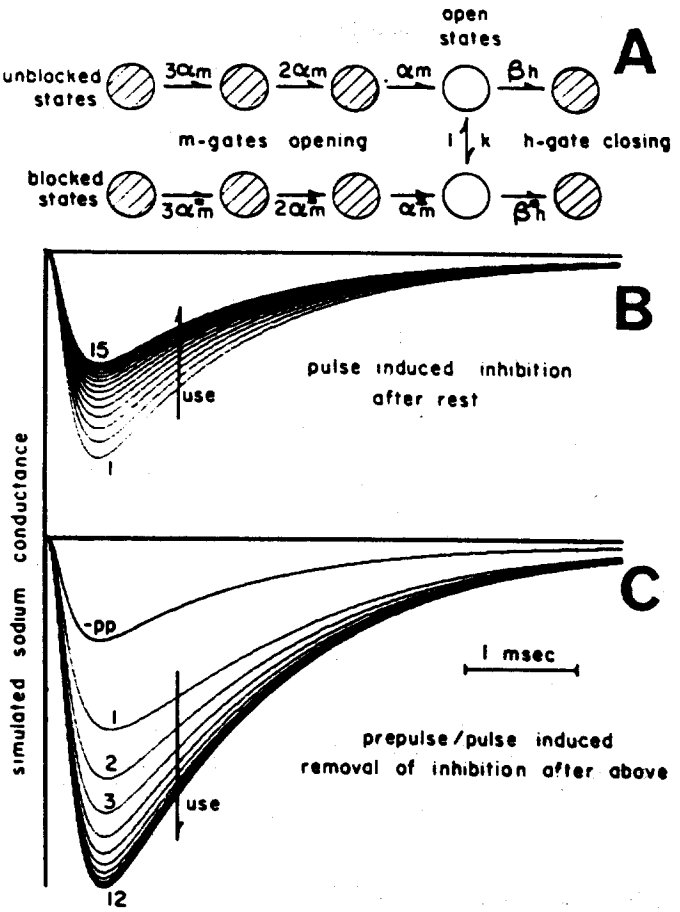


FIG. 8. Computer simulation of conductance changes with the proposed hypothesis. A. Hodgkin-Huxley sodium conductance simulating program (top row of five states in illustration) was modified so that a fraction of the channels could be bound by GEA 968 and thereby have the voltage dependence of sodium inactivation shifted by 40 mV. The bottom row of five states indicates bound states and their altered transition constants (*), m gating (α^*_m) assumed normal but h gating given a shifted voltage dependence. Drug binding is assumed to occur between the open states only by rate constants k = 0.15 msec^{-1} and l = 0.15 msec^{-1} respectively. B. Appearance and disappearance of unblocked open state (conducting state) plotted against time. Five-millisecond pulses to −14 mV (from holding potential of −74 mV) simulated from time 0 at 2 sec^{-1}. During the rest period between pulses, the fraction of unblocked states relaxed toward the resting available conductance fraction, 0.4 for 0.6 mM GEA, with a time constant of 10 seconds. First through 15th pulses shown. C. After 30 pulses as in B, inhibition removal induced by introducing a prepulse to −134 mV ahead of depolarizing pulse. The 30th trace without prepulse is shown (−PP) and then 12 conductance traces with the prepulse are shown. The first trace with prepulse shows about a 20% increase in time-to-peak conductance. The rate constants k and l were selected to simulate a typical block accumulation rate of 0.33 sec^{-1} and block removal rate of 0.76 sec^{-1} for this concentration of GEA 968.

normal. Drug binding and unbinding is assumed to occur only between the open states, the determining rate constants being k and l, respectively. Simulation of 5-msec depolarizing pulses occurs at a rate of 2 sec^{-1} with parameters selected for a 0.6 mM GEA concentration. According to the Hodgkin-Huxley equations used, 40% of the normal fraction of sodium channels are inactivated (far right state) and 99.7% of the blocked fraction of channels are inactivated at the resting potential adopted for this simulation. As repetitions of the depolarizing pulse are simulated, more and more channels move into the blocked states (fig. 8B) until only 20% of all channels remain unblocked (compared to 40% on the first pulse). After this steady state is reached, a large hyperpolarizing prepulse is introduced, removing sodium inactivation from all normal channels and from 95% of the bound channels. Use-dependent removal of inhibition then occurs (fig. 8C). The simulated conductance changes overtime can be compared with experimentally monitored current traces since currents are proportional to conductances with a constant driving potential being maintained by the voltage clamp. The simulation of figure 8B clearly resembles the photographed data presented as figure 2 and figure 8C resembles the photographed data of figure 3 in this paper, even with regard to increased times-to-peak currents. These increased times-to-peak for sodium currents corresponds to the block "recovery currents" conceptualized by the hypothesis in figure 7C; with the help of a hyperpolarizing prepulse, blocked channels open and then unblock, providing a kinetically slower source of conducting channels. Therefore, this computer simulation shows that the molecular hypothesis can generate many of the use-dependent features of GEA 968 action.

The peaked curve relating initial rate of block onset to prepulse level, depicted in figure 5B, can also be explained by the hypothesis. The hypothesis proposes that channels become blocked as they open. Thus, using the Hodgkin-Huxley gating variables m and h (m^* and h^* for bound channels), the change in conductance that occurs during a depolarizing pulse is given by

$$dg/dt = l\, m^{*3} h^* (1 - g) - k\, m^3 h\, (g) \quad (2)$$

where k and l are rate constants governing binding and unbinding of the GEA molecule to the sodium channel, respectively, and g represents the unblocked conductance fraction. The change in conductance that occurs during a given pulse is found by integrating

$$\Delta g = \int (dg/dt)\, dt = l \int m^{*3} h^* (1 - g)\, dt - k \int m^3 h\, g\, dt \quad (3)$$

Thus the maximum rate of onset of block will require prepulses to voltages where h is high, but h^* is still low. This condition is best realized where the difference between the two steady-state inactivation curves has a maximum, for potentials of about −95 mV in figure 5A; the block onset curve in figure 5B also peaks for this prepulse potential level.

The hypothesis developed in this paper does not deal with the slow reversal of inhibition that occurs at rest (10-second time constant for 0.6 mM GEA). The random openings of sodium channels at the resting potential between depolarizing pulses may account for this loss of inhibition and for the inhibition that is observed in the absence of repetitive depolarization. Alternatively, some binding of the GEA molecule to closed sodium channels may be occurring.

Other hypotheses. Other explanations of the use-dependent mode of action of GEA 968 must be considered. It is clear that some process is changing the sodium conductance, a process that takes many depolarizations (not a single long depolarization) to reach completion. This process is *fully* effected by short pulses only a few milliseconds long and by prepulses of approximately 20-msec duration. These pulse durations are like those needed to effect changes in the Hodgkin-Huxley activation and inactivation processes, respectively. The open-channel requirement of the hypothesis easily accounts for these findings.

It is also possible that drug binding may not actually block sodium channels but may act by merely shifting the inactivation curve. This altered hypothesis still fits the loss of conductance with repetitive pulses when no prepulses are applied since essentially all altered channels are inactivated. However, the altered hypothesis seems contradicted by other observations. Large hyperpolarizing prepulses remove inhibition in a use-dependent manner (fig. 3). This would not be the case if both normal and altered "open-channel" states could conduct (see fig. 7C); it would then only be necessary to open the

altered channels not open and then "unblock" them.

An alteration of the normal inactivation process in all sodium channels (such as a prolonged inactivation time constant) might be thought to account for these experimental findings. However, several arguments can be presented against such a hypothesis. First of all, figure 4 shows measurements of the steady-state inactivation curve for a node before and during treatment with GEA 968. As this figure illustrates measurements taken close to each other with no intervening pulses reveal a normal h_∞. Measurements taken many depolarizing uses apart reveal a shift h_∞, but this apparent inactivation curve includes the contribution of use-dependent inhibition which involves movement of normal channels into the blocked population of channels. Also, if the time constant for inactivation is lengthened for the sodium channels, then long hyperpolarizing prepulses would suffice to remove the inactivation. Figure 6B illustrates experiments that indicate that prepulses by themselves do not cause a large amount of block removal *unless* these prepulses are followed by channel-opening pulses; these depolarizing pulses would by themselves increase inactivation whereas they are observed in these experiments to aid in recovery from the GEA-induced block. Finally, recall that prepulse durations as short as 20 msec (for −125 mV prepulse) were adequate to produce the full reported recovery in these experiments, and that inactivation occurred during depolarizing pulses with a fairly normal time constant. These findings all argue against a lengthened inactivation time constant hypothesis.

Related phenomena. Other instances of use-dependent blockage of excitability mechanisms have recently been reported. Marquis and Mautner (1974) found that conduction block of squid axons by thiol reagents is enhanced by repeated electrical stimulation. Dulhunty and Gage (1971), in studies with Maculotoxin extracted from the salivary glands of octopus, also report action potential blockage that requires several uses of the excitability mechanism before the effect develops. A somewhat analogous use-dependent block of potassium channels by internally applied ammonium compounds has also been reported (Armstrong, 1971; Armstrong and Hille, 1972).

The frequency-dependent block of sodium conductance described in this paper may bear some relationship to similar phenomena observed in cardiac tissue, particularly when under the influence of antiarrhythmic drugs. For instance, Johnson and McKinnon (1957), in studies on rabbit ventricular fibers, showed that the maximum rate of rise of the action potential, $(dV/dt)_{max}$, was depressed by quinidine according to the driving rate; the rate of rise of these cardiac action potentials is presumed to be a measure of the availability of the sodium conductance mechanism (see Weidmann, 1955); thus the sodium conductance increase that occurs upon stimulation depends dramatically on the frequency of stimulation. Tritthart *et al.* (1971) have also described a frequency-dependent reduction of the upstroke velocity of action potentials in isolated papillary muscles of guinea pigs after lidocaine treatment. According to the model presented in this paper, such phenomena might be accounted for by the existence of parallel sodium "inactivation" states from which recovery occurs very slowly compared to recovery from normal sodium inactivation. The blocked channel states indicated in figure 7 can be considered as such "parallel inactivation states" from which recovery occurs with about a 10-second time constant for experimental conditions reported here. Such a slow recovery would yield a relative refractory period far exceeding the period needed for removal of inactivation from the normal sodium channels and allow for the possible accumulation of such refractoriness from action potential to action potential (see also Haas *et al.*, 1971).

Active form of local anesthetics. Arguments have been advanced for the cationic form of local anesthetics being the active form and for this form acting on the internal membrane surface of nerves (Ritchie and Greengard, 1966; Narahashi *et al.*, 1970, 1972; Frazier *et al.*, 1970. Part of the evidence involves internal perfusion with quaternary forms of local anesthetics. However, one can argue that quaternary anesthetics are chemically modified forms of the true or tertiary anesthetics and may, therefore, have a different mode of action from true anesthetics. Evidence in this paper provides a new line of argument that tertiary and quaternary anesthetics share some common mode of action. Namely, I have shown that the tertiary local anesthetics, GEA 968, lidocaine, procaine and procaine amide, all show use-dependent

inhibition of the sodium conductance mechanism of a type similar to that discovered for a quaternary derivative of lidocaine by Strichartz (1973). The conclusions that the active form of local anesthetics is the cation and that the site of action is on the internal membrane surface are strengthened by the finding that tertiary and quaternary forms of local anesthetics share this use-dependent mode of action.

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