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Interactions of Lidocaine and Calcium in Blocking the Compound Action Potential of Frog Sciatic Nerve

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The exact role of calcium in nerve conduction in neurons that have been blocked by local anesthetics remains controversial. Recently, attention has been drawn to the importance of examining both frequency-dependent and nonfrequency-dependent conduction block, since it is felt that frequency-dependent block provides a model that more closely approximates the normal physiologic state. The present study was designed to examine the effects of calcium on both the nonfrequency-dependent and frequency-dependent components of lidocaine nerve block. Desheathed, whole sciatic nerves from frogs were placed in a sucrose gap chamber and stimulated by trains of 20 impulses at frequencies from 3 to 90 Hz at supramaximal intensity for activation of the compound action potential. After control studies, the nerve was bathed by a frog Ringer's solution containing calcium concentrations, which increased from 0.0 mM to the physiologic value of 2.0 mM with or without 0.5 mM lidocaine. Compound action potentials were measured, and both frequency-dependent block and nonfrequency-dependent block were compared in each solution. Low calcium concentrations significantly enhanced both nonfrequency- and frequency-dependent lidocaine block. The effect of low concentrations of calcium was greater at higher frequencies of stimulation. (Key words: Anesthetics, local: lidocaine. Ions: calcium. Nerves: block; conduction.)

MANY FACTORS, including ionic content, type of local anesthetic, and frequency of nerve action potential, are capable of producing or modifying neuronal blockade. It is well recognized that calcium plays an important role in nerve excitability. Increasing the external calcium concentration was found to cause a reversible decrease in the inward sodium current in squid axon.[1] Increasing the calcium concentration also caused an increase in firing threshold in myelinated fibers.[2] Apparently, calcium is capable of changing nerve conduction without greatly affecting the resting membrane potential, and thus its actions are similar to those of local anesthetics.[3]

It also has been shown that calcium can modulate the effects of local anesthetics. Blaustein and Goldman[4] reported that high calcium concentrations restored the action potential of a single lobster giant axon that had been blocked by procaine and also antagonized the effect of procaine on membrane conductance.

It is well recognized that local anesthetic action is enhanced by repetitive stimulation.[5,6] Courtney, using the voltage clamp method, confirmed the importance of stimulus frequency to the production of local anesthetic nerve block.[7] He demonstrated the existence of a block that was enhanced by high stimulus frequencies and named it frequency-dependent block.[7]

Strichartz reported on the effect of calcium on conduction blocks produced by a lidocaine derivative (GEA 968).[8] The purpose of the present investigation was to examine the interaction of calcium, lidocaine, and stimulus frequency and their influence on compound action potentials of whole nerves.

Materials and Methods

The sciatic nerves of large northern Rana-pipiens-frogs were removed, desheathed, and placed in a sucrose gap chamber (fig. 1). The stimulating and recording chambers were filled with frog Ringer's solution. The sucrose chamber was filled with 10% sucrose to enhance the amplitude of the recorded compound action potential. The section of nerve in the drug chamber, which was 10 mm in length, was perfused either with frog Ringer's (115 mM NaCl, 2 mM $CaCl_2$, 2.5 mM KCl, and TRIS buffer to maintain normal *p*H). This solution was modified to contain calcium concentrations of 0.0, 0.2, or 2.0 mM without lidocaine or 0.0, 0.2, 0.5, 1.0, or 2.0 mM calcium with lidocaine (0.5 mM). This concentration of lidocaine was shown in this study and by others[9] to produce an appropriately large depression of the compound action potential in the desheathed, bathed nerve preparation. All experiments were done at room temperatures of 20–22° C, and the *p*H of the solutions was maintained at 7.1–7.3.

The nerves were stimulated by square wave pulses, which were 0.05 ms in duration and twice supramaximal intensity. The stimuli were presented in trains. Each train was composed of 20 impulses at each of the following frequencies: 3, 10, 20, 50, and 90 Hz (100 stimuli per

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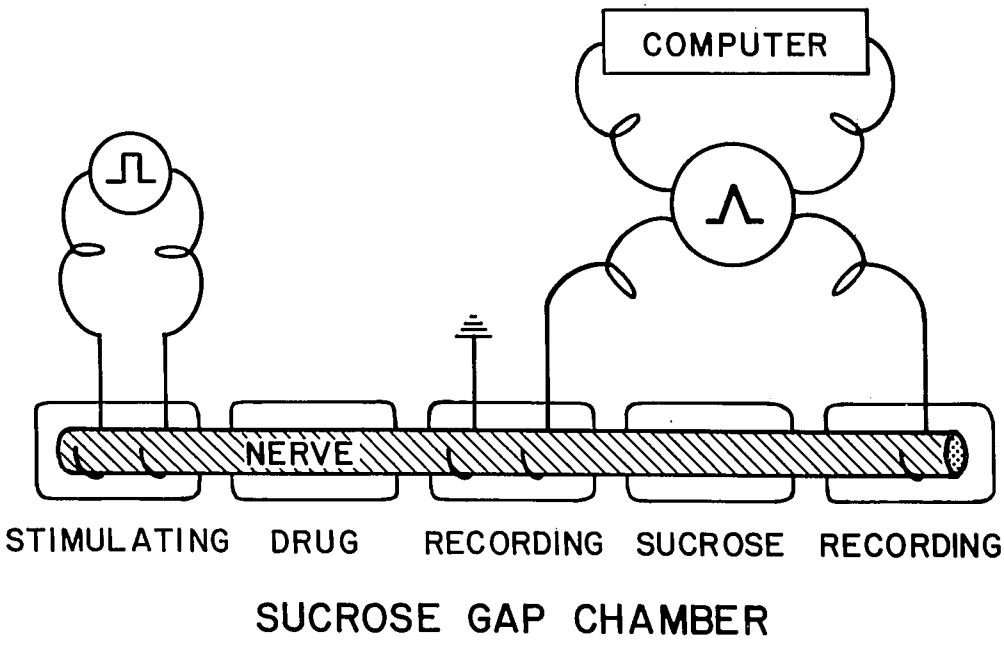

FIG. 1. This figure is a diagrammatic representation of the sucrose gap chamber. The chamber was constructed of plexiglass with wells (rectangles) connected by a channel (location of the nerve). This channel was filled with petroleum jelly in the segments between each well (in order to isolate the solution in each well). The stimulating and recording wells were filled with frog Ringer's solution. The drug chamber and sucrose chamber contained both inlet and outlet ports through which substances could be perfused. The sucrose chamber was perfused constantly with a 10% sucrose solution. The drug chamber was perfused with frog Ringer's during control studies. The perfusate of the drug chamber was changed to the appropriate solution during the experimental protocol. The chamber was sealed so that there was no evaporation from or passage of solutions between each well.

set of trains). A 20-s interval occurred between each train. Fifteen such sets were presented. The first set was control, during which the drug chamber was perfused by frog Ringer's solution. After the first set, frog Ringer's was replaced by the experimental solution and the remaining 14 trains were given. A complete stimulus series lasted approximately 30 min; a duration greater than that required for drug equilibration as found in this study and reported by others.[9]

The area of the compound action potentials was measured and recorded by a PDP 11/40 computer. An algorithm was developed that made it possible to reproducibly measure the area under the curve for each compound action potential. Data analysis divided the effect of local anesthetics into basal (nonfrequency) and frequency-dependent conduction block.

Both the nonfrequency- and frequency-dependent block are expressed as a per cent of control action potential. The value of the nonfrequency-dependent block was determined by measuring the difference between the area of the first action potential of the control and the area of the first action potential of the last train following drug equilibration. This difference is assumed to be due only to drug effects and not frequency effects, since the first action potential of each train is free from the effect of the preceding impulse. The frequency-dependent block was determined by calculating the difference between the area of the first and last action potentials of the last train. This is frequency dependent because it reflects the influence of frequency on the block.

TABLE 1. Effect of Different Calcium Concentrations on Compound Action Potential of Whole Desheathed Frog Sciatic Nerve*

Calcium Concentration	Nonfrequency-dependent Block	Frequency-dependent block at 90 Hz
2.0 mM (9)	2.0 ± 1.1	6.5 ± 1.2
0.2 mM (6)	1.7 ± 1.8	5.3 ± 1.6
0.0 mM (9)	2.5 ± 1.0	6.0 ± 0.5

* Values are expressed as mean percentage change from control ± SEM. Numbers in parentheses indicate the number of experiments at each concentration.

TABLE 2. Effect of Different Calcium Concentrations on the Compound Action Potential of Whole Desheathed Sciatic Nerve in the Presence of 0.5 mM Lidocaine*

Calcium Concentration	Nonfrequency-dependent Block	Frequency-dependent Block at 90 Hz
2.0 mM (9)	8.3 ± 0.7	22.2 ± 2.3
1.0 mM (9)	13.1 ± 1.2†	27.1 ± 2.4
0.5 mM (6)	17.8 ± 3.0†	42.3 ± 2.3‡
0.2 mM (6)	32.3 ± 3.7‡	53.7 ± 2.7‡
0.0 mM (7)	76.3 ± 2.5‡	21.7 ± 2.4§

* Values are expressed as mean percent change from control ± SEM. Numbers in parentheses indicate the number of experiments at each concentration.

† $P < 0.01$.

‡ $P < 0.001$.

§ The value of frequency-dependent block at 0.0 mM is small because of a large nonfrequency-dependent block (*i.e.*, the total block cannot exceed 100%).

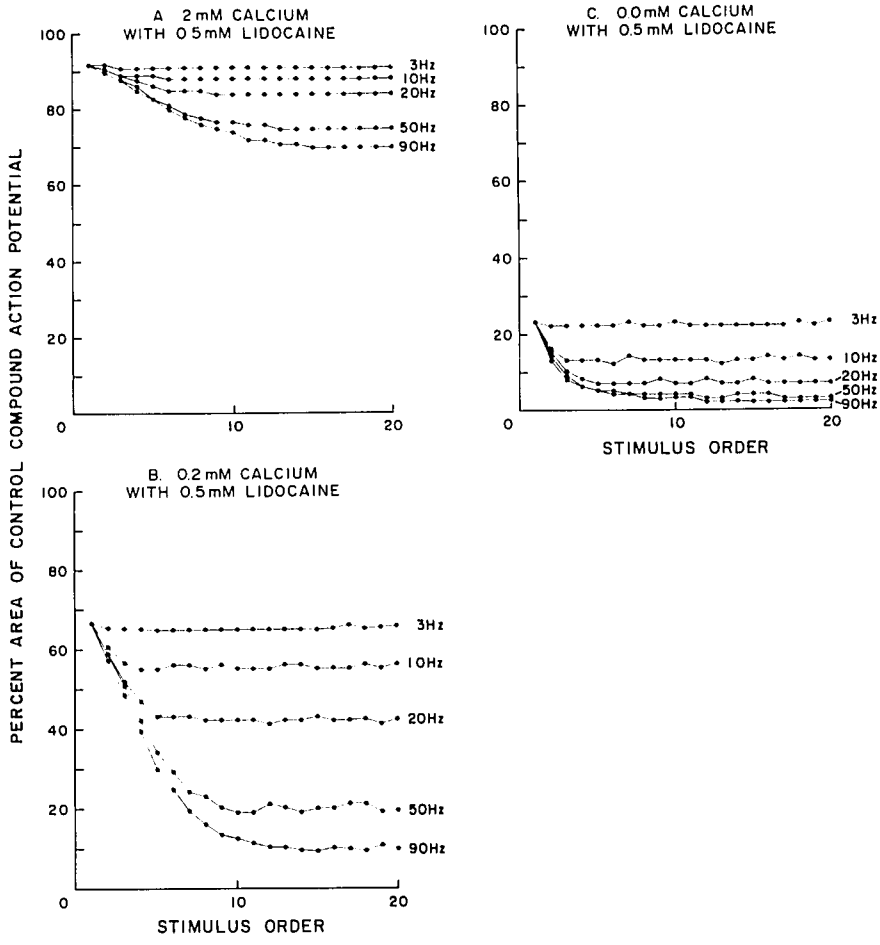

FIG. 2. Area of compound action potentials in the presence of different calcium concentrations and different stimulus frequencies and 0.5 mM lidocaine (abscissa, stimulus order during the last train at 90 Hz). Each point shows the mean area of the action potential for each stimulus elicited by the last train (at a time after the drug has equilibrated with the nerve) at each frequency and is presented as the per cent area of the control compound action potential for each frequency. *A.* An 8% nonfrequency-dependent block (reduction in the first stimulus at each frequency when compared with control, *i.e.*, no lidocaine) was caused by 0.5 mM lidocaine in frog Ringer's solution (containing 2 mM calcium). There was little change in the area of the compound action potential from stimulus 1 to stimulus 20 at 3 Hz, but as the frequency of stimulation increased, the area of the compound action potential was reduced (frequency-dependent block). For example, the area of the compound action potential following the last stimulus at 90 Hz was 30% less than control. Eight per cent of that was due to the nonfrequency-dependent block, and the remaining 22% was the frequency-dependent block. *B.* A 10-fold reduction in the calcium concentration (to 0.2 mM) in the presence of 0.5 mM lidocaine enhanced both the nonfrequency- and frequency-dependent block. The nonfrequency-dependent block was 32%. The total block following the last stimulus at 90 Hz was 86%, which indicates that the frequency-dependent block had been increased to 54%. *C.* Calcium-free frog Ringer's solution with 0.5 mM lidocaine dramatically enhanced the nonfrequency-dependent block. The frequency-dependent block was also greater (*i.e.*, close to 100%), although, by our definition, the total amount of frequency-dependent block was smaller due to the very large nonfrequency-dependent block (*i.e.*, the block could not be greater than 100%).

Results

In the absence of lidocaine, changing the calcium concentration from 0.0 mM to 2.0 mM did not cause a statistically significant difference in either frequency- or nonfrequency-dependent block (table 1). In contrast, in the presence of 0.5 mM lidocaine, changes in the calcium concentration caused a significant difference in both types of block (table 2). The nonfrequency-dependent block, which is free from the effect of preceding impulses, increased from 8 to 76%, with decreasing calcium concentration in the presence of lidocaine.

The extent of the calcium lidocaine interaction is seen in figure 2 for three concentrations of calcium. At 3 Hz, the area of the action potentials tended to be the same from the first to the last stimulus in all cases. However, the area of the action potential following the last stimulus of the last train at the other frequencies decreased as the stimulus frequency increased in the presence of lidocaine. In the presence of lidocaine, 90 Hz stimulation caused a profound block of the action potentials following the last stimulus when compared with both control and the last stimulus at 3 Hz. This block, caused by high-frequency stimulation, was increased by decreasing calcium concentrations. Frequency-dependent block in the presence of 2.0 mM calcium solution was 22% (fig. 2*A*). This frequency-dependent block increased up to 54% in 0.2 mM calcium (fig. 2*B*). In 0.0 mM calcium solution, the frequency-dependent block appears to become smaller (fig. 2*C*). However, this is due to the large increase in nonfrequency-dependent block at that same calcium concentration (*i.e.*, the area cannot fall below 0%).

Discussion

Our method for the determination of frequency dependent block is different from that of Courtney *et al.*[9] According to their method, frequency-dependent block was assessed by measuring the decrease observed in the last response of the train compared with the same response

under control conditions. Thus, when the nonfrequency-dependent block is large, a large component of the frequency-dependent block would be due to the nonfrequency-dependent block. Because of this problem, we devised a new method for determining frequency-dependent block in a way that does not permit such a problem to arise.

The enhancement of both the nonfrequency- and frequency-dependent lidocaine block by lowered calcium may be due to many factors. One possible explanation is that sodium inactivation was altered. Frankenhaeuser[2] examined the effect of altering external calcium concentration on sodium inactivation in frog nerves. Lowering the external calcium concentration from 1.08 mM to 0.27 mM enhanced sodium inactivation. Raising the external calcium concentration to 4.32 mM decreased the sodium inactivation. It has also been reported that sodium inactivation is enhanced by local anesthetics (cocaine, procaine, lidocaine, and benzocaine).[10,11] This combined effect of both decreased external calcium concentration and the presence of lidocaine could act in the following way: Normally approximately 60% of the sodium channels are open during the resting state.[12] When the sodium inactivation is enhanced, the number of available channels would be expected to decrease. This decrease in the number of available sodium channels would decrease the nerve excitability, since fewer channels would be open for the ion movements essential for an action potential. Thus low external calcium concentrations in the presence of a local anesthetic may enhance nerve block by amplifying the sodium inactivation process.

The above explanation does not provide an adequate reason for the lack of block seen in the absence of lidocaine and is not, at first glance, compatible with the enhancement of sodium channel inactivation in low calcium reported by Frankenhaeuser and Hodgkin.[1] Lowering the calcium concentration without lidocaine did not cause a significant change in either frequency- or nonfrequency-dependent block. A possible reason for this apparent discrepancy is that in low calcium, the firing threshold is lower[2] and more fibers can be activated by a given strength of stimulation than in higher calcium. This may compensate for the decrease of the action potential height in a single fiber caused by enhanced sodium channel inactivation and thus cause the compound action potential to maintain a normal height.

The enhancement of frequency-dependent block in the presence of lidocaine may be explained by the modulated receptor hypothesis of Hille.[11] According to the hypothesis, repetitive stimulation in the presence of a local anesthetic enhances sodium inactivation. Hille postulated that a receptor inside of the sodium channel only can be reached by hydrophylic local anesthetics when both inner sodium channel gates are open. When a nerve is stimulated repetitively, both gates open to respond to the depolarization, and more and more local anesthetic molecules enter the open sodium channels. These molecules bind the receptor inside the sodium channel and, therefore, the number of available sodium channels is decreased, and as a result, the compound action potential gets smaller with increasing stimulation frequency.

Although the above discussion of possible mechanisms to explain the results of this study are speculative, the data clearly indicate that there are important interactions between stimulus frequency, ionic content, and local anesthetics. Further studies of such interactions may reveal information that can be employed in the production of better, longer-acting local anesthetics.

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