Competitive Action of Calcium and Procaine on Lobster Axon

A study of the mechanism of action of certain local anesthetics

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ABSTRACT Voltage clamp studies with the squid giant axon have shown that changes in the external calcium concentration (Frankenhaeuser and Hodgkin, 1957) shift the sodium and potassium conductance versus membrane potential curves along the potential axis. Taylor (1959) found that procaine acts primarily by reducing the sodium and, to a lesser extent, the potassium conductances. Both procaine and increased calcium also delay the turning on of the sodium conductance mechanism. Calcium and procaine have similar effects on lobster giant axon. In addition, we have observed that the magnitude of the response to procaine is influenced by the external calcium concentration. Increasing external calcium tends to reduce the effectiveness of procaine in decreasing sodium conductance. Conversely, procaine is more effective in reducing the membrane conductance if external calcium is decreased. The amplitude of the nerve action potential reflects these conductance changes in that, for example, reductions in amplitude resulting from the addition of procaine to the medium are partially restored by increasing external calcium, as was first noted by Aceves and Machne (1963). These phenomena suggest that calcium and procaine compete with one another with respect to their actions on the membrane conductance mechanism. The fact that procaine and its analogues compete with calcium for binding to phospholipids in vitro (Feinstein, 1964) suggests that the concept of competitive binding to phospholipids may provide a useful model for interpreting these data.

INTRODUCTION

Studies by Frankenhaeuser and Hodgkin (1957) on the voltage-clamped squid giant axon and by Frankenhaeuser (1957) on the myelinated nerve fiber have shown that external calcium is intimately associated with the nerve membrane conductance mechanism. The voltage clamp studies demonstrate that increasing the calcium in the external medium affects both the...
sodium and potassium conductance versus voltage curves by shifting these curves along the voltage axis so that a larger membrane depolarization step is needed to reach a given conductance. The level of maximum conductance is essentially unchanged. Decreasing the external calcium shifts the curves in the opposite direction along the voltage axis so that a smaller depolarization step is necessary to reach a given conductance. These shifts are of the order of 10 to 15 mv for a 5-fold change in calcium concentration. Taylor's (1959) studies on the effect of procaine on the voltage-clamped squid axon demonstrate that this agent also influences the membrane sodium and potassium conductances (see also Shanes et al., 1959). Unlike calcium, however, procaine acts primarily by reducing the magnitude of the sodium and potassium conductances for a given step depolarization.

The studies by Taylor on procaine and by Frankenhaeuser and Hodgkin on calcium also demonstrate an affect of these agents on the time parameters of the sodium conductance changes. Thus, treatment with either high calcium or procaine results in an increase in the time-to-peak of the initial (sodium) current, for a given membrane depolarization step.

The fact that both calcium and procaine affect the time parameters for the sodium conductance mechanism, as well as the conductance versus voltage parameters, suggests that they may both exert their actions by affecting the same membrane site.

One of the earliest suggestions of an interaction between calcium and procainelike local anesthetics may be found in the studies of Simon and Szelöczey (1928). These workers found that significantly more calcium was released from rabbit sciatic nerve preparations treated with cocaine than from the control preparations. More recently, Aceves and Macne (1963) demonstrated that, in single cells of frog spinal ganglia, the normal action of the local anesthetics procaine and xylocaine, in reducing the action potential amplitude, is partially antagonized by increasing the concentration of calcium in the bathing medium. They also noted that the excitation threshold was higher in the presence of high calcium plus procaine than with either high calcium alone, or procaine in normal calcium.

The present study was undertaken using voltage clamp techniques in order to define further the interrelationship between calcium and procaine with respect to the membrane conductances. A preliminary report has been published (Blaustein and Goldman, 1965b).

**METHODS**

Single giant axons measuring approximately 80 to 100 μ in diameter, obtained from the circumesophageal connectives of the lobster, *Homarus americanus*, were used for all experiments. The voltage clamp experiments were carried out using the sucrose-gap techniques described in detail by Julian, Moore, and Goldman (1962a, b). A
single giant axon was freed from most of its surrounding connective tissue and was pulled into the sucrose-gap chamber. Two streams of flowing, high-resistance, isotonic sucrose were used to isolate electrically three regions of the axon. One end was depolarized in isotonic potassium chloride. The central region of the axon (about 50 to 60 μ long) was bathed with sea water or the test solution. The other end of the axon was bathed in sea water, and current for stimulation of an action potential or clamping was injected via this end. The membrane potentials ($E_M$) were measured as the potential of the electrode in the isotonic KCl pool minus the potential in the solution bathing the central node. The central pool was a virtual ground, and current flow from the sea water side pool to the central pool was taken as a measure of current flow through the "nodal" membrane. As was noted by Julian et al. (1962a) the sucrose-gap method causes the membrane to be hyperpolarized by some 20 to 50 mv. Thus, our resting membrane potential ($E_R$) values ranged from $-85$ to $-130$ mv as compared with the normal resting membrane potential of about $-70$ mv measured with micropipettes in the absence of a sucrose-gap (Julian et al., 1962a). This hyperpolarization appears to be due to local currents arising at the junction of sucrose, sea water, and axon as a result of liquid junction potentials (Blaustein and Goldman, 1965a). As a consequence of this hyperpolarization phenomenon, action potentials with amplitudes of 140 mv or more are quite common when the sucrose-gap method is used, although there is no evidence of significant effects on the sodium or potassium currents.

In order to avoid the possibility of inactivation of the sodium current-carrying system in the resting state (Hodgkin and Huxley, 1952; Frankenhaeuser, 1959), the steady membrane potential at which the axons were clamped, i.e. "the holding potential" ($E_h$), was usually 5 to 10 mv higher than the already hyperpolarized membrane potential.

The ionic composition of the several solutions tested is shown in Table I. In any single experiment, the concentration of sodium in the bathing medium was maintained constant by the substitution of appropriate concentrations of choline chloride where necessary. The various concentrations of crystalline procaine HCl added to these solutions will be noted below. The isotonic sucrose solution containing 725 mM

### Table I

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</table>

* In addition, all sea waters contained (in mEq/liter): K⁺, 10; Mg⁺⁺, 16; SO₄⁻, 8. Solutions were unbuffered, and pH ranged from 6.2-6.5.
sucrose was prepared according to the methods of Julian et al., (1962 a). All experiments were conducted at temperatures ranging from 4° to 8°C. Most of our experiments with the voltage clamp were performed in the usual manner. The axon was stimulated and an action potential observed. The membrane potential was then clamped and a series of depolarization steps was applied. Upon completion of this sequence, the voltage clamp was turned off, the resting membrane potential was then recorded, the axon was stimulated, and an action potential was again recorded. Following this, a solution change was made in the central pool. After sufficient time for stabilization, usually of the order of 2 to 4 min, the same sequence, of action potential, voltage clamp, then action potential, was again repeated. In some of our experiments, however, we did not turn off the clamp during the solution changes. In the latter experiments step depolarizations to a constant level every 4 to 6 sec during the solution change period enabled us to watch for sudden changes in current due to changes in the length of the central node as a result of flow rate variations which may occur when the solutions are switched. This method also permits a direct comparison of time-to-peak of the initial currents at a constant step depolarization for the various test solutions.

CALCULATIONS The slope of the line passing through zero current at the "holding-potential" and through the steady-state current value at a hyperpolarization step of 30 to 50 mv was taken as a nonspecific leakage conductance \( g_{\text{leak}} \) and all currents were corrected for this leakage. For positive membrane potentials, a second leakage conductance \( g'_{\text{leak}} \) was defined by the slope of the line passing through the origin (zero voltage, zero current) and through the residual peak initial current (following correction for \( g_{\text{leak}} \)) at the potential at which this current reversed direction. Only the peak initial current was corrected for \( g'_{\text{leak}} \) so that the sodium current was taken as the peak initial current minus a \( g_{\text{leak}} \) correction for negative membrane potentials, and the peak-initial current minus both a \( g_{\text{leak}} \) and a \( g'_{\text{leak}} \) correction at positive membrane potentials. The \( g'_{\text{leak}} \) correction was used to account in part for the nonlinear leak characteristics of the membrane (Adelman and Taylor, 1961), and in part for potassium current flow during the early transient (Frankenhaeuser and Moore, 1963; Meves and Chandler, 1965).

The membrane potential at which the net sodium current reversed direction was taken as the sodium equilibrium potential \( E_{Na} \). Membrane sodium conductances \( (g_{Na}) \) were then calculated from the corrected peak initial currents \( (I_{pi}) \) according to the equation: \( g_{Na} = I_{pi}/(E_{M} - E_{Na}) \), where \( E_{M} \) is the value of the membrane potential during the step. Due to the difficulties inherent in the usual methods for determining the value of the potassium equilibrium potential, steady-state slope conductances were used as a measure of the potassium conductance \( (g'_{K}) \), where: \( g'_{K} = \frac{dI'_{K}}{dE_{M}} \), where \( I'_{K} \) is the corrected steady-state current.

RESULTS

1. Effects of Calcium and of Procaine on Lobster Axon Sodium Conductance

The data shown in Fig. 1 compare the effects of low calcium, of high calcium, and of procaine in normal calcium on the sodium conductance versus voltage.
curve of the lobster giant axon. Since not all of the observations were made on a single axon, the control and test curves from several axons have been normalized so that all the control curves are superimposable. In accordance with the observations of Frankenhaeuser and Hodgkin (1957), reduction of external calcium can be seen to shift the curve to the left along the voltage axis while increasing external calcium shifts the curve to the right. Observe that the curve shifts by about 8 mv for the 3.7-fold increase in calcium concentration. There appears to be a negligible effect of the calcium concentration on the level of the maximum sodium conductance reached, i.e., the horizontal plateau. This was not always the case, however, and we often noticed a decrease in the level of the maximum sodium and potassium con-
ductances when the external calcium concentration was increased. The average decrease in maximum sodium conductance, calculated according to the methods described above, was about 8 to 10%, for a 4-fold increase in calcium concentration. A qualitatively similar observation, also on the lobster axon, has been noted by Moore and Takata (personal communication).

Looking at the curve for the procaine-treated axon, we note that, as shown by Taylor (1959), the obvious effect is a reduction in conductance all along the curve. The apparent shift of this curve to the right may be partly explained on the basis of a decrease in conductance at all membrane potentials.

2. Interactions between Calcium and Procaine with Respect to the Action Potential

The action potentials shown in Fig. 2 demonstrate the effect of varying calcium concentrations on the response to procaine. These data are very similar to the action potentials exhibited by Aceves and Machne (1963). It should be noted that in our experiments, probably as the result of the hyperpolarizing effect of sucrose-gap techniques noted above, there is minimal inactivation of the sodium current-carrying system in the resting state. Thus,
action potential amplitudes are negligibly affected by 4- to 5-fold variations in external calcium concentration in the absence of procaine.

The upper row in Fig. 2 (odd numbers) are photographs of the action potential records before the voltage clamp was turned on. The lower row (even numbers) were photographed immediately after a voltage clamp series, and just before the solution in the central pool was changed. The entire sequence was carried out on a single “node.” The column at the left (3A) shows the action potential with the axon in the control sea water. In column 3B (photographs 3 and 4), we see that there is only a slight reduction in the amplitude and overshoot in the presence of 7.3 μM of procaine HC1 with approximately 3.7 times the normal calcium concentration. When the calcium concentration is now brought back to normal (3C), without changing the procaine concentration, there is no overshoot and the amplitude of the action potential is markedly decreased. Increase in the external calcium concentration (3D) results in a partial reversal, i.e., the amplitude is increased and an overshoot is observed despite the continued presence of procaine. In the last column (3E) with the axon once again in control sea water, there is further increase in the amplitude of the action potential and in the overshoot.

3. Interactions between Calcium and Procaine with Respect to Current Voltage Relations

Membrane current versus potential curves for the same axon as shown in Fig. 2 are represented in Fig. 3. The curves from the control sea water portions of the experiment have been omitted to minimize the number of curves on the graph for the sake of clarity. It can be readily seen that the peak initial (sodium) current is markedly reduced when the external calcium concentration is decreased in the presence of a constant concentration of procaine (compare curves 3B and 3C with respect to peak initial current). Furthermore, this effect is partially reversed when the external calcium concentration is again increased as shown in curve 3D. A similar effect appears to be true of the steady-state (potassium) current when the calcium concentration is varied in the presence of a constant procaine concentration; these changes are, however, somewhat less obvious than the changes in the sodium current curves. These effects of calcium and procaine on the sodium current curves would appear to account for the observed changes in action potential amplitudes seen in Fig. 2.

4. Interactions between Calcium and Procaine with Respect to Membrane Conductances

Curves of the logarithm of the sodium conductance versus membrane potential for the current voltage data of Fig. 3 are shown in Fig. 4. The con-
ductances were calculated according to the methods described above. The horizontal plateau region of the respective curves will be referred to as the "maximum sodium conductances" ($\tilde{g}_{Na}$). The curves in Fig. 4 demonstrate that there is a significant reduction in $\tilde{g}_{Na}$ when the external calcium concentration is decreased from 183 to 50 mEq/liter in the presence of a constant procaine concentration and that this effect is partially reversible when the external calcium concentration is then increased.

Similar conductance data for an axon treated with procaine in which the calcium concentration was reduced and then brought back to normal are
shown in Fig. 5. Again, the same effect is observed; a decrease in the external calcium concentration increases the effectiveness of procaine in reducing $g_{Na}$.

Table II summarizes the action potential and conductance data relative to the interaction between calcium and procaine for several axons. In all

![Diagram](image)

**Figure 4.** Log sodium conductance versus membrane potential curves for same data as in Fig. 3.

these axons we see that the reduction in $g_{Na}$ due to treatment with procaine is inversely affected by the calcium concentration in the bathing medium. In most axons the same phenomenon is observed with respect to the maximum potassium conductance ($g'_{K}$). This was not always the case, however, and may in part be due to some difficulties in being certain of the correct potassium current values which are somewhat more difficult to measure than are the sodium current values. Furthermore, if the effects of increased external calcium in reducing the membrane conductances (referred to above)
were taken into account, $g'_{Na}$ would be greater in the higher calcium plus procaine situation, than in the lower calcium plus procaine condition in all instances. A correction for this effect of increased calcium might also increase the observed differences between $g'_{Na}$ in the higher calcium plus procaine solutions as compared to the lower calcium plus procaine solutions. These effects of calcium have not been taken into account in the computations of the data presented in Table II or any of the figures.

Where the data are available, they show that the reduction in the action potential overshoot due to procaine treatment also bears an inverse relationship to the calcium concentration in the sea water (see Table II).

5. Other Effects of Procaine

A few other qualitative or semiquantitative observations deserve mention. As may be seen by a comparison of the sodium conductance data of experi-

![Figure 5. Log sodium conductance versus membrane potential curves for another axon. Normal calcium before treatment (2A) and during recovery (2D); low calcium plus procaine (2B); normal calcium plus procaine (2C).](image-url)
M. P. Blaustein and D. E. Goldman  
**Action of Calcium and Procaine on Nerve**

Treatment of an axon with procaine during and especially before removal of calcium usually decreases the magnitude of the rise in leak conductance associated with marked reductions of external calcium (compare leak conductance data from experiments 42-64 and 94-64 with that of 103-64 in Table II). Probably as a consequence of this "protection" against the development of large leak conductances, axons tend to deteriorate much less rapidly in low calcium solutions when procaine is present than in its absence. For example, the data from axon 103-64 in Table II demonstrate that, following exposure to low calcium, a procaine-treated axon may withstand several solution changes. The large leak conductances here are likely due to exposure to low calcium before the procaine was introduced. We have never observed a node which could withstand more than one or two solution changes following a marked reduction in external calcium unless the axon was treated with procaine. A similar type of protection by procaine or cocaine against the effects of calcium depletion has been noted in squid axons by Shanes (1958) and in frog sciatic nerve bundles by Straub (1956).

**DISCUSSION**

1. **Competitive Interaction between Calcium and Procaine**

The foregoing data demonstrate that the magnitude of the reduction of maximum peak sodium conductance (and possibly also maximum potassium conductance) due to treatment with the local anesthetic procaine is a function of the calcium concentration in the bathing medium. Increasing the external calcium concentration reduces the effectiveness of a given concentration of procaine in lowering membrane conductance while, conversely, lowering of the external calcium concentration enhances the effectiveness of procaine with respect to the membrane conductance. A qualitatively similar phenomenon was noted with respect to the action potential amplitudes (Fig. 1 and Table II). Furthermore, Aceves and Machne (1963) found a similar kind of antagonism between the local anesthetic agents, procaine and lidocaine, and calcium. It appears unlikely that these phenomena are related to the so called "resting membrane sodium current inactivation" because, as Taylor has shown (1959), procaine does not affect sodium current inactivation, and because, as noted in the Methods section above, the axon is normally hyperpolarized under the sucrose-gap, so that resting sodium inactivation is negligible.

In attempting to interpret the data presented here, several other observations seem pertinent. As noted in the Introduction, both procaine (Taylor, 1959) and calcium (Frankenhaeuser and Hodgkin, 1957) have been shown...
### Table II

**Effect of Varying Calcium Concentration on Membrane Conductances of Procaine-Treated Axons**

<table>
<thead>
<tr>
<th>Experiment*</th>
<th>Sea water†</th>
<th>Procaine concentration</th>
<th>Duration of application</th>
<th>Action potential overshoot§</th>
<th>( g_{\text{Na}} )</th>
<th>( g_{\text{K}} )</th>
<th>( g_{\text{leak}} )</th>
<th>( \gamma'_{\text{leak}} )</th>
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<td>43</td>
<td></td>
</tr>
</tbody>
</table>

* Each experiment represents a single "node" while the code letters refer to voltage clamp sequences following solution changes. Thus, e.g., 37-64-2A to 2D was performed on a single node.

† See Table I for composition of test solutions.

§ Overshoot is here taken to be the same as the absolute value of the membrane potential at the peak of the action potential.

‖ See text for description of conductance symbols.

†† No action potentials obtained between voltage clamp runs for these axons.
in the squid axon to affect the membrane sodium and potassium conductances as well as the time parameters at least for the turning on of the sodium conductance mechanism. We have made similar observations on the membrane conductances (Fig. 1 and Table II) and the time parameters (unpublished) for both procaine and calcium in the lobster giant axon.

Possibly related to our observation that procaine may protect against the development of large leakage conductances when calcium is removed, are the reports that procaine is also effective in slowing the rate of depolarization attendant upon increasing external potassium (Straub, 1956; and see Shanes, 1958, for a review of this subject). Likewise, increasing external calcium also reduces or partly reverses the depolarizing effect of increased external potassium (Höber and Strohe, 1929; Guttman, 1940; see also, Shanes, 1958). Furthermore, procaine is effective in preventing or reversing the depolarization which results from removal of external calcium (Straub, 1956; Shanes, 1958; Davis and Dettbarn, 1962).

The sum of these data on the effects of calcium and of procaine on the axon membrane, and of the interactions between calcium and procaine suggests that procaine may compete for nerve membrane sites normally occupied by calcium. When these sites are occupied by procaine molecules they would be less readily available than the calcium-occupied sites for undergoing the reactions which normally follow membrane depolarization.

2. **Effects of Calcium and Procaine on Threshold Phenomena**

One significant problem needs to be dealt with. As was mentioned above, Aceves and Machne (1963) observed that, despite this apparent antagonism
between calcium and procaine with respect to the action potential amplitude, there appeared to be an additive effect or synergism between these two agents with respect to threshold phenomena. Thus, treatment with high calcium plus procaine resulted in a higher threshold for excitation than either high calcium alone or procaine in normal calcium. On the basis of

this apparent paradox, these authors concluded that calcium and procaine affect different membrane sites.

Because a net inward current is prerequisite to the development of an action potential, inspection of the uncorrected peak initial current versus voltage curves in Fig. 6 readily demonstrates that the axon in high calcium plus procaine should have a higher threshold than in either high calcium alone, or procaine in normal calcium. This is a consequence of the fact that either procaine or increased calcium tends to shift the position of net current

---

**Figure 6.** Uncorrected (net) peak initial current versus membrane potential to show relative positions of cross-over of zero net current line (i.e., "threshold" potential). LC, normal calcium; LC and LE, normal calcium plus procaine; LF, high calcium; LD, high calcium plus procaine. Negative current is inward.
curve so that the inward current occurs at a higher membrane potential by comparison with the normal.

Since calcium sets the position of the membrane conductance curves with respect to the voltage axis, the relative effect of a given procaine concentration may be a function of the position of the conductance curves as determined by the calcium concentration; the farther to the right the curve is situated, the smaller the effect a given amount of procaine will have. If this is the case, the magnitude of the increase in threshold due to adding procaine to an axon already in high calcium should be less than that which results from adding the same amount of procaine to an axon in low or normal calcium.

If we consider the excitation threshold to be associated with the attainment of a specific sodium conductance level (as arbitrarily represented by the horizontal, dashed line at a log conductance of 1.5 in Fig. 7, for example), then the log conductance plot (Fig. 7) of the data from the same axon as in Fig. 6 shows the effect of the calcium concentration on the response to procaine. The shift of the conductance curve to the right in high calcium plus procaine (1D), using the high calcium curve as a baseline (1F) is less than
the shift when procaine is added to a normal calcium medium (compare curves 1C and E, and 1G).

3. Kinetics of the Calcium-Procaine Interaction; Membrane Phospholipid as a Model System

Of interest with respect to the concept of competitive interaction between calcium and procaine are the in vitro studies by several workers (Kimizuka and Koketsu, 1962; Woolley and Campbell, 1962; Koketsu et al., 1964; and Rojas and Tobias, 1965) demonstrating calcium binding to phospholipids. Recently, Feinstein (1964) has shown that the procainelike local anesthetics such as procaine and tetracaine interfere with calcium binding by phospholipids and that, in fact, the anesthetic molecules may themselves bind to phospholipids. It was on the basis of these studies that Feinstein suggested competitive binding between local anesthetics and calcium as a possible way of accounting for the mechanism of action of local anesthetics.

Data from chemical analyses and electron micrograph studies suggest that the axon membrane is a protein-coated bimolecular lipid layer some 100 Å thick, and that a significant proportion of the lipid present is phospholipid (see, for example, Robertson, 1960; Folch-Pi and LeBaron, 1957; Roots and Johnston, 1965). The polar heads of the phospholipid most likely face the inner and outer protein coats. It has been proposed (Goldman, 1964) that the polar heads of membrane phospholipids may serve as a gating mechanism which controls transient axon membrane permeability changes. According to this theory, the phospholipid polar groups may be oriented in any of several configurations, the predominant configuration being determined by the electric field across the membrane. One of the configurations binds calcium preferentially and two other configurations prefer sodium and potassium, respectively. Fig. 8 shows three possible phospholipid polar group

\[ \text{Co} - I \rightleftharpoons \text{Co}^+ + \text{I} \]

\[ +\text{Pr} \]

\[ \text{Pr} - \text{I} \]

\[ \text{II} \rightleftharpoons \text{II} - \text{Na}^+ \]

\[ \text{III} \rightleftharpoons \text{III} - \text{K}^+ \]

\text{FIGURE 8. Model reaction network for interactions between calcium and procaine. See text for description of symbols (adapted from Goldman, 1964).}
configurations, labeled I, II, and III, which bind or adsorb calcium, sodium, and potassium, respectively. The change from the I to the II and III configurations depends upon an appropriate alteration in the electric field across the membrane. The changes in configuration proceed in a clockwise manner when the axon is put through the usual depolarization-repolarization cycle. This model provides a useful basis for the interpretation of the data on the competitive interaction between calcium and procaine which we have presented here. The data may be interpreted as suggesting that procaine competes with calcium for binding to phospholipid in configuration I. If the procaine phospholipid complex (Pr-I in the diagram) dissociates slowly compared to the Ca-I complex, it would not be readily available for the usual depolarization reactions.

Using this model, we can write the equations for the binding constants of calcium \(K_{Ca}\) and of procaine \(K_{Pr}\) to the phospholipid in configuration I:

\[
K_{Ca} = \frac{[Ca - I]}{[Ca^{++}][I]} \tag{1}
\]

\[
K_{Pr} = \frac{[Pr - I]}{[Pr][I]} \tag{2}
\]

Since Feinstein has shown that local anesthetic molecules may act similarly to divalent cations and since both calcium and the local anesthetics bind a pair of phospholipid molecules in vitro, it is possible that in the above equations the I stands for a pair of phospholipid molecules. However, such an assumption makes little difference to the basic argument. In fact, the kinetic analysis is really independent of the exact nature of the material labeled I, II, and III.

If we now define a new constant, \(\bar{K}\), as the ratio of \(\frac{K_{Ca}}{K_{Pr}}\), we may combine the two equations for the binding constants (1 and 2) into a single equation:

\[
\bar{K} \cdot \frac{[Ca^{++}]}{[Pr]} = \frac{[Ca - I]}{[Pr - I]} \tag{3}
\]

The experimental observation that procaine decreases the maximum sodium and potassium conductances implies that for this model system much of the phospholipid bound by procaine (Pr-I) is not readily available for the usual depolarization reactions. By contrast, the calcium-bound phospholipid (Ca-I) readily provides, by dissociation, a supply of free phospholipid in configuration I to undergo changes subsequent to a change in the electric field. One further assumption is that with moderate amounts of calcium present, very little of the phospholipid in configuration I is unbound, so that virtually all the phospholipid for the depolarization reactions
is present as Ca-I. Equation (3) then states that the ratio of available to unavailable phospholipid is proportional to the ratio of calcium to procaine in the bathing medium. Thus, normally the gates are maintained in a closed configuration as a result of calcium binding at the gate sites. If this is so, removal of most of the external calcium from the bathing medium would result in significant depolarization of the membrane, because of large increases in potassium and sodium conductances (see Shanes, 1958). Furthermore, excitation fails rapidly when all the external calcium is removed (Frankenhaeuser, 1957; also see Adelman and Adams, 1959).

The threshold phenomena discussed earlier may also be examined from the point of view of the proposed model system. It can be shown that the number of gate sites changing from configuration I to configuration II is a function of the number of unbound sites in configuration I and of the membrane potential (Goldman, 1964). The excitation threshold is then directly related to the change in configuration of a certain minimum number of sites from I to II as well as to the rate of this change. Inspection of equations (1) and (2) readily reveals that either high calcium or the addition of procaine will reduce the number of unbound sites in configuration I. Furthermore, in the presence of high calcium plus procaine the number of these sites will be still less than with either high calcium alone or with procaine in normal calcium. Thus, the threshold potential for the axon in high calcium plus procaine should be higher than with either procaine in normal calcium or high calcium alone as the bathing medium, despite the fact that procaine and calcium compete for binding to the same site.

One other result of this kinetic analysis is that the addition of procaine to this system should increase the ratio of free calcium to unbound phospholipid I and thus make the system act as if the concentration of calcium had been increased slightly. It is therefore interesting to note that the shift of the conductance curves to the right along the voltage axis is somewhat more than can be accounted for solely by a constant-factor decrease in the conductance all along the curve (see Figs. 1, 5, and 7 above, as well as Fig. 3 in Taylor, 1959).

On the basis of the foregoing analysis, we would expect the concentration of (Ca-I) to be proportional to the maximum peak sodium conductance at any procaine concentration, and the concentration of (Pr-I) to be proportional to the magnitude of the decrease in maximum sodium conductance brought about by a given concentration of procaine. A corresponding relationship between the Ca-I concentration and the action potential amplitude would be anticipated. Thus, (Pr-I) + (Ca-I) is equal to (Ca-I)* where (Ca-I)* is the maximum amount of (Ca-I) available in the absence of procaine. Under these conditions, rough estimates of the procaine inhibition of either action potential amplitude or of maximum peak sodium conductance may be ob-
tained from experimental data and put back into equation (3). This calculation has been carried out with data from several different preparations, both vertebrate and invertebrate, and the results are summarized in Table III. As can be seen from the table, there is some variation in the calculated values of $K$, the estimates ranging from 0.2 to 0.66. The variance is quite small, however, considering the large range of calcium and procaine concentrations from which this data was obtained, as well as the somewhat crude methods of analysis employed. Furthermore, this manner of looking at the data may help to explain why, in vertebrates, where the extracellular concentration of calcium is roughly one-tenth of that in marine invertebrates, only about one-tenth the concentration of procaine is needed to reduce the height of the action potential by about the same amount in frog node, for example, as compared to squid or lobster.

4. Effect of Procaine on the Potassium Conductance

A final word should be said concerning the interactions of procaine and calcium with respect to the potassium conductance. The fact that the potassium conductance is less affected by procaine than is the sodium conductance might appear, at first glance, to contradict the model for the mechanism of procaine action suggested above (see Fig. 8) since the percentage decreases in the two conductances would be expected to be the same. However, if the rate constant for the dissociation of the Pr-I complex is significantly smaller than for the Ca-I complex (see above), we would expect the early (sodium current) transient to be over before a significant amount of Pr-I has dissociated. We would then expect procaine to have a greater effect on the time constant for the turning on of the potassium conductance than on the "sodium-on" time constant. Indeed, Taylor’s (1959) data on the effects of

<table>
<thead>
<tr>
<th>Preparation</th>
<th>pH (range)</th>
<th>$\text{Ca}^{++}$ (range)</th>
<th>Procaine (range)</th>
<th>$K$ (avg)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobster axon</td>
<td>6.3-6.5</td>
<td>5.0-91.5</td>
<td>4.0-7.3</td>
<td>0.64</td>
<td>(17) Present study</td>
</tr>
<tr>
<td>Squid axon</td>
<td>7.9</td>
<td>9.5</td>
<td>0.9-3.7</td>
<td>0.23</td>
<td>(7) Taylor (1959)</td>
</tr>
<tr>
<td>Squid axon</td>
<td>8.0*</td>
<td>10.0*</td>
<td>3.7</td>
<td>0.20</td>
<td>(2) Shanes et al. (1959)</td>
</tr>
<tr>
<td>Frog sciatic single</td>
<td>7.9</td>
<td>2.2</td>
<td>0.3-0.5</td>
<td>0.20</td>
<td>(4) Bloom and Schoepfle (1963)</td>
</tr>
<tr>
<td>node</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frog spinal ganglion</td>
<td>7.2</td>
<td>1.8-9.0</td>
<td>0.2-1.6</td>
<td>0.66</td>
<td>(10) Aceves and Machne (1963)</td>
</tr>
</tbody>
</table>

* Estimated.
† Number of values in parentheses.
procaíne on the time parameters in the squid axon are in agreement with this concept. He commented that if both the sodium-on and sodium-off time constant curves were shifted a few millivolts to the right along the voltage axis, these shifts could explain both the increase in time-to-peak of the early sodium transient, and the greater percentage reduction in peak sodium conductance for moderate depolarizing steps than for large depolarizing steps in the presence of procaine. Both these effects would be the expected consequence of a relative increase in the external calcium concentration (discussed above). On the other hand, Taylor observed a large increase in the composite sodium-off plus potassium-on time constant and this could be the effect of a relatively slow dissociation of the Pr-I complex which might nevertheless be rapid enough to affect the measured potassium currents.

This work was performed under Research Task MR-005.08-0020.02. The authors are grateful to Dr. Richard FitzHugh for his help in interpreting threshold phenomena in the Hodgkin-Huxley equations and for suggesting the approach used in Fig. 6. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.

Received for publication 17 August 1965.

BIBLIOGRAPHY