# ANESTHETIC AND CALCIUM ACTION IN THE VOLTAGE CLAMPED SQUID GIANT AXON

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## ABSTRACT

Changes in spike configuration and in the inward and outward currents of voltage-clamped axons agree in indicating that the increases in permeability to sodium and potassium ions during activity are depressed by procaine and cocaine and augmented by calcium. At low levels of depolarization, the effect of the multivalent ion is similar to that of the local anesthetics, in keeping with their similar effects on the threshold of excitability. The reduction of membrane conductance at rest requires a higher concentration of the drugs than that needed to affect the *increase* in permeability with activity.

## INTRODUCTION

The technique of fixing the membrane potential at predetermined levels and of following the time course of membrane current in the squid giant axon (1, 7, 8) has provided a powerful tool for analyzing the electrochemical events associated with the action potential (3–8). This technique, referred to as the "voltage clamp," has revealed an initial inward surge of current followed by a sustained outward current after the sudden application of a maintained depolarization. For a variety of reasons, recently summarized (2 a, 10), the inward current is considered to be primarily the transfer of sodium ions into the fiber and the outward current at late times that of potassium ions carried out. Such ionic movements are the result of an increase in permeability first to sodium and then to potassium. The initial inward and late outward currents will be designated $I_{Na}$ and $I_K$, respectively, for convenience to facilitate evaluation of the action of the agents studied.

Observations on the squid axon (11) and especially on vertebrate nerve, sum-

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marized recently (9), have indicated that a local anesthetic such as cocaine reduces the permeability of the unexcited membrane to sodium and potassium. There is reason to believe that it interferes more strikingly with the increase in permeability to these ions during excitation (10). The results obtained in the research to be described confirm this by the more direct measurements obtainable with the voltage clamp as well as by analysis of the action potential.

## Methods

The voltage clamp procedure, essentially as described by Hodgkin et al. (8), was applied to giant axons isolated from the hindmost stellar nerves of the squid, Loligo pealii. The chamber had approximately the same dimensions as those described by Hodgkin et al., but the axon was mounted horizontally, both ends being cannulated, and a two-wire electrode assembly was inserted through one of the cannulas.[1] Use of a dual beam oscilloscope allowed simultaneous observation of the clamping voltage and of the current flow; the latter was measured as the potential drop across a calibrated length of sea water. Only partial compensation (8) for the effect of resistances in series with the membrane was employed.

In several cases axons were immersed first in sea water, then in sea water plus drug or excess calcium, and then in sea water again. With these, the mean values for the controls were compared with the experimental results. In other instances the preparation was treated first with the experimental solution and the results compared with those following recovery in sea water.

The most successful series provided data on membrane current as a function of clamp depolarization and hyperpolarization as well as on spike configuration and on threshold shock strength. As noted by Frankenhaeuser and Hodgkin (2) and Tasaki et al. (13, 14), in the range of clamp voltage corresponding roughly to threshold depolarization we frequently observed a transitory atypical inward and outward current that became negligible at stronger depolarizations. This gave the appearance of one region of the clamped fiber being more responsive than the rest of the axon, especially since on occasion the inward current was seen to arise with an appreciable latent period as in simple excitation; such a conclusion is also suggested by the appearance of a similar transitory sequence of inward and outward current upon cessation of hyperpolarization, much as in simple break response, again occasionally with an appreciable latent period. High concentrations of local anesthetics applied to the unclamped ends of the axon did not prevent this behavior, as observed by Tasaki and Bak (13). Lower concentrations of these anesthetics added to the clamped regions likewise did not prevent these responses, also as noted by Tasaki and Bak (13).

The observations by Tasaki and Spyropoulos (14) clearly show the presence of non-uniformity in the clamped region that may have been the source of these threshold responses. Other possibilities have been discussed by Frankenhaeuser and Hodgkin

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[1] It is a pleasure to acknowledge our indebtedness to the Instrument Section of the National Institutes of Health for assistance in the design and for the construction of the chamber and electrodes. The chamber was made possible by Mr. L. R. Crisp, Mr. J. DeBrosky, Mr. G. C. Riggle, and Mr. George Norris, and the electrodes by Mr. K. Bolen and Mr. J. Gilliam.

(2). The significance of the cut branches present in these preparations remains to be evaluated. As noted by Frankenhaeuser and Hodgkin (2) and Tasaki and Bak (13), the membrane response is uniform at greater depolarizations. The results to be described are based chiefly on studies with larger depolarizations.

The gain of the clamping amplifier was about 100 and the rise time as measured across the membrane was 20 $\mu$sec. All experiments were carried out at a room temperature of about 25°C.

<div align="center">RESULTS</div>

*Threshold and Action Potential.*—The rising and falling phases of the spike and the following positive "overshoot" or "positive potential" (*PP*) have been related to the increases in sodium and potassium permeability as observed during the voltage clamp (10). Their changes therefore provide a basis for evaluating the action of drugs (10) and can be compared with the results obtained with the voltage clamp technique.

The action potentials studied were those observed, routinely, with stimuli twice or three times threshold before and after a clamp series. The data in Table I are the averages of such action potentials. The same region as that clamped was stimulated with a 100 microsecond pulse delivered through a 200 $\mu\mu$F. condenser to the current electrode; the voltage wire served for observation of the impulse. Since the stimulus was divided between the capacitance of the membrane and the coupling condenser, we were able to observe the strength of this stimulus across the membrane when an all-or-none response was just barely evoked; this has been designated the threshold, *T*, in Table I.

At 0.1 per cent, procaine and cocaine raise *T* by a third, but at half this concentration the threshold is unchanged. Elevation of calcium fivefold is two-thirds as effective in raising threshold as the higher concentration of the local anesthetics.

Calcium resembles the local anesthetics in increasing threshold and positive potential, but differs in its action on other characteristics. Thus, procaine and cocaine reduce spike amplitude, especially at the higher concentration, still more markedly reduce the rate of rise ($\dot{S}$) and fall ($-\dot{S}$) of the spike, especially the latter, and lengthen the half-time of decline of *PP*. Calcium does not significantly alter spike amplitude or the *PP* half-time; more striking is its opposite effect on the rising and falling phases, the latter being accelerated more than the former.

Since the rate of change of membrane potential is proportional to membrane current and inversely to membrane capacitance, $\dot{S}$ and $-\dot{S}$ in Table I can be converted to the maximum inward and outward current during depolarization and repolarization per square centimeter by multiplication by the membrane capacitance of 1 square centimeter. Membrane capacitance is about 1 $\mu$F./cm.$^2$ (9), hence the figures for $\dot{S}$ and $-\dot{S}$ are actually the same as the currents, the units being $\mu$a./cm.$^2$.

The decrease in $\dot{S}$ and $-\dot{S}$ in the local anesthetics indicates interference with

the increase in sodium and potassium conductances, $G_{Na}$ and $G_K$, respectively. The validity of this interpretation is supported by the more direct observations carried out with the voltage clamp. A decrease in $-\dot{S}$ may also be brought about by a delay in inactivation, but no evidence is available that this occurred.

TABLE I

Threshold voltage, $T$, spike amplitude, $S$, maximum rate of development of the spike, $\dot{S}$, the maximum rate of repolarization, $-\dot{S}$, maximum amplitude of the positive potential, $PP$, and half-time of recovery from the latter, $t_{1/2}$, compared for the same axon in sea water (S.W.) and in sea water containing either 0.05 to 0.10 per cent cocaine or procaine (A) or fivefold excess calcium (B). Fiber diameters, $d$, are also given.

| Preparation | Agent | Concentration | $d$ | $T$ | | $S$ | | $\dot{S}$ | | $-\dot{S}$ | | $PP$ | | $t_{1/2}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | S.W. | A | S.W. | A | S.W. | A | S.W. | A | S.W. | A | S.W. | A |
| | | per cent | micra | mv. | | mv. | | v./sec. | | v./sec. | | mv. | | msec. | |
| 14* | Cocaine | 0.1 | 403 | 31.2 | 44.0 | 109 | 87 | 481 | 282 | 380 | 205 | 16.8 | 19.8 | 2.5 | 3.5 |
| 22 | Cocaine | 0.1 | 433 | 29.6 | 41.0 | 113 | 75 | 516 | 239 | 541 | 164 | 13.3 | 17.8 | 2.7 | 4.7 |
| 23 | Cocaine | 0.1 | 402 | 34.4 | 45.0 | 104 | 54 | 628 | 400 | 534 | 209 | 12.1 | 16.0 | 3.4 | 5.0 |
| 27 | Procaine | 0.1 | 586 | 42.6 | 56.4 | 121 | 95 | 582 | 353 | 399 | 205 | 16.2 | 19.1 | 3.6 | 4.0 |
| 29 | Procaine | 0.1 | 470 | 29.0 | 43.0 | 102 | 87 | 452 | 265 | 344 | 201 | 11.0 | 17.0 | 3.8 | 5.1 |
| Average | | | 459 | 33.4 | 45.9 | 110 | 80 | 532 | 308 | 440 | 197 | 13.9 | 17.9 | 3.2 | 4.5 |
| 7* | Cocaine | 0.05 | 392 | 33.3 | 33.3 | 100 | 89 | 342 | 325 | 424 | 279 | 17.5 | 17.9 | 2.4 | 3.3 |
| 9* | Cocaine | 0.05 | 390 | 38.4 | 35.5 | 117 | 108 | 540 | 508 | 391 | 277 | 17.3 | 17.8 | 4.5 | 4.9 |
| Average | | | 391 | 35.9 | 34.4 | 109 | 99 | 441 | 417 | 408 | 278 | 17.4 | 17.9 | 3.5 | 4.1 |
| | | mM | | S.W. | B | S.W. | B | S.W. | B | S.W. | B | S.W. | B | S.W. | B |
| 7* | Ca++ | 50 | 392 | 33.3 | 41.0 | 96 | 95 | 313 | 357 | 290 | 355 | 14.2 | 18.9 | 2.4 | 2.7 |
| 9* | Ca++ | 50 | 390 | 37.6 | 44.9 | 114 | 115 | 510 | 533 | 377 | 488 | 14.2 | 18.4 | 4.6 | 4.5 |
| 14* | Ca++ | 50 | 403 | 32.3 | 37.0 | 108 | 105 | 445 | 450 | 339 | 418 | 16.4 | 18.8 | 3.0 | 2.9 |
| Average | | | 395 | 34.4 | 41.0 | 106 | 105 | 423 | 447 | 335 | 420 | 14.9 | 18.7 | 3.3 | 3.4 |

* Controls in these cases are the averages of values obtained before application and following removal of the experimental agent.

*Voltage Clamp.*—Fig. 1 shows typical curves of peak inward ($I_{Na}$) and outward ($I_K$) current plotted against membrane depolarization ($V$) as affected by cocaine and calcium. These were obtained with one particularly hardy axon.

In cocaine $I_{Na}$ and $I_K$ at each depolarization, as well as the maximum $I_{Na}$ obtained, are smaller than in the controls. $V$ at which $I_{Na}$ reverses direction ($V_{Na}$) is changed but little.[2] In excess calcium, $I_{Na}$ and $I_K$ are smaller than in

[2] Since the peak inward current normally occurs while the potassium current is still negligible (6), little error is involved unless the time of the $I_{Na}$ peak is retarded experimentally more than the rise in potassium current. This is discussed later in reference to shifts that may occur in the membrane potential at which $I_{Na}$ reverses.
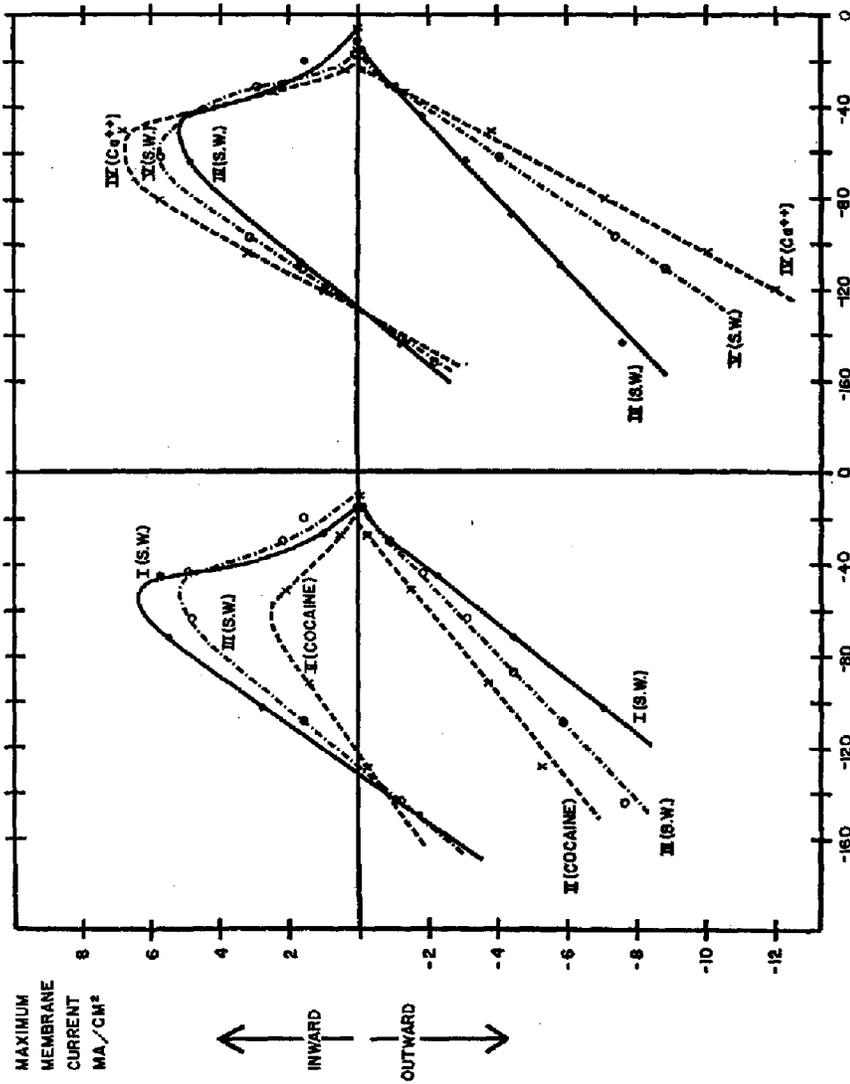
FIG. 1. Peak initial inward current and maximum final outward current as functions of the maintained depolarization. Roman numerals give the sequence in which the indicated solutions were applied. S.W. = sea water, cocaine = sea water with 0.05 per cent cocaine, and Ca++ = sea water containing a total of 50 mM calcium. All curves obtained with axon 9.

the controls only at small depolarizations, whereas over most of the depolarization range $I_{Na}$ and $I_K$ are appreciably larger. Thus, the clamp and spike data are consistent in indicating that the cocaine hampers the increases in $G_{Na}$ and $G_K$. Calcium, on the other hand, favors the increases except at depolarizations below about 40 mv. The last is of interest in view of the threshold data and of other evidence (10) that excitation as well as spike production is dependent on the increase in $G_{Na}$.

## TABLE II

Comparison of Variables Measured on the Same Voltage-Clamped Axons in Sea Water (S.W.) and in Sea Water with Either a Local Anesthetic (A) or Fivefold Elevated Calcium (B)

| Prep-ara-tion | Agent | Con-cen-tra-tion | $MI_{Na}$ | | $V_{Na}$ | | $G_{Na}$ | | $i_{Na}$ | | $MI_K$ | | $G'_K$ | | $i_K$ | | $G_m$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | S.W. | A | S.W. | A | S.W. | A | S.W. | A | S.W. | A | S.W. | A | S.W. | A | S.W. | A |
| | | per cent | ma./cm.² | | mv. | | mmho/cm.² | | A/sec. cm.² | | ma./cm.² | | mmho/ cm.² | | A/sec. cm.² | | mmho/ cm.² | |
| 14 | Cocaine | 0.1 | 3.00 | 0.84 | 112 | 105 | 62.0 | 23.9 | 16.8 | 6.4 | 4.74 | 2.32 | 43.6 | 24.1 | 17.7 | 8.15 | 0.63 | 0.35 |
| 22 | Cocaine | 0.1 | 2.04 | 0.52 | 120 | 110 | 41.4 | 9.8 | 13.0 | 3.1 | 2.66 | 0.56 | 25.4 | 4.9 | 9.22 | 3.60 | 1.79 | 0.63 |
| 23 | Cocaine | 0.1 | 1.90 | 0.50 | 124 | 100 | 38.5 | 17.1 | 70.0 | 6.3 | 1.56 | 0.76 | 14.8 | 7.6 | 5.07 | 2.34 | 1.45 | 1.27 |
| 27 | Procaine | 0.1 | 1.28 | 0.52 | 134 | 136 | 36.7 | 13.0 | 11.6 | 4.0 | 0.79 | 0.47 | 7.6 | 4.5 | 4.30 | 2.23 | 0.95 | 0.70 |
| 29 | Procaine | 0.1 | 0.95 | 0.30 | 129 | 110 | 23.8 | 8.6 | 52.0 | 3.3 | 1.23 | 0.42 | 12.2 | 3.5 | 3.49 | 1.16 | 0.73 | 0.55 |
| Average | | | 1.83 | 0.54 | 124 | 112 | 40.5 | 14.4 | 30.7 | 4.6 | 2.20 | 0.91 | 20.7 | 8.9 | 7.96 | 3.54 | 1.11 | 0.70 |
| 7 | Cocaine | 0.05 | 3.06 | 1.08 | 115 | 105 | 59.5 | 31.6 | 51.5 | 20.0 | 6.98 | 4.30 | 64.8 | 38.8 | 15.3 | 8.4 | 1.06 | 1.10 |
| 9 | Cocaine | 0.05 | 5.47 | 2.90 | 130 | 124 | 86.7 | 69.0 | 30.5 | 15.7 | 7.10 | 5.00 | 67.4 | 53.8 | 26.3 | 16.4 | 0.42 | 0.52 |
| Average | | | 4.26 | 1.99 | 123 | 115 | 73.1 | 50.3 | 41.0 | 17.9 | 7.04 | 4.65 | 66.1 | 46.3 | 20.8 | 12.4 | 0.74 | 0.81 |
| | | mM | S.W. | B | S.W. | B | S.W. | B | S.W. | B | S.W. | B | S.W. | B | S.W. | B | S.W. | B |
| 7 | Ca⁺⁺ | 50 | 2.34 | 3.10 | 108 | 109 | 46.2 | 72.4 | 44.0 | 38.0 | 5.65 | 7.75 | 54.5 | 75.0 | 9.8 | 11.6 | — | — |
| 9 | Ca⁺⁺ | 50 | 5.60 | 6.76 | 126 | 129 | 78.8 | 125.0 | 25.0 | 44.0 | 6.50 | 12.00 | 62.5 | 123.0 | 19.2 | 35.8 | 0.65 | 0.82 |
| 14 | Ca⁺⁺ | 50 | 2.24 | 2.20 | 118 | 112 | 53.5 | 53.5 | 13.2 | 13.3 | 3.04 | 3.52 | 25.2 | 30.7 | 9.8 | 9.4 | 0.59 | 0.71 |
| Average | | | 3.39 | 4.02 | 117 | 117 | 59.5 | 83.6 | 27.4 | 31.8 | 5.06 | 7.76 | 47.4 | 76.2 | 12.9 | 18.9 | 0.62 | 0.77 |

Table II summarizes the various measurements made from such curves and others made directly from our voltage clamp records. For comparative purposes the following are given: The resting conductance, $G_m$, calculated from the inward current during a hyperpolarization of 50 to 100 mv.; the maximum value of $I_{Na}$, $_MI_{Na}$; the maximum rate of rise of $I_{Na}$, $\dot{I}_{Na}$, during a depolarization of 55 mv.; and the maximum value of $I_K$, $_MI_K$, and the maximum rate of rise $I_K$, $\dot{I}_K$, during a depolarization at which $I_{Na}$ is close to zero—about 100 to 120 mv. (the value of $V_{Na}$). From the linear slope of the $I_{Na}$ — V curves at large depolarizations, the maximum sodium conductance, $G_{Na}$, was computed. Similarly a maximum potassium conductance, $G'_K$, was calculated from the linear slope of the $I_K$ — V curves. This is the steady state conductance, not the conductance defined by Hodgkin and Huxley (4), given by $G_K$ =

$I_K/(E_m - E_K)$, which approaches $G'_K$ as the magnitude of clamp depolarization increases. Or, put another way, $G'_K$ and $G_K$ differ in being slope and chord conductances measured in the linear part of the $I_K - V$ curve.

All the measurements are seen to be reduced by procaine and cocaine, the effectiveness of cocaine being about twice as great at 0.1 per cent as at 0.05 per cent. $G_m$ is affected least, being reduced by a third at the higher concentration and affected but little at the lower. Whether the increase in $G_m$ at low cocaine concentrations is significant is doubtful (*cf.* reference 2). The other figures are reduced by more than 50 per cent at higher concentrations of local anesthetic, $G_{Na}$ and $G_K$ being affected to about the same extent, and $\dot{I}_{Na}$ being decreased more than any other characteristic. Only $V_{Na}$ underwent a nearly negligible change in the local anesthetics. Indeed, application of the drugs occasionally left $V_{Na}$ unchanged, whereas removal of the agents caused a small increase. These small changes may not reflect an actual change in $V_{Na}$ but rather a change in the degree of interaction between $I_{Na}$ and $I_K$. Since $\dot{I}_{Na}$ is affected far more than $\dot{I}_K$, the extent to which $I_K$ contributes to a reduction of inward current (since our $I_{Na}$ is uncorrected for a small $I_K$ component), and hence to a shift of the intersection of the latter on the $V$ axis, may vary. The largest shifts in $V_{Na}$ occur in the two preparations (23 and 29) that had the largest changes in $\dot{I}_{Na}$. Earlier studies have shown a negligible effect of cocaine on the resting potential (8 *a*).

In keeping with expectations from the alterations it caused in the spike, excess calcium increased all the variables measured with strong clamping voltages but had no consistent effect on $V_{Na}$. The resting potential is unaffected by increased extracellular calcium (8 *a*). $G_m$ may also have been increased, but additional data are needed to establish this since in another preparation not listed the reverse was observed; the findings of Frankenhaeuser and Hodgkin (2) indicate a reduction in $G_m$ by elevated calcium levels.

## DISCUSSION

Our measurements are consistent with the view that the increase in threshold in local anesthetics and in excess calcium is a consequence of a reduced sensitivity of $G_{Na}$ to depolarization. The drugs differ from calcium in exerting this action at all levels of depolarization and thereby influence spike generation; with calcium not only does this effect disappear at higher depolarizations but also the extent of increase of $G_{Na}$ is substantially greater, in keeping with Frankenhaeuser and Hodgkin's finding that elevated calcium reduces inactivation (2).

While these effects on $G_{Na}$ are no doubt of primary importance in excitation phenomena, the similarity of the effects on $G_K$ is significant on theoretical grounds. For it suggests that the increases in permeability to $Na^+$ and $K^+$ are not as different in mechanism as one might infer from their different time

courses as described by Hodgkin and Huxley (4)—a similarity also evident in the action of $Ca^{++}$ on $G_K$ and $G_{Na}$ (2).

Our results provide further support for the proposal that the locus and mechanism of action of a local anesthetic differ from those of a multivalent ion (10). The latter interacts with the membrane so as to reduce the sites available for passage of monovalent ions at lower depolarizations, yet more sites apparently become available with larger depolarizations. This would be accounted for if (a) $Ca^{++}$ occupies sites that normally become available to the monovalent ions with activity, (b) in its interaction with the membrane, $Ca^{++}$ causes more such sites to appear and occupies them prior to activity, and (c) when depolarization occurs, particularly above a certain level, $Ca^{++}$ is removed from these sites, which then function for the transfer of monovalent ions. The local anesthetics, on the other hand, can cause only a reduction in the availability of sites during depolarization.

It is noteworthy that 0.05 per cent cocaine and 50 mm calcium interfere with the *increase* in permeabilities but not obviously with the resting permeability as reflected by $G_m$ measurements. As proposed on other grounds for cocaine and other agents (9), the action of these agents on the unexcited membrane may not be reflected by changes in $G_m$ because an alteration occurs which involves only a superficial layer of the membrane. It should be remembered, too, that $G_{Na}$ at rest is so small that its modification would not be detectable. Another possibility is that at low concentrations lipide-soluble compounds such as the local anesthetics penetrate the membrane in regions *between* the channels utilized by the ions during activity, thereby exerting a restraint on configuration changes, such as an increase in pore size or pore number, without greatly affecting the channels in the resting membrane (10). The available data cannot serve to distinguish these possibilities.

Tables I and II serve to show that the changes in the spike and positive potential can be qualitatively accounted for in terms of $G_{Na}$ and $G_K$ as defined by voltage clamp measurements and, conversely, that the rising and falling phases of the spike, and the positive potential when present, afford an estimate of the behavior of these variables. It is noteworthy that, in keeping with the principles as enunciated by Hodgkin and Huxley (6), spike amplitude approaches but never exceeds $V_{Na}$. Moreover, spike amplitude, $S$, and the rates of rise and fall of the spike, $\dot{S}$ and $-\dot{S}$, may be reduced without significantly affecting $V_{Na}$. The reduction in $S$ and in $\dot{S}$ in the local anesthetics is attributable to a slowing of the increase in $G_{Na}$ and to its more limited maximum magnitude (inactivation), so that $I_{Na}$, which discharges and reverses the charge on membrane capacitance, is smaller and overtaken by $I_K$ (resulting from the increase in $G_K$, even though this, too, is retarded), which counteracts $I_{Na}$ before it is fully effective and recharges the membrane. A quantitative analysis of the situation requires additional data, particularly in respect of the time course of

inactivation. Our results on this process were not sufficiently extensive to merit presentation.

It was pointed out under Results that $\dot{S}$ and $-\dot{S}$, when multiplied by membrane capacitance, give the maximum net inward and outward currents. If the first is designated $i_{Na}$, since membrane capacitance is about $1\mu F./cm.^2$ (9), we obtain from the controls in Table I a value of about 0.45 ma./cm.$^2$ for $i_{Na}$. The corresponding maximum value under voltage clamp—$_MI_{Na}$, given in Table II—is five to ten times larger. A substantial safety factor is therefore present in ordinary spike production, for only one-fifth to one-tenth of the current (and $G_{Na}$) capabilities revealed during voltage clamps appears to be utilized. Obviously, then, a lowering of $_MI_{Na}$ by a factor of 2 or perhaps somewhat more need not greatly affect $\dot{S}$, but as $_MI_{Na}$ is limited to the values required by the spike, the latter will be depressed. This provides a qualitative basis for the much smaller effect of cocaine, especially at 0.05 per cent, on $\dot{S}$ than on $_MI_{Na}$. A quantitative analysis of the situation is possible by application of the Hodgkin-Huxley relationships. This has not been attempted, but Dr. John Moore (personal communication) reports that such computations do indeed show the much lower sensitivity of spike parameters compared to those measured with the voltage clamp.

The larger positive potential when the calcium of the medium is elevated is consistent with a larger increase in $G_K$. Hodgkin and Huxley (6) point out that the positive potential represents a closer approach to $E_K$ by virtue of $G_K$ exceeding $G_{Na}$ more than in the resting state, hence the membrane, shortly following the spike, more closely approximates a "potassium electrode." A faster shutting off of elevated $G_{Na}$, such as observed by Frankenhaeuser and Hodgkin (2), may also contribute to the larger $PP$ in elevated extracellular calcium; however, a quantitative analysis employing the Hodgkin-Huxley formulation is required to establish its importance.

The larger positive potential obtained in the local anesthetics may also be due to a faster shutting off of increased $G_{Na}$ or to a lower resting level of $G_{Na}$, but direct information on these is not at hand. The larger $PP$ is associated with a lengthening of the half-time of subsidence. The latter may be a consequence of the slowing of the increase in $G_{Na}$ during the decline in positive potential, as suggested by impedance (12) and potential (10) studies. On the other hand, the lengthening of the membrane time constant to be expected from the decreased membrane conductance, $G_m$ (Table II), will account for the behavior of the half-time.

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