Effect of procaine on electrical properties of squid axon membrane

ROBERT E. TAYLOR
Laboratory of Biophysics, National Institutes of Health, Bethesda, Maryland


Procaine (0.025–0.1%; pH 7.9) caused a reduction in the amount and rate of development of the early transient (sodium) and late steady state (potassium) currents which occur during a depolarizing voltage step applied to the excised, voltage-clamped squid axon. Consistent results were obtained by holding the membrane potential at a hyperpolarized value prior to the applied step. No effect was seen on the resting potential, on the sodium equilibrium potential, or on the proportion of the sodium carrying system which was 'inactive' at any membrane potential. The blocking action of procaine is a result of the inhibition by the drug of the sodium carrying system. The effect of procaine on the potassium conductance is such as to oppose the blocking action.

Procaine is a member of a large group of chemically diverse substances which at certain concentrations reversibly depress the activity of peripheral nerve with little or no effect on the resting membrane potential (see, e.g. 1–4). Many general reviews exist (e.g. 5–11) in which references to the voluminous literature may be found. In general, these substances decrease the resting permeability of the membrane to salts and water and decrease the depolarization resulting from increased potassium or rubidium in the medium (12–15). For those which are substituted tertiary amines, as procaine, eserine, etc., the potency increases with pH in a manner which suggests that only the free base is active (16–18 and others). Some of these considerations led to early attempts to relate permeability properties to 'narcosis' (e.g. 6, 12, 19), to later 'permeability theories of narcosis' (7, 8), and to a characterization of the effects of 'anesthetics' as a kind of stabilizing influence on the plasma membrane (6). Lillie stated, (20, p. 393) 'In anesthesia, it is to be assumed that the membrane is so altered that it fails to respond to a change in its electrical potential by an increase in its permeability.' This statement could not be properly understood until it was shown that the essential event in excitation is an initial increase in the permeability of the membrane to a certain ion species rather than a general increase in permeability to all ions. In the case of the squid nerve, under normal conditions, this particular ion species is sodium (21–27). In terms of the work of Hodgkin, Huxley and Katz, the action of a nondepolarizing blocking agent which does not increase the resting permeability must be on the mechanism which controls the relation between the membrane potential and the sodium permeability. For procaine, the possibility that the block could be caused by a large increase in the permeability to potassium for moderate depolarizations in spite of a decrease in the resting permeability, can be seen to be unlikely by the above-mentioned observations that procaine decreases the depolarization resulting from an increase in the external potassium ion concentration.

Results reported by Weidmann (28) indicated that, in Purkinje fibers, procaine amide, as well as cocaine, quinidine sulphate and an antihistaminic, increased the proportion of the sodium carrying system which was inactive in the resting state. Since this was not the result of a simple lowering of resting potential, some relief of the blocking action would be expected from hyperpolarization and 'anodal break' stimulation should be favored. Both results were found by Weidmann for the Purkinje fibers, but for other tissues the reports are not constant (see 10, 11). Since very low procaine concentrations were used by Weidmann—0.005%—and applied for rather long times, the effects he found were perhaps caused by procaine inside the fiber. It will be seen that procaine does not act this way on the squid axon.

The aim of the present paper is to report in some detail the action of procaine on the relations between the membrane potential and the permeabilities to sodium and potassium in the squid giant axon which have been observed by the use of the space and voltage clamp techniques developed by Marmont (29). Cole (30) and Hodgkin, Huxley and Katz (21–27). Results similar to those reported here were found independently by Shanes, Freygang, Grundfest and Amatniek (11, p. 208).
METHODS

The hindmost stellar nerve of the squid (Loligo pealeii) was dissected out and the giant axon either partially cleaned of the surrounding small nerve fibers (1957 experiments), or carefully cleaned of all fibers and much of the loose connective tissue (1956 experiments). Careful cleaning of the axon usually reduced the subsequent survival time, probably as a result of the pulling of small branches, or cutting them off too close. Accurate comparisons are difficult since the methods of measurements used in 1957 were improved over those of 1956, but it appeared that the results obtained with partially cleaned axons would not be greatly changed by careful cleaning.

The object of these experiments was to measure the effect of procaine on the ionic currents which occur during short times following a step change of membrane potential. The technique was essentially that introduced by Marmont (29) and Cole (30) for obtaining a short stretch of nerve in which the current density is uniform and measurable. The manner in which the experiments were done was similar to that described by Hodgkin, Huxley and Katz (22). The analysis of the ionic current during the potential step was not as complete as that of Hodgkin and Huxley (23-26), and many of their results were used in the interpretation of the data presented here.

The principal way in which the technique used in this investigation differed from previous ones was in the use of a single axial electrode (platinized nichrome or platinum) and Ag-AgCl2 external electrodes for supplying current, while the membrane potential was monitored by means of an impaled microelectrode with tip diameter of about 2 microns, filled with 3 M KCl, which had a resistance of about 0.5 megohms, and an external electrode filled with KCl-agar. Each of these electrodes was connected through a calomel half cell to the input of a feedback system which regulated the current in such a way that a step change in membrane potential would be produced (Cole and Moore, in preparation). The same system was used to set the membrane potential to any desired value between pulses.

The chief advantage in using calomel cells and liquid junctions is in the stability of the potential measurements over a considerable period of time. No corrections have been applied for liquid junction potentials (see above) since for most purposes the only relevant assumption made is that they did not change during the course of an experiment. The only check on the latter point came with measuring the potential between the impaling microelectrode and the external reference electrode before penetration and at the end of the experiment when it was withdrawn.

Uncompensated feedback (22) was used throughout, and no corrections are made for any resistance in the neublurea in series with the capacity, which is assumed to be present but small.

Flowing, oxygenated solutions, adjusted to pH 7.9 were used routinely. The temperature was controlled to within 0.5° (31). Because of other, concomitant experiments, two different artificial sea waters were employed. Their compositions are listed in Table I. In these, and other unpublished experiments, no effect of the lack of sulphate ions in the artificial sea water was observed. It is presumed that the membrane of the squid giant axon is impermeable to sulphate ions as is that of the frog skeletal muscle (32).

In all of the experiments reported here the membrane potential was continuously held at a hyperpolarized value. The membrane potential at which the nerve was held between pulses will be referred to as the holding potential, $E_H$, the membrane potential during the pulse will be called $E_p$. The resting potential, $E_R$, is that potential at which the membrane current is zero in the steady state. All of these potentials are absolute, but with no corrections for liquid junction potentials (see above).

# Table I. Solutions Used. Normal Artificial Sea Water and Sulphate-Free Artificial Sea Water

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Sulphate Free</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>431. mM/L</td>
<td>404. mM/L</td>
</tr>
<tr>
<td>KCl</td>
<td>9.1</td>
<td>8.6</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>23.4</td>
<td>21.0</td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>28.0</td>
<td>26.0</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>2.15</td>
<td>2.15</td>
</tr>
<tr>
<td>pH</td>
<td>7.9</td>
<td>7.9</td>
</tr>
<tr>
<td>Axon</td>
<td>57-68</td>
<td>57-68</td>
</tr>
</tbody>
</table>

![Graph of membrane current over time](http://ajplegacy.physiology.org/)

**FIG. 1.** Illustration of the effect of 0.1% procaine at pH 7.9 on the membrane currents following a depolarizing step. In this case membrane was hyperpolarized initially and the step was to an absolute value of $E_p$ equal to $-10$ mV. (A) before, (D) during, and (A) after the application of procaine. Arrows indicate the time of the peak inward current. Axon 57-60, 12.5° C.

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Sign conventions: There is a movement at the present time to adopt the convention of expressing membrane potentials in the same internal potential minus external potential (27). This convention is adopted here for the sake of uniformity. Thus, depolarizing pulses, outward membrane currents and the usual action potential will be positive quantities. Such terms as anodal, cathodal and positive and negative after potential, when used at this time must refer to the opposite convention. It is hoped that these terms will eventually be abandoned.
that the system as affected by procaine was not yet in a steady state, if indeed such exists, and in at least one case the axon was still recovering 40 minutes after return to artificial sea water. It is thus not appropriate at this time to attempt any quantitative correlation between concentration and effect or to place too much emphasis on recovery or lack of it (cf. 28).

RESULTS

Resting potential. In no experiment with procaine was there any effect on the resting potential that could reasonably be ascribed to the drug. For three experiments in which it was carefully measured after about 20 minutes in 0.1% procaine, the change in resting potential was depolarization of 1.3, 1.5 and 2.5 mv. This is well within the range of depolarization which occurs normally during this time with excised squid axons. There was some indication that the presence of the procaine slowed down the rate of deterioration usually found. Such a result need not be surprising in view of the fact that deterioration is in part caused by the leakage of sodium ions into the nerve at a higher rate than they are actively extruded and there there is evidence that the resting inward leakage of sodium is decreased by nerve depressants of this type (e.g. 15, 34).

Ionic current and membrane potential. Figure 1 illustrates the effects of 0.1% procaine on the membrane currents following a depolarizing step. The most striking action is a marked reduction in the amplitude of the early transient peak. In addition, the time to this peak is delayed, the steady state current is delayed and its final value decreased.

The value of the current at the transient peak, \( I_N \), and during the steady state, \( I_N' \), for various values of the absolute membrane potential during the step, \( E_p \), is represented for a typical experiment in figure 2. Also indicated in this figure is the value of the membrane potential just prior to the step, \( E_{m} \), and the resting potential, \( E_R \). It is immediately clear that the action of procaine is to reduce the amount of membrane current at all values of membrane potential, both during the transient phase and the steady state. It might be emphasized that this axon was sufficiently hyperpolarized during the entire experiment so that none of the sodium carrying system was in a refractory condition just prior to the applied depolarizing step.

Peak sodium conductance. The sodium conductance is defined (23) as

\[
\delta S_N = \frac{I_N}{E_p - E_{m}},
\]

where \( I_N \) is the current carried by the sodium ions, \( E_p \) the value of the membrane potential during the voltage step and \( E_{m} \) the membrane potential at which no net sodium current flows. We are interested in the value of the sodium conductance at the time of the peak transient current as seen in figure 1 and represented as \( I_N' \) in

![Diagram](image-url)
Neglect of the potassium current component at the time of the peak transient current could have more serious consequences, but will be unlikely to affect the results by more than a small percentage. In this case also the procedure of hyperpolarizing the membrane prior to the application of the depolarizing step has desirable features, since the resting potassium conductance is normally less under these conditions than it is at the resting potential. This has the effect of reducing the size of the step of membrane current which occurs at the beginning of the step of applied potential and to delay the onset of the potassium current increase (35).

Under these conditions a good approximation to the sodium conductance at the time of the transient peak is assumed to be given by

$$g_{Na}' = \frac{I_{Na'}}{E_p - E_0}$$

where $I_{Na'}$ is the total current at the peak and $E_0$ is the value of $E_p$ such that $I_{Na'}$ is zero. In addition to the above considerations, it may be noted that both $I_{Na'}$ and $(E_p - E_0)$ are numerically smaller than the corresponding $I_n$ and $E_n - E_0$. So that the errors which do occur cancel to some extent. It is further assumed that the total current contains negligible contributions from the active movements of sodium and potassium. This would be the case if these movements were rigidly coupled to form an electrically neutral transfer system, a possibility discussed by Hodgkin and Keynes (36), or if the normal secretory mechanisms were not operating very efficiently in these excised axons. Certainly excised squid axons gain sodium (37-38) when kept in sea water. In any event a direct indication that the secretory mechanisms are not involved in any direct way is given by the result of Hodgkin and Keynes (36) that the prevention of sodium extrusion by dinitrophenol does not alter the

At this time (26) in addition to the sodium current, the total current is composed of a potassium current which is increasing with time as well as a small contribution of all of the other permeable ions. The last is designated the 'leakage' current and is assumed to have an equivalent conductance which does not vary with membrane potential. If so, then the leakage conductance in the present experiments could not have exceeded 1.0-2.0 mmho/cm² because conductances of this order were measured under certain conditions, viz. during the step return of the membrane potential at the end of an applied pulse from about 60 mv to $E_n$, and thus may be neglected.
resting membrane potential or the rapid sodium movements associated with the conduction of impulses.

Effects of procaine on $g_{Na}'$. The conductance at the peak of the sodium current during a potential step as computed by the above relation is plotted for a typical experiment in figure 4, against the absolute value of the membrane potential during the step, $E_p$.

Two different effects are apparent from this graph. The main effect of the procaine ($0.1\%$) is to decrease the value of $g_{Na}'$ for all values of $E_p$, but also the curve is shifted slightly in such a direction that larger depolarizations are required in the procaine to produce a given fraction of that maximum conductance found at large depolarizations.

The effect of procaine on $g_{Na}'$ is shown in table 2, for seven experiments, and the shift of the value at which $g_{Na}'$ was one-tenth of the maximum in table 3, for three experiments. The recovery was never complete. On the average the conductance decreased in the procaine to 0.39 and recovered to 0.74 of its initial value. The average shift of the one-tenth maximum point was 6 mv in the experiments. The recovery was never complete. On the average the conductance decreased in the procaine to 0.39 and recovered to 0.74 of its initial value. The average shift of the one-tenth maximum point was 6 mv in the procaine to 0.39 and recovered to 0.74 of its initial value. The average shift of the one-tenth maximum point was 6 mv in the procaine to 0.39 and recovered to 0.74 of its initial value. The average shift of the one-tenth maximum point was 6 mv in the procaine.

Restoration of procaine was an accelerating process, which makes comparison difficult. However, the lack of full recovery seen in these experiments could fall within the normal range.

<table>
<thead>
<tr>
<th>Axon</th>
<th>$E_h(1/10)$</th>
<th>Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>D</td>
</tr>
<tr>
<td>57-36</td>
<td>-38</td>
<td>-34</td>
</tr>
<tr>
<td>57-60</td>
<td>-37</td>
<td>-27</td>
</tr>
<tr>
<td>57-68</td>
<td>-51</td>
<td>-47</td>
</tr>
</tbody>
</table>

Aver. 6 3

Table 4 shows the effect of $0.1\%$ procaine at pH 7.9 on Relation Between Steady State Inactivation and Membrane Potential in 2 Experiments

<table>
<thead>
<tr>
<th>Axon</th>
<th>Temp. °C</th>
<th>$E_h$</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>D</td>
<td>A</td>
</tr>
<tr>
<td>57-36</td>
<td>20</td>
<td>-41</td>
<td>-41</td>
</tr>
<tr>
<td>57-68</td>
<td>10</td>
<td>-52</td>
<td>-52</td>
</tr>
</tbody>
</table>

$E_h$ and $k$ obtained by fitting normalized inactivation curve to $h = 1/[1 + \exp. (E - E_h)/k]$. B, before; D, during; A, after procaine.

Huxley (26) the transient sodium current during a potential step is characterized by the product of three parameters, i.e. $g_{Na}E_ph$, where $g_{Na}$ is a constant and the time courses of $m$ and $h$ depend on potential. This method of breaking the transient current down into an on ($m$) and an off ($h$) process is not unique, as they pointed out, but has been a useful and successful way of fitting the data. It is not possible without further systematic work to completely specify how the effects of procaine on the sodium conductance at the peak and the time to this peak are to be described in terms of the effects on $g_{Na}$ and the time constants, $\tau_m$ and $\tau_h$ associated with the rates at which $m$ and $h$ assume their new values. The simplest conclusion which is consistent with the data is that for rather large depolarizing steps the time constants, $\tau_m$ and $\tau_h$ are not greatly changed by procaine but that the value of $g_{Na}$ is decreased.

On this view the increase in the time to the peak inward current for depolarizing steps of moderate size would be the result of changes in the time constants $\tau_m$ and $\tau_h$. While the data do not demand it, an attractive possibility is that the curves of $\tau_m$ and $\tau_h$ versus membrane potential are shifted slightly in the direction of depolarization. If these curves have the form given by the analysis of Hodgkin and Huxley (26) a shift of some 5 mv would be sufficient to not only increase the time to the peak inward currents by the amounts found, but would also yield a greater percentage reduction in the magnitude of the peak current for moderate depolarizing steps than for large, as noted above.

Inactivation in steady state. In two experiments the fraction of the sodium carrying system which was not refractory in the steady state, $h$ was measured in the usual way ($\Delta V$) by observing the peak inward current at some convenient $E_p$ as affected by the value of the membrane potential before application of the pulse.

No shift of the inactivation curve was found in these experiments. In figure 5, the results are given of one experiment (57-36 20°C). The normalized peak inward current during a potential step to $-7$ mv is plotted against the value of the membrane potential before the step. Table 4 shows the effect of $0.1\%$ procaine at 10°C and 20°C. $E_h$, the potential at which inactivation is 50%,
where \( I_K \) is the magnitude of the current carried by potassium ions, \( E_p \) the membrane potential during the step and \( E_K \) the membrane potential at which no net potassium current flows. During the steady state, an approximation to \( g_K \) is given by

\[
g_K = \frac{I_K}{E_p - E_K},
\]

and \( k \), an inverse measure of the steepness of the inactivation curve, were obtained by fitting the experimental points with the equation used by Hodgkin and Huxley (25), which with the present sign convention is

\[
h = \frac{1}{1 + \exp [(E - E_0)/k]}.
\]

The difference of the control value of \( E_h \) for these two nerves is probably not due to the fact that the solution bathing one nerve contained no sulphate, or that the temperature differed. In a total of seven experiments in which the control value of \( E_h \) was measured in solutions without sulphate, \( E_h \) varied from 41 mv to 53 mv with an average of 48.2 mv. These seven axons were also measured at temperatures from 10°C to 20°C and no correlation with temperature existed. In one (unpublished) experiment—in sulphate containing artificial sea water, axon 57-60—the temperature was shifted from 15°C to 22.5°C with no consequent change in the value of \( E_h \), in agreement with the result of Hodgkin and Huxley (33).

It is thus seen that procaine has no effect on the inactivation in the resting state such as that seen by Weidmann on Purkinje fibers, or that seen on the squid axon in the resting state such as that seen by Weidmann. Thus a slow recovery is superimposed on a normal decline. The fall of \( g_K \) in procaine, however, is much too large to be accounted for by a deterioration process in excised axons. This particular point clearly needs further systematic work.

**Potassium conductance.** As for the sodium conductance, the potassium conductance is defined by

\[
g_K = \frac{g_K'}{E_p - E_h},
\]

where \( g_K' \) is the total membrane current in the steady state and \( E_h \) is the resting membrane potential. This approximation is rather good for large depolarizations but can be quite inaccurate in the range \( E_h < E_p < -30 \) mv.

**Effect of procaine on \( g_K \).** The approximate potassium conductance in the steady state during a depolarizing step of voltage is plotted for a typical experiment in figure 6, against the membrane potential during the step. In general, the effect of procaine is similar to that seen for the sodium conductance, but smaller. The results of six such experiments are shown in table 5. The conductance as calculated in the manner described above is listed for values of voltage during the step, \( E_p \), of 0 and +60 mv. It appears from the table that on the average, \( g_K' \) is reduced by 20% and the recovery is not very complete. Two different effects probably contribute to the lack of complete recovery. The recovery from procaine seems to be a slow process and the normal deterioration process in excised axons is associated with a decrease in the conductance to potassium for large depolarizations. Thus a slow recovery is superimposed on a normal decline. The fall of \( g_K' \) in procaine, however, is much too large to be accounted for by a deterioration process. This particular point clearly needs further systematic work.

**Potassium current time constant.** A conspicuous feature of the effect of procaine on the ionic current during a potential step is the increase in the time required for the steady state to become established. That part of the current time curve after which the steady state current has reached about 75% of its final value was approximated by an exponential and the time constants, \( r_{na} \), thus determined are shown for a typical experiment, plotted against the membrane potential during the step, in figure 7. The increase in the time constant thus determined was greater for depolarizing pulses near \( E_p = 0 \) than for pulses near the sodium potential. The time constants

**TABLE 5. Effect of Procaine at pH 7.9 on Approximate Value of Potassium Conductance in the Steady State (gK'), for Values of Membrane Potential (Eh) of Zero and 60 mv**

<table>
<thead>
<tr>
<th>Eh (mv)</th>
<th>B</th>
<th>D</th>
<th>A/B</th>
<th>Ratio</th>
<th>B</th>
<th>D</th>
<th>A/B</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
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<tr>
<td>60</td>
<td>0.66</td>
<td>0.70</td>
<td>1.0</td>
<td>0.94</td>
<td>0.70</td>
<td>0.94</td>
<td>1.0</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Figures in parentheses are in arbitrary units. B, before; D, during; A, after procaine. For concentrations of procaine and temperatures see table 1.
which were found at $E_p = 0$ and $+50$ mV arc listed in Table 6. The average increase at zero is about 70% as compared to about 40% at $E_p = +50$ mV.

A complication in the interpretation of these results arises from the fact that the development of the steady state ionic current is determined by the simultaneous increase in the potassium current and decrease in the sodium current. Thus, the time constant $\tau_{th}$ as determined here is a composite of the potassium on, $\tau_k$, and the sodium off, $\tau_s$, time constants. Since these two time constants arc of the same order of magnitude, the composite time constant, $\tau_{th}$, will be influenced more by the value of $\tau_k$ when the peak inward current is large compared to the steady state outward current (for moderate depolarizing steps) and by $\tau_s$ where the reverse is true (for larger depolarizing steps). At the sodium potential, no sodium current will be flowing and the increase in the time constant in this region must indicate an increase in the time constant of the process which is responsible for turning the potassium conductance on.

The greater increase in the time constant under discussion at moderate depolarizations than at large could be accounted for by the above-mentioned possibility that the curve relating the time constant for the sodium off process to membrane potential was shifted in the direction of depolarization.

**DISCUSSION**

It is assumed in this paper that adequate methods were used to obtain a short length of squid axon over which the membrane potential was controlled. It is also assumed that any variation in the current density over this region resulting from inhomogeneities in the properties of the membrane were not large enough to introduce any serious errors in the interpretation of the data. Both of these assumptions have been questioned in a recent series of articles (39-41); neither can be completely defended at this time. Further investigations are in progress bearing on these points and the matter will not be pursued here.

Assuming, then, that the currents as measured are the average ionic currents through a voltage controlled region of membrane, one may state, without recourse to any other theories or empirical formulations, that the blocking action of procaine, and presumably of any non-depolarizing blocking agent, is explained by the reduction of the transient inward current which occurs following a depolarizing step of membrane potential. This reduction is not a result of a change in the sodium equilibrium potential (cf. Fig. 9), which means that the forces tending to drive sodium inward are unchanged.

A reduction in the magnitude of this inward current with no change in the relationship of ‘inactivation’ to membrane potential and with only minor alterations in the time constants involved suggests a simple reduction in the carrying capacity of the system which allows sodium ions to be transported across the membrane in response to concentration and electrical potential gradients. Increase in the concentration of sodium ions in the external medium should partly counteract the effects of procaine, as happens in frog nerve (42, 43), but this would be expected regardless of the reason for the reduction of inward current. Partial relief of the depressing effects of low concentrations of procaine would be expected with electrical hyperpolarization of the nerves used in this study in spite of the fact that the level of ‘inactivation’ was unchanged. There are two reasons for this: a) electrical hyperpolarization (in this case) would reduce the resting potassium conductance, which, according to the empirical equations of Hodgkin and Huxley, would lower the threshold for excitation; b) at the resting potential, before the application of procaine,
the nerves were in a state of partial inactivation and, although this level was not changed by the procaine, the removal of this inactivation by hyperpolarization could counteract depressing effects however caused. It is thus important to know, not only the effect of hyperpolarization on the depressing effect of a drug, but also the relation of this effect to the effects of hyperpolarization before the drug was applied before any conclusions regarding ‘inactivation’ may be drawn. However, even with these factors in mind, it would appear that procaine does effect the curve of inactivation versus membrane potential in Purkinje fibers (28) and frog nerve (42, 44–46). For further discussion of these points and other references, see Shanes (11, pp. 184 and 228). Whether this difference between the squid axon and other excitable cells is fundamental or trivial remains to be seen.

The situation with regard to the effect of procaine on the potassium conductance is considerably more straightforward. In this case the predominant effect is simply a reduction in the permeability of the membrane by a factor almost independent of membrane potential. This could be a result of the complete removal of a certain proportion of the sites through which potassium ions flow, but the increase in time constant associated with changes in conductance with membrane potential makes it appear more likely that each individual site is affected. Beyond this point, only speculation is possible. Certainly the effects of procaine are completely different from those of increased external calcium ion concentration where the potassium conductance for large depolarizations is virtually unchanged (39, and ourselves, unpublished), as is the case for sodium as well.

In the squid axon, excitation following depolarization occurs when the inward sodium current exceeds the outward potassium plus chloride current. It would be expected, then, that a reduction of the potassium conductance by procaine would tend to lower the threshold and thus partly counteract the blocking action of the drug. Dr. FitzHugh has recently examined this effect in detail, with the aid of the analog computer, for the set of empirical equations derived by Hodgkin and Huxley (26), using the values they gave for a temperature of 6.3°C. A 20% decrease in the potassium conductance ($g_K$ in their terminology) reduced the threshold depolarization considerably; a reduction of 30% resulted in the nerve model being just on the edge of spontaneous firing.

Considerably more experimental work is needed before results of this kind can suggest any mechanism for the action of procaine on the processes which control the sodium and potassium conductances. When a physical or molecular theory of the relation between membrane potential and ion conductances is forthcoming the mechanism of action of many agents, including procaine, will probably be understood almost at once. It is hoped that studies of the kind reported here will aid in the formulation of such a theory.

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