1968

Effect of Low Sodium, Tetrodotoxin, and Temperature Variation upon Excitation

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ABSTRACT The lowering of external sodium raised both the constant quantity threshold, Q_o, and the rheobase, I_o, in both real space-clamped squid axons and the theoretical axon as computed on the basis of the standard Hodgkin-Huxley equations. In both real and theoretical axons the minimum intensity for excitability for short pulses, which occurs at about 15°C, was still present when low sodium replaced seawater. Low sodium did not affect the temperature dependence of the strength-duration relationship in the range, 5° to 25°C. The excitability of tetrodotoxin-treated real axons was found to be more temperature-dependent than that of normal real axons. Also the data on dosage-response to TTX of real axons fit the dose-response relationship of a hypothetical system in which one TTX ion binds reversibly to its receptor to produce a fraction of the inhibitory effect, the curve being identical to a simple adsorption isotherm. The Hodgkin-Huxley equations describe the broad outline of events occurring during excitation quite well.

INTRODUCTION

In 1962 the effect of temperature upon threshold membrane voltage and current of space clamped squid axons was investigated, using the double sucrose gap technique (Guttman, 1962). When it became apparent that pulse duration was of considerable significance in any such inquiry, the work was extended (Guttman, 1966) and strength-duration curves were obtained for square wave current pulses from 10 μsec to 10 msec and at temperatures from 5° to 35°C.

The change in threshold potential, at which an action potential separated from a subthreshold response, averaged 17 mv at 20°C with a Q_{10} of 1.15. The average rheobase current was 12 μamp/cm^2 at 20°C with a Q_{10} of 2.35 compared to 2.3 obtained previously. At short times the threshold charge was $1.5 \cdot 10^{-8}$ coul/cm^2. This was relatively independent of temperature and sometimes showed a minimum in the temperature range. At intermediate times and at all temperatures the threshold currents were less than for both the single time constant model and the two factor excitation process as developed

by A. V. Hill. FitzHugh performed computer investigations on the effect of temperature on the excitation of the squid axon membrane as represented by the Hodgkin-Huxley equations (FitzHugh, 1966). These were in general in good qualitative agreement with our experimental results but showed significant quantitative differences.

The present experiments are an attempt to ascertain the effect of decreased sodium concentrations upon the strength-duration relationship, especially the temperature dependence of this effect since both Hoyt (1965) and Stein (personal communication) have emphasized the importance of testing any physical model with temperature data. The present report summarizes the results obtained during the summer of 1965 in Woods Hole, where low sodium and later tetrodotoxin, "puffer fish poison," were used to bathe the outside of space-clamped squid axons. The results on real axons are compared with the computed results upon the standard theoretical axon of Hodgkin and Huxley. Wherever calculated values are presented in the figures or discussed in the text, they refer to those computed by Cooley and Dodge at our request and described in the Appendix.

METHOD

The apparatus and procedures were essentially the same as those described in the previous paper (Guttman, 1966). The experimental arrangement was as shown in Fig. 1 of that paper. Square wave pulses were provided by a Tektronix 161 pulse generator at one pulse per second and were applied to platinized platinum electrodes located in compartments A and C of a nerve chamber divided internally into five compartments, A, B, C, D, and E. Current was indicated on the upper beam of a Tektronix model 502 dual beam cathode ray oscilloscope connected across a 1 kΩ resistor in series with the active current lead. The membrane potentials were measured between the two Ag/AgCl electrodes in compartments C and E and recorded on the lower beam of the oscilloscope. Resting potentials were monitored by a DC millivoltmeter. The length of the central seawater compartment, C, was 0.8 mm, and the two sucrose compartments (B and D) were each 6.5 mm long. The extreme compartments (A and F), each 15 mm long, were filled with 200 mM KCl, in order to give zero potential at the ends of the axon in these compartments. The solutions in the seawater and sucrose compartments were continuously circulated, flowing up from below, and being sucked off from above, to maintain constant levels above the axon. The sucrose levels were slightly higher than the seawater level to provide a higher hydrostatic pressure on the sucrose and thereby reduce the contamination of the sucrose by seawater. Vaseline seals separated the sucrose from the seawater compartments.

Only two complete strength-duration runs were carried out since their characteristics—a constant quantity threshold at short durations (cf. Gildemeister and Weiss, 1909) and a constant current threshold (rheobase) at long durations—agreed with the results previously published (Guttman, 1962, 1966) and the calculations of Cole et al. (1955). Thereafter, determinations of values for short pulses (50 μsec) and long rheo-

basic pulses (5 msec) were considered sufficient in lieu of entire runs in the interest of saving time and sparing the axons.

In the first series of experiments, Na^+ was partially replaced with $choline^+$, leaving the Cl^- concentration unchanged. Artificial seawater solutions were made up and isosmotic ChCl was substituted for various percentages of NaCl therein. The formula for the artificial seawater solution used was as follows (mM): 430 NaCl, 10 KCl, 10 $CaCl_2$, 50 $MgCl_2$, and 5 Tris, adjusted to pH 7.3–7.5.

RESULTS

Experiments with Low Sodium The importance of the sodium ion in spike generation was shown by Hodgkin and Katz (1949 *a*) and confirmed by Cole

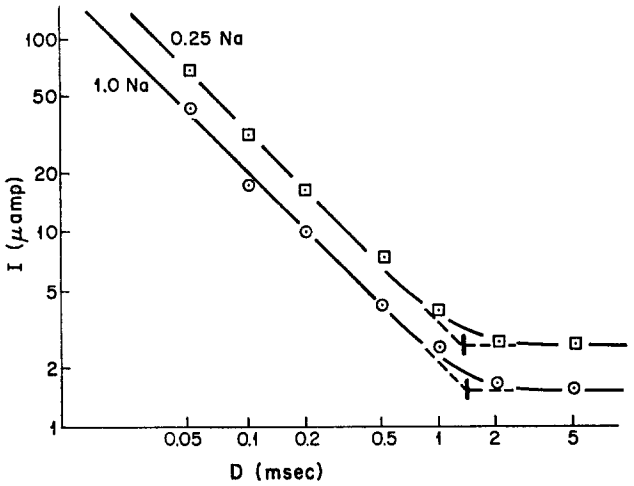

FIGURE 1. Strength-duration curves for axon in seawater (⊙), and in seawater in which 75% of the sodium has been replaced by choline (⊡). Duration of square wave pulses in milliseconds, D, is plotted against the current in microamperes, I, both on log scales. All points taken on the same axon at 5°C. Vertical bars represent τ, as defined in the text, for the two different concentrations of sodium.

(1953, 1955) who established that the rate of rise and amplitude of the squid axon spike depends upon the extracellular sodium concentration. By comparing voltage clamp records for the squid giant axon surrounded by seawater and by sodium-free solutions respectively, Hodgkin and Huxley (1952 *a*) were able to demonstrate that the initial phase of the inward current associated with activity was normally produced by the sodium ion. Since the significance of the sodium ion in nerve activity had been so clearly demonstrated, the effect of lowering the sodium content of the external medium upon excitability in a space-clamped squid axon has been investigated. A study of the temperature dependence of the observed effects should prove valuable in understanding the basic processes involved.

The same method of plotting the data was used in this series of experiments (both pulse duration and threshold current were logarithmically plotted, Fig. 1) as in the 1962 paper. This method is helpful for demonstrating the constant quantity threshold for short pulses and the constant current threshold for long rheobasic pulses. It is also advantageous since the point of intersection of the lines representing the constant quantity threshold for short pulses, Q_o, and for long rheobasic pulses, I_o, is a measure of $\tau = Q_o/I_o$, the time constant of excitation.

The data presented in Fig. 1 show that although low sodium raises the current threshold for both short and rheobasic pulses, it does not affect the

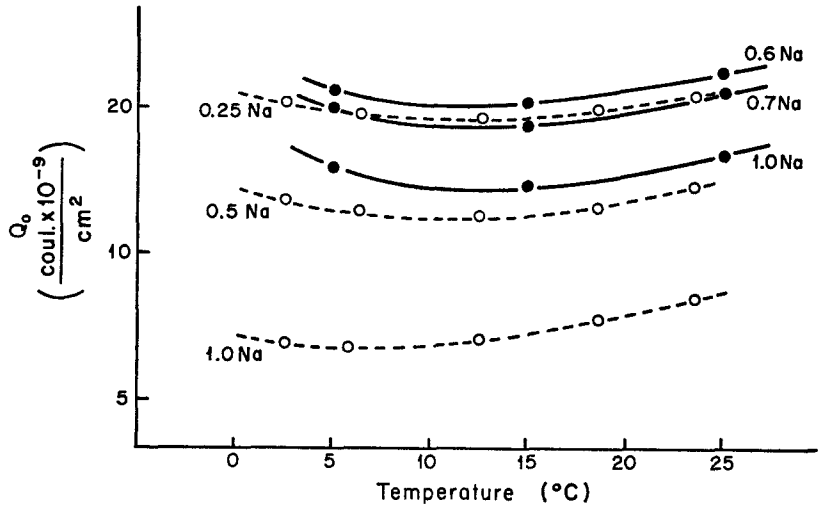

FIGURE 2. Threshold quantity of current for short shocks, Q_o, in coulombs $\times 10^{-9}$/cm on a logarithmic scale vs. temperature in °C on a linear scale at different sodium concentrations for the experimental and theoretical axon. The solid lines and points are all experimental results on the same axon. Dashed lines and open circles are computed.

strength-duration relation; i.e., the shape of the curve, nor τ, the time constant of excitation.

It is possible to estimate ΔV, the point where the trace of the local subthreshold response deviates from the action potential, directly from the photographic records (cf. Fig. 2, Guttman, 1966) where a just subthreshold pulse is followed by a threshold pulse and the two records are superimposed in a double exposure. But this point is somewhat arbitrary in both experimental records and computations and the ΔV so obtained is not a particularly accurate measure. Therefore, it seemed better to use quantities which were measured directly with some precision; viz., the threshold current of the stimulus and its duration. From these the constant quantity at short shocks,

Q_o, may be calculated:

$$Q_o = \frac{I_s\, t}{A}$$

where I_s is the short shock threshold current, t is pulse duration, and A is the effective area of membrane in the experimental compartment, C. The deter-

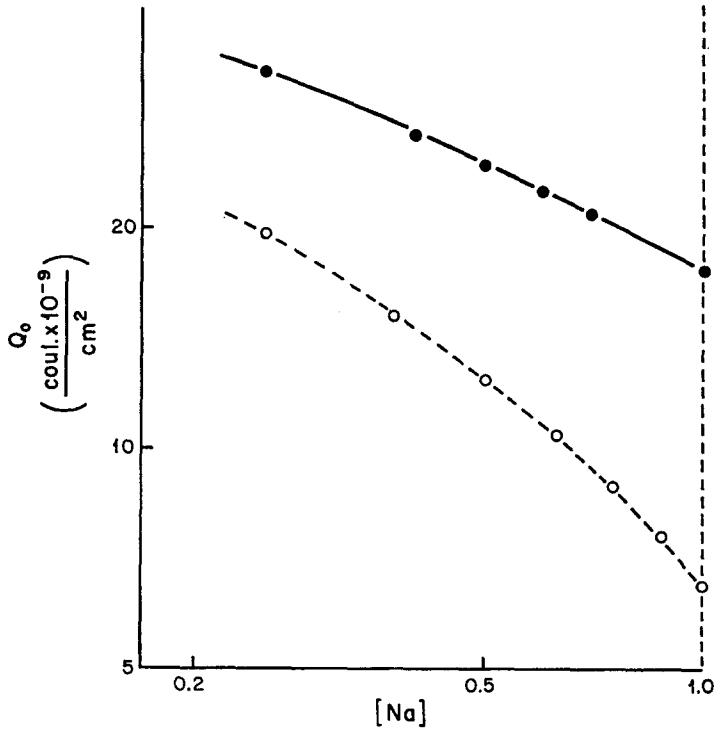

FIGURE 3. Constant quantity threshold at short shocks (50 μsec), Q_o in coulombs × $10^{-9}/\text{cm}^2$, vs. Na concentration both on logarithmic scales. Solid line and points are experimental averages from 16 runs on 8 axons. Dashed line and open circles are computed values.

mination of the effective area, A, presented some problems since vaseline seals (later abandoned) were used in this series of experiments. The method used to estimate it was the same as that described in the earlier paper.

In Fig. 2, the temperature dependence of Q_o for the experimental axon at different sodium concentrations is compared with a similar study for the theoretical axon. The calculated ΔV's, the sudden displacement in membrane potential which results in an action potential, were multiplied by $1\mu\text{F}/\text{cm}^2$, the established capacity of the squid axon membrane, to give Q_o for the theoretical axon.

Our experimental results, based on an assumption of 0.06 cm^2 of effective membrane area, show that decreasing the sodium content of the bathing solutions does not affect the temperature dependence of Q_o. The shape of the curve remains unaltered and the minimum still appears at about 15°C in the low sodium curves.

In Fig. 2, all points were taken on the same experimental axon and to reduce deterioration sodium concentrations below 0.6 of the normal concentration of Na were nol used. It was found, however, that for lower sodium concentrations

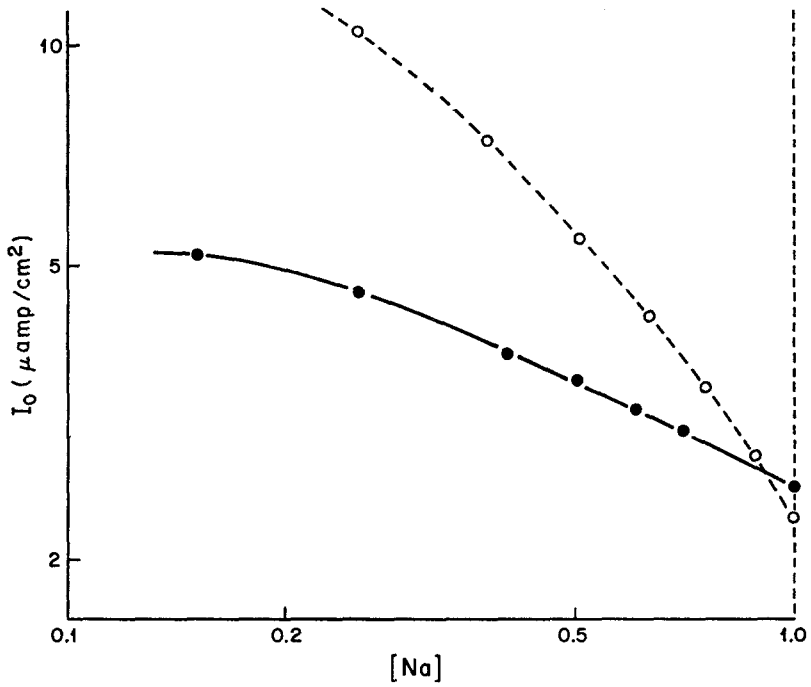

FIGURE 4. Rheobasic threshold (I_o/cm^2) vs. Na concentration both on logarithmic scales. Solid line and points are experimental averages from 12 runs on 8 axons. Dashed line and open circles are computed values.

on the theoretical axon the curves became flatter and the minimum shifted to a somewhat higher temperature value as sodium was decreased (Fig. 2).

In a total of eight runs on three experimental axons where Q_o was tested at 5°, 15°, and 25°C, a minimum was found at 15°C whether the axon was in seawater (three runs) or in low sodium solutions (five runs). This confirms the experimental results published in the 1966 paper and also the theoretical findings reported by FitzHugh (1966). He found that the temperature minimum shifted from 6° to 19°C as the condition of the theoretical axon was improved by a factor of four (from $A = 1$ to $A = 4$, in his notation). Our result is thus well within these limits.

It is interesting, incidentally, that Cooley and Dodge (1966) report that computations on the propagating axon have shown that short shock threshold decreased monotonically with temperature. Thus a qualitative difference between the space-clamped and the free axons in this respect is predicted but there seem to be no experiments for comparison.

In Fig. 3, the effect of variation in sodium concentration upon Q_o for both experimental and theoretical axons is presented somewhat differently. Aver-

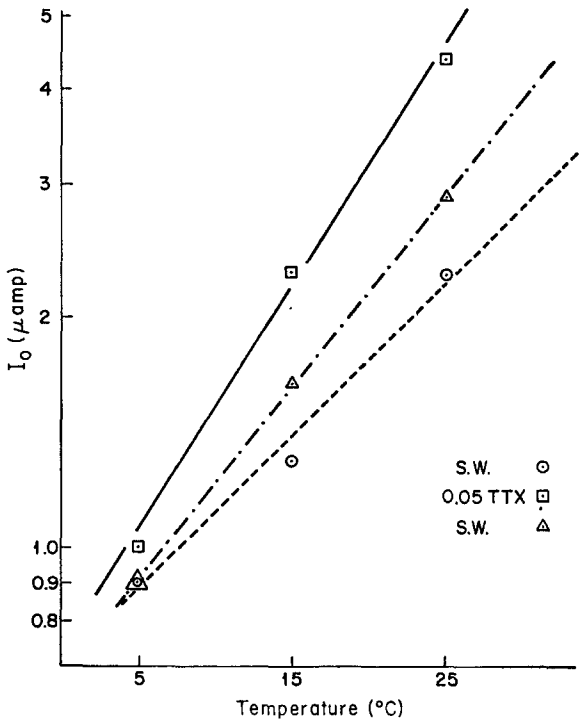

FIGURE 5. Temperature dependence of tetrodotoxin effect upon rheobase. Rheobasic current, I_o, in microamperes, is plotted against temperature in °C. First data were taken with the axon in seawater (⊙). Then the axon was treated with an 0.05 μg/ml solution of tetrodotoxin (⊡). Then partial recovery in seawater (△) is shown. All data taken on the same axon. One of two similar experiments.

ages of experimental results from six axons at a number of temperatures are presented assuming an effective area of 0.06 cm². Variation of temperature (between 5° and 25°C) did not affect the shape of the curve significantly.

The effect of low sodium upon Q_o for the standard theoretical axon at the standard temperature of 6.3°C was examined at higher resolution and these results are likewise presented in Fig. 3.

The effect of variation of sodium concentration upon rheobasic threshold for both the experimental and theoretical axon is shown in Fig. 4. Data from

six experimental axons at a number of temperatures are presented and the curves again slid vertically for best fit. The composite plot was positioned as as in the case of Fig. 3. Here again temperature variation does not affect the shape of the experimental curve significantly. Rheobasic values for the theoretical axon were obtained at 6.3°C. It is clear from Figs. 3 and 4 that decreasing sodium raises both the constant quantity threshold and rheobasic threshold in both experimental and theoretical fibers.

If Figs. 2 to 4 had been plotted as the change in threshold, rather than the absolute stimulus intensities, then for Fig. 2 the experimental curves would be pretty well bracketed by the theoretical computations and Figs. 3 and 4 would show that the theoretical axon is about twice as sensitive to sodium reduction as the average experimental axon.

Experiments with Tetrodotoxin Since it has been claimed that tetrodotoxin (TTX) affects specifically the inward sodium current in voltage-clamped axons (Narahashi et al., 1964; Nakamura et al., 1965; Watanabe et al., 1967 *a*; Moore et al., 1967; Watanabe et al., 1967 *b*), we investigated whether it could be substitued for low sodium in our experiments and give similar results. This was indeed found to be the case.

A decrease in threshold related to concentration occurred with each treatment when sufficiently high concentrations were used. In most cases, the rise of threshold due to drug treatment was faster than the recovery in seawater.

In the case of four fibers, although the action potential spike was markedly diminished with tetrodotoxin treatment, corroborating the work of Kao and Fuhrman (1963), the reversal of the effect with seawater was amazingly successful even for quite high concentrations, associated with much diminished spike heights.

In the case of two other fibers it was not possible to treat the axon repeatedly with tetrodotoxin. The initial exposure to the drug (0.01 and 0.05 μg/ml concentration, respectively) and the related increase in threshold resulted in an irreversible loss of excitability, and the diminished spike was finally replaced by a graded response.

In Fig. 5, the temperature dependence of the tetrodotoxin effect upon rheobasic threshold is illustrated. All points are taken on the same axon. Rheobasic threshold was determined in each run at 5°, 15°, and 25°C, respectively. In the first run, the axon was immersed in seawater, in the second run it was in 0.05 μg/ml tetrodotoxin, and in the third run partial recovery in seawater occurred. It is apparent that the tetrodotoxin effect upon threshold is much greater at higher temperatures. An experiment of the same design was carried out on a second axon, with similar results.

The data presented in Fig. 5 indicate that TTX has a positive temperature coefficient for blocking. In recently published work, Narahashi and Anderson

(1967) have been able to establish that allethrin, a potent blocking agent which affects both Na and K conductance, has a negative temperature coefficient as far as blocking is concerned.

The dosage response to tetrodotoxin is shown (Fig. 6) using the method of presentation utilized by Hille for TEA (cf. Fig. 6, Hille, 1967) and for saxitoxin (cf. Fig. 4, Hille, 1968). In Fig. 6, experimental data from four axons all at 20°C are presented and the increased rheobase, I_o', compared to normal I_o is shown as an excitability, I_o/I_o', and is compared with a simple adsorption

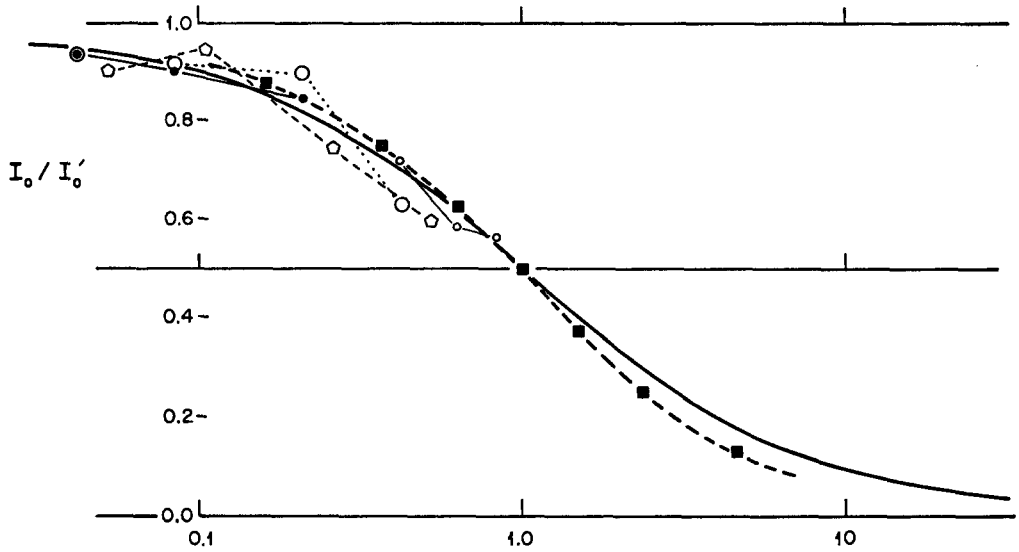

FIGURE 6. Dosage response to TTX. Experimental data from four axons in which excitability (1/normalized rheobase) is compared with a simple adsorption isotherm (heavy solid line). 1/normalized rheobase on a linear scale vs. relative concentration of TTX on a logarithmic scale. The data are slid horizontally for best fit. The concentrations for 50% excitability (or doubling of the rheobase) were 0.23, 0.23, 0.19, and 0.12 μg/ml TTX. Computed curve (dashed line, solid squares), based on assumption that TTX affects sodium conductance, normalized by sliding horizontally 0.7 unit to the left. See text for details.

isotherm:

$$N = \frac{\beta N_o}{\alpha T + \beta}$$

where N equals the number of open sites, N_o equals the number of sites, T equals the concentration of TTX, and α and β are forward and reverse reaction rates. The concentrations of TTX at which a doubling of the rheobase (50% excitability) occurred were 0.23, 0.23, 0.19, and 0.12 μg/ml.

Computed $\bar{g}'_{Na}/\bar{g}_{Na}$ data were normalized (solid squares, dashed line) on the relative TTX concentration curve (Fig. 6) by assuming that 50% decrease in Na conductance corresponds to 100% increase in rheobase. This is not strictly the case, as can be seen in Fig. 7 where $\bar{g}'_{Na}/\bar{g}_{Na}$ for the computed axon on a linear scale is plotted against I_o/I'_o on a linear scale. If such normalization is carried out, however, the computed points (squares) fit quite well (Fig. 6) on the simple adsorption isotherm curve.

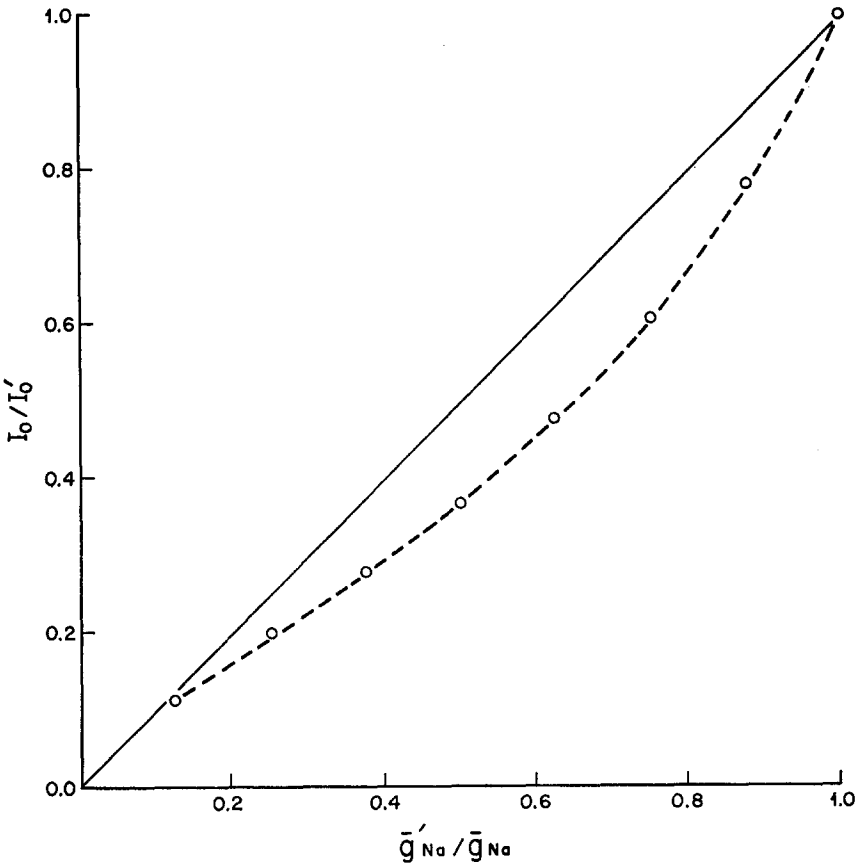

FIGURE 7. I_o/I_o' vs. $\bar{g}'_{Na}/\bar{g}_{Na}$ for calculated axon, dashed line. See text for details.

DISCUSSION

Certain conclusions emerge from the comparison of the effect of treatment of experimental and theoretical axons with low sodium and tetrodotoxin.

In the case of both the experimental and computed axons, lowering the sodium content of the bathing solution raises both constant quantity and rheobasic thresholds but does not affect the shape of the strength-duration curve nor τ, the time constant of excitation.

In real axons the blocking effect of TTX has a positive temperature coefficient. Also the data on dosage-response to TTX of real axons fit the dose-response relationship of a hypothetical system in which one TTX ion binds reversibly to its receptor to produce a fraction of the inhibitory effect, the curve being identical to a simple adsorption isotherm. When changes in rheobase values are plotted against sodium conductance changes for the computed axon, these computed data also fit fairly well on the adsorption isotherm curve.

It has been suggested that the guanidinium group of the TTX molecule or of saxitoxin becomes lodged in the "gate" of the early transient channel on the nerve membrane surface, thereby blocking the movement of any ions (cf. Kao and Nishiyama, 1965; Narahashi, Anderson, and Moore, 1967), although an alternate hypothesis is suggested by Nakamura et al. (1965).

Narahashi et al. (1960) suggested that in muscle fibers, tetrodotoxin blocks excitability through selective inhibition of the sodium-carrying system without affecting the potassium-carrying system. Tetrodotoxin blockade of sodium conductance increase was demonstrated in voltage-clamped lobster axons by Narahashi et al. (1964) and in clamped squid axons by Nakamura et al. (1965).

Selective inhibition of the sodium-carrying system was obtained with urethane in voltage clamp experiments on the nerve cells of *Onchidium* (Hagiwara and Saito, 1959). Narahashi (1964) found that *N*-butanol blocks the sodium mechanism rather selectively, for the maximum rate of rise of the action potential is decreased more than is the rate of fall without accompanying appreciable change in the resting potential. (The evidence that the maximum rate of rise may be regarded as a measure for the inward current was described by Hodgkin and Katz (1949 *b*) and by Narahashi (1961) for the propagated action potential, and by Hodgkin and Huxley (1952 *b*) for the space-clamped membrane action potential.)

In our experiments with space-clamped axons tetrodotoxin also decreased the maximum rate of rise of the spike more than the rate of fall. In addition, very little change in resting potential accompanied the action of moderate doses of tetrodotoxin. This corroborates the findings of many (Narahashi et al., 1964). Loss of excitability (replacement of the action potential by a graded response) well preceded disappearance of the resting potential when lethal doses were used.

Decrease in maximum rate of rise of the spike also occurred in our experiments when low sodium seawater was used on space-clamped axons, utilizing the double sucrose gap technique. This corroborates the work of Hodgkin and Katz (1949 *b*) with internal microelectrodes.

In general, the experiments presented here are not inconsistent with the belief that the primary action of local anesthetics is not on the mechanism

that determines resting potentials but on the mechanism that produces action potentials. It was shown long ago (Guttman, 1939) that narcotics (isoamyl carbamate, chloroform, etc.) affect membrane resistance but not membrane capacitance. Shanes, Freygang, Grundfest, and Amatniek (1959) and Taylor (1959) by means of voltage clamp experiments on squid axons showed that cocaine and procaine suppressed the rise of sodium and potassium conductances which occurs upon depolarization.

To summarize, the experiments reported here indicate that the Hodgkin-Huxley equations describe the broad outlines of events occurring during excitation very well. The computations based on the Hodgkin-Huxley equations are indeed in good qualitative agreement with data from real axons but, as is not surprising, they cannot mimic every aspect of the excitation process without some further refinement.

Thanks are due to Dr. C. Y. Kao for the sample of purified crystalline tetrodotoxin used in these experiments. We are also grateful to Dr. K. S. Cole for many helpful suggestions.
This work was aided by Grant GB 3301 from the National Science Foundation and Grant NB 07259-01 from the National Institutes of Health.

Received for publication 19 October 1967.

Appendix

Computation of Threshold for the Hodgkin-Huxley Model of the Squid Axon

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Solutions of the Hodgkin-Huxley (1952 *b*) equations, modified as described below, were computed for the membrane action potential (space-clamp constraint) by standard procedures (FitzHugh and Antosiewicz, 1959).

In order to simulate the effect of reduced sodium ion concentration it is necessary to take into account not only the reduction of the sodium equilibrium potential but also a reduction in the effective sodium conductance. The attenuation of the sodium component of the ionic current measured in the voltage clamp was quantitatively accounted for with an equation derived from the "independence principle" by Hodgkin and Huxley (1952 *a*, Equation 12). This was used in the present computations with the following procedure: denoting the standard sodium equilibrium potential by V_{Na}, and the equilibrium potential in reduced sodium concentration V'_{Na}; then at each time step of the integration $g_{Na}(V, t)$ was calculated; the value of the sodium current (I_{Na}) which would have been obtained under standard conditions was calculated from $I_{Na} = g_{Na}(V - V_{Na})$; and from this the sodium current for the reduced sodium concentration (I'_{Na}) was determined from the independence principle rewrit-

ten in the form:

$$I'_{Na} = I_{Na} \cdot \frac{\exp\{(V'_{Na} - V) F/RT\} - 1}{\exp\{(V_{Na} - V) F/RT\} - 1}$$

For the computations plotted in Figs. 6 and 7 we have followed FitzHugh (1966) in multiplying all the ionic conductances by a factor of four (designated $A = 4$) to more accurately represent the ionic current densities measured in voltage clamp experiments at Woods Hole. For the computations plotted in Figs. 2–5, the standard Hodgkin-Huxley axon was used, where $A = 1$. We have assumed that the only effect of temperature is to change the empirical rate constants with a Q_{10} of 3.

REFERENCES

COLE, K. S. 1953. Ions, potentials and the nerve impulse. *Naval Med. Res. Inst. Lecture and Rev. Series No. 53–57*, 89.

COLE, K. S. 1955. Ions, potentials and the nerve impulse. *In* Electrochemistry in Biology and Medicine. T. Shedlovsky, editor. John Wiley and Sons, Inc., New York. 129.

COLE, K. S., H. A. ANTOSIEWICZ, and P. RABINOWITZ. 1955. Automatic computation of nerve excitation. *J. Soc. Ind. Appl. Math.* **3**:153.

COOLEY, J. W., and F. A. DODGE, JR. 1966. Digital computer solutions for excitation and propagation of the nerve impulse. *Biophys. J.* **6**:583.

FITZHUGH, R. 1966. Theoretical effect of temperature on threshold in the Hodgkin-Huxley nerve model. *J. Gen. Physiol.* **49**:989.

FITZHUGH, R., and H. A. ANTOSIEWICZ. 1959. Automatic computation of nerve excitation—detailed corrections and additions. *J. Soc. Ind. Apol. Math.* **7**:447.

GILDEMEISTER, M., and O. WEISS. 1909. Über indirekte Muskelreizung durch Stromstösse und Strompausen. *Arch. Ges. Physiol.* **130**:329.

GUTTMAN, R. 1939. The electrical impedance of muscle during the action of narcotics and other agents. *J. Gen. Physiol.* **22**:567.

GUTTMAN, R. 1962. Effect of temperature on the potential and current thresholds of axon membrane. *J. Gen. Physiol.* **46**:257.

GUTTMAN, R. 1966. Temperature characteristics of excitation in space-clamped squid axons. *J. Gen. Physiol.* **49**:1007.

HAGIWARA, S., and N. SAITO. 1959. Voltage-current relationships in nerve cell membrane of *Onchidium verruculatum*. *J. Physiol., (London)*. **148**:161.

HILLE, B. 1967. The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. Gen. Physiol.* **50**:1287.

HILLE, B. 1968. Pharmacological modifications of the sodium channels of frog nerve. *J. Gen. Physiol.* **51**:199.

HODGKIN, A. L., and A. F. HUXLEY. 1952 *a*. Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol., (London)*. **116**:449.

HODGKIN, A. L., and A. F. HUXLEY. 1952 *b*. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol., (London)*. **117**:500.

HODGKIN, A. L., and B. KATZ. 1949 *a*. The effect of temperature on the electrical activity of the giant axon of the squid. *J. Physiol., (London)*. **109**:240.

HODGKIN, A. L., and B. KATZ. 1949 *b*. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol., (London)*. **108**:37.

HOYT, R. 1965. Discussion report. *J. Gen. Physiol.* **48**(5, Pt. 2): 83.

KAO, C. Y., and F. A. FUHRMAN. 1963. Pharmacological studies on tarichatoxin, a potent neurotoxin. *J. Pharmacol. Exptl. Therap.* **140**:31.

KAO, C. Y., and A. NISHIYAMA. 1965. Actions of saxitoxin on peripheral neuromuscular systems. *J. Physiol.*, (*London*). **180**:50.

MOORE, J. W., T. NARAHASHI, N. C. ANDERSON, and M. P. BLAUSTEIN. 1967. Tetrodotoxin: comments on effects on squid axons. *Science.* **157**:220.

NAKAMURA, Y., S. NAKAJIMA, and H. GRUNDFEST. 1965. The action of tetrodotoxin on electrogenic components of squid giant axons. *J. Gen. Physiol.* **48**:985.

NARAHASHI, T. 1961. Effect of barium ions on membrane potentials of cockroach giant axons. *J. Physiol.*, (*London*). **156**:389.

NARAHASHI, T. 1964. Restoration of action potential by anodal polarization in lobster giant axon. *J. Cellular Comp. Physiol.* **64**:73.

NARAHASHI, T., and N. C. ANDERSON. 1967. Mechanism of excitation block by the insecticide, allethrin, applied externally and internally to squid giant axons. *Toxicol. Appl. Pharmacol.* **10**:529.

NARAHASHI, T., N. C. ANDERSON, and J. W. MOORE. 1967. Comparison of tetrodotoxin and procaine in internally perfused squid giant axons. *J. Gen. Physiol.* **50**:1413.

NARAHASHI, T., T. DEGUCHI, N. URAKAWA, and Y. ONKUBO. 1960. Stabilization and rectification of muscle fiber membrane by tetrodotoxin. *Am. J. Physiol.* **198**:934.

NARAHASHI, T., J. W. MOORE, and W. R. SCOTT. 1964. Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. *J. Gen. Physiol.* **47**:965.

SHANES, A. M., W. H. FREYGANG, H. GRUNDFEST, and E. AMATNIEK. 1959. Anesthetic and calcium action in the voltage clamped squid giant axon. *J. Gen. Physiol.* **42**:793.

TAYLOR, R. E. 1959. Effect of procaine on electrical properties of squid axon membranes. *Am. J. Physiol.* **196**:1071.

WATANABE, A., I. TASAKI, I. SINGER, and L. LERMAN. 1967 *a*. Effects of tetrodotoxin on excitability of squid giant axons in sodium-free media. *Science.* **155**:95.

WATANABE, A., I. TASAKI, I. SINGER, and L. LERMAN. 1967 *b*. Tetrodotoxin: comments on effects on squid axons. *Science.* **157**:221.