Some Relations between Action Potential and Resting Potential of the Lobster Giant Axon

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ABSTRACT Experiments were performed to determine the quantitative relation existing between action potential and resting potential of the lobster giant axon. Resting potential changes were induced by either increasing the external potassium concentration or by reducing the external calcium concentration. For either treatment the action potential amplitude is proportional to the logarithm of the resting potential minus a constant. This constant is equivalent to the minimum resting potential at which a propagated spike is possible, and is larger for depolarization in low calcium than in high potassium. Thus the change in action potential per unit change in resting potential is greater in low external calcium than in high external potassium. Analog computer solutions to the Hodgkin-Huxley equations for squid axon membrane potentials show that, if the initial conditions are properly specified, the action potential is proportional to the logarithm of the potassium potential minus a constant. The experimental results and the analog computations suggest that reducing external calcium produces changes in the invertebrate axon that cannot be accounted for solely on the basis of alterations in the potassium potential.

A reduction in the concentration of external calcium ion invariably results, in the lobster giant axon, in a reduction in both resting potential and action potential (Dalton (1958)). Whether these two processes are absolutely linked has not been clear. There have been, however, certain indications that calcium may have at least a dual role in nerve membrane function (Adelman and Adams (1959); Dalton (1959)). The object of the present studies was to inquire further into this question, and in particular to attempt to determine whether one of these roles may be associated with the resting potential and another with the action potential. As a first approximation, the problem may be stated as a rather simple question: "May the reduction in action

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potential in low calcium solutions be attributed solely to the simultaneously occurring reduction in resting potential?"

The main difficulty in distinguishing between effects on the resting potential on the one hand and effects on the action potential on the other is the absence of adequate information as to the relationship between these two potentials. Intuitively, one might expect that a given change in resting potential would be reflected by an equal change in action potential, since the action potential must "start" from the level of the resting potential. However, where data are available for this preparation (Dalton (1958) and unpublished data), substances which reduce resting potential effect a disproportionately greater reduction of the action potential. In high external potassium, for example, the action potential is progressively reduced to zero when the resting potential is reduced by about 15 to 20 per cent. At least two interpretations are possible for such results: (1) that the primary action is on the resting potential, and that the magnitude of the resting potential governs the magnitude of the action potential, or (2) that direct effects are exerted on both the resting potential and the action potential. These two possibilities might be stated in a more operational way as (1) that the action potential is the same function of the resting potential for all agents, or (2) that different agents give different relations between the two potentials.

Changes in external potassium concentration have classically been regarded as primarily affecting the resting potential.[1] Although there seems to be no reason to expect that changes in external potassium should have a direct effect on the action potential process (other than effects governed by resting potential changes), this possibility cannot be excluded. As an approach to the problem of a possible dual role for calcium, however, one can make the initial assumption that the primary effect of changes in potassium is only on the resting potential. One can then compare the effects of high external potassium with those of low external calcium in reducing transmembrane potentials. If the resulting changes in action potential per unit change in resting potential are the same for potassium and calcium, no information concerning a dual role for calcium is obtained. If, however, low calcium should produce a greater reduction of action potential per unit change in resting potential than does high potassium, there is a strong suggestion that there is a completely different relation between the resting potential and action potential processes when calcium is involved than there is when potassium is involved. This argument will be discussed more fully after presentation of the results.

Apparently, no careful analysis has been made of the relation between

[1] Inasmuch as there is a voluminous literature dealing with the effects of high external potassium on a wide variety of neural tissues, the reader is referred to the exhaustive review article by Shanes (1958).

resting potential and action potential while both are being reduced by some external factor. Before examining the effects of reduced external calcium, therefore, it was necessary to determine the relationship between resting potential and action potential in high external potassium solution.

Materials and Methods

Conventional recording techniques were used for intracellular recording of resting and action potentials by means of a capillary microelectrode. The preparation consisted of one of the giant axons from the circumesophageal connectives of the lobster (*Homarus americanus*). Lobsters were obtained fron the local fish market; they arrive there daily from Nova Scotia, packed in iced boxes.

Details of the dissection and recording techniques have been previously described (Dalton (1958)). The present apparatus did not differ significantly.

"Normal" lobster perfusion solution was a slight modification of that of Cole (1941) (see Dalton (1958)). Experimental solutions containing high potassium were kept isotonic by a corresponding reduction in sodium. The percentage change necessary for sodium in such cases has been shown to be too small to produce an appreciable change in transmembrane potentials. For low calcium solutions, isotonicity was maintained by the addition of dextrose. Solutions were continuously circulated through the system at a rate of about 2 cc./min. The temperature in the chamber was maintained at 10–12°C.

Because of an appreciable "dead space" in the circulating system and the nerve chamber, and because the nerve chamber contained the whole nerve bundle, an appreciable time (about 10 minutes) was required for the nerve to reach a new steady state for changes in external potassium concentration. For this reason, only one experimental solution was required to obtain a range of measurements of changing potentials. Measurements were made at 1 minute intervals (or less) during the time between the introduction of the high potassium solution and the reaching of a new steady state. No difference was noted in the ratio of potentials measured in this manner from those measured at a steady state in various external potassium concentrations.

Resting potential was monitored continuously by means of a strip chart recorder. The record was marked at each measurement of the action potential, so that measurements of the two potentials could be exactly correlated. Magnitudes of action potential were estimated to the nearest millivolt from projected film records. Resting potential measurements were estimated to the nearest tenth of a millivolt by microscopic examination of the strip chart record. We do not maintain, of course, that the absolute magnitude of the resting potential can be measured to the nearest tenth of a millivolt (or even to the nearest millivolt), but we do believe that changes of potential may be validly estimated to the nearest tenth of a millivolt.

RESULTS

Relation between Resting Potential and Action Potential in High External Potassium

Measurements of resting and action potentials in high external potassium were made on seventeen axons. In ten of these axons the effects of changes in potassium ion concentration were the only studies made. For these axons, the external potassium concentration was varied between 2.5 and 35 mM (10 mM being the "normal" potassium concentration). In the other seven axons, the effects of high potassium were compared with the effects of low calcium, and an external concentration of 15 mM potassium was the only experimental concentration used.

The results of a typical experiment using one of the higher values (25 mM)

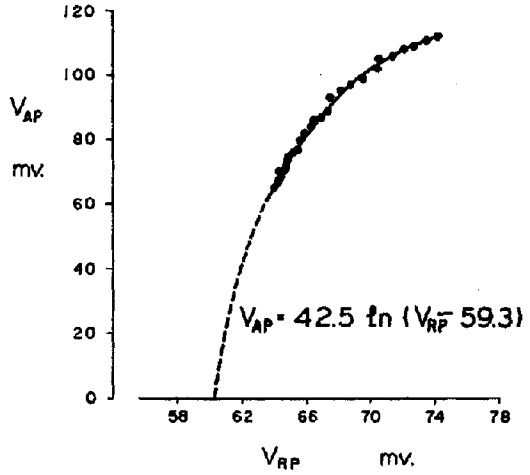

FIGURE 1. The relation between action potential (V_{AP}) and resting potential (V_{RP}) for changes in potentials induced by high external potassium. The curve drawn through the points is calculated according to the equation given. Results from a typical experiment.

of external potassium are shown in Fig. 1. Here the magnitude of the action potential (V_{AP}) is plotted as a function of the magnitude of the resting potential (V_{RP}). In such experiments the action potential was not allowed to be reduced to zero, because of the appearance of irreversible effects at such high potassium concentration. However, it was evident from an extrapolation of such curves that the action potential is related to the logarithm of the resting potential above a certain minimum value (that is, the logarithm of the resting potential minus a constant). Such constants may be approximately deter-

mined by extrapolation, and a more exact value determined by trial and error for the best fit of the points. The points shown in Fig. 1 are replotted in Fig. 2 as the magnitude of the action potential *versus* the natural logarithm of the resting potential minus this constant (V_0). An equation describing this relationship may then be determined. The curve drawn through the points in Fig. 1 is the calculated curve according to the equation given. The general empirical equation expressing the relationship between resting potential and action potential is given by

$$V_{AP} = k \ln[(V_{RP} - V_0)/V_1]$$

in which V_0 is the constant which must be subtracted from the resting potential to obtain a linear relationship between the action potential and the logarithm of the resting potential, and V_1 is a scale factor having a value of 1 mv. The constant V_0 is obviously related to the magnitude of resting potential at which the action potential goes to zero, and actually, because of the proper-

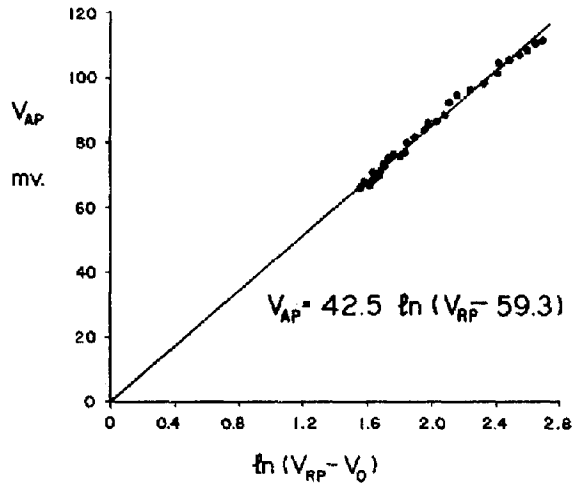

FIGURE 2. Data from the same experiment as shown in Fig. 1. The data are replotted to show the relation between the magnitude of the action potential (V_{AP}) and the natural logarithm of the resting potential minus a constant ($V_{RP} - V_0$).

ties of the logarithmic term, equals the value of the resting potential at which the action potential disappears, minus 1.

Such constants can be determined for a narrower range of change of potentials as well; no essential difference was noted whether the range of change was large or small. In a few preliminary determinations of such constants for low external potassium (increasing potentials), the same general relationship was shown.

Twenty-five determinations of such constants (V_0) and the slopes of the

lines so obtained (k) were made on seventeen axons (all for high external potassium), and are shown in Table I. Calculations of means and standard errors of the means (S.E.M.) give a value for V_0 of 54.6 ± 0.9 and for k of 35.8 ± 0.6. Although standard deviation determinations indicate a moderately small variability (see Table I), it should be pointed out that the equation so derived is not particularly useful as a "predictor" for the actual value expected for a given resting potential (or conversely). The slope of the curve (V_{AP} *versus* V_{RP}) is quite steep, particularly at lower potential values, so that small

TABLE I

Axon No.	V_0	k	Axon No.	V_0	k
1	61	41	10	47	36
	62	42	11	50	33
	59	41		52	35
2	58	37	12	57	32
	58	37	13	60	35
	58	39	14	48	37
3	54	32		50	36
4	53	37	15	50	33
5	59	33		54	35
6	48	38	16	55	37
7	55	37		51	33
8	50	33	17	59	33
9	57	32			
			Mean	54.6	35.8
			Standard error of mean	0.9	0.6
			Standard deviation	4.6	3.1

changes in either V_0 or k produce relatively large changes in the ratios of the two potentials.

Relation between Resting Potential and Action Potential in Low External Calcium

A nominal zero calcium concentration was used for these studies. The effects produced on transmembrane potentials by such a solution do not tend to reach a steady state in a relatively short time as do the effects produced by high external potassium. The resting potential, in low calcium solutions, shows an approximately exponential decline during the first 10 to 12 minutes' exposure, followed by an approximately linear decline. Because of the differences in the time course of effects in low calcium and in high potassium, 15 mM external potassium was chosen as the concentration which gave approximately the same reduction in resting potential as did the nominal zero calcium

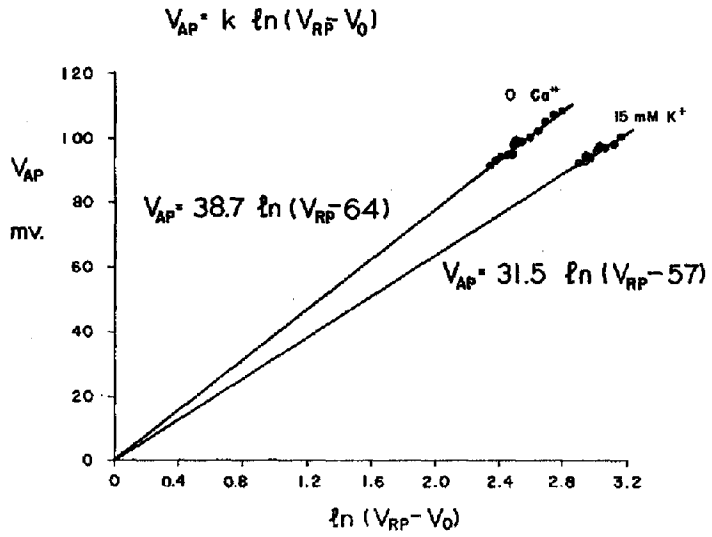

FIGURE 3. A comparison of data for high external potassium and low external calcium. Lines drawn through the points are according to the equations given. Results from a typical experiment.

TABLE II

Axon No.	$V_0(Ca^{++})$	$V_0(K^+)$	Ratio (Ca:K)	$k(Ca^{++})$	$k(K^+)$	Ratio (Ca:K)
11	64	50	1.28	46	33	1.39
	70	52	1.35	69	35	1.97
12	64	57	1.12	39	32	1.22
13	65	60	1.08	41	35	1.17
14	62	48	1.29	53	37	1.43
	—	50	—	—	36	—
15	61	50	1.22	40	33	1.21
	63	54	1.17	45	35	1.28
16	65	55	1.18	48	37	1.30
	66	51	1.29	44	33	1.33
17	65	59	1.10	35	33	1.06
Mean	64.5	53.3	1.21	46.0	34.4	1.34
S.E.M.	0.8	1.2	0.03	3.0	0.5	0.08

solution acting for a period of 10 minutes. To eliminate the problem of variability from axon to axon, both solutions were tested on each axon.

The results from a typical experiment of this sort are shown in Fig. 3. Although this figure has compressed scales to show the extrapolation to zero action potential, it can be seen that the points for low calcium do represent a different population from those for high potassium. A more careful examination on expanded scales has shown that the same general relationship between

the action potential and the resting potential is obtained for low calcium and for high potassium. However, in every case tested (seven axons, with a total of ten determinations for low calcium and eleven for high potassium), the values for V_0 and k are markedly different for the two types of ionic variations. The values so obtained for the seven axons are shown in Table II. The mean and S.E.M. for the V_0 values are 53.3 ± 1.2 for high potassium and 64.5 ± 0.8 for low calcium. Similar differences are obtained for the proportionality constant k, inasmuch as k and V_0 are related, for a given axon with V_{AP} and V_{RP} specified. Application of the conventional "t" test shows that the differences between the two populations of V_0 values or the two populations of k values are highly significant. Another approach is to compare such values for each axon (thus eliminating any problem of variability from axon to axon). If one considers the ratio of V_0 for low calcium to V_0 for high potassium for each pair of runs on a particular axon, the ratio is always greater than 1 (mean 1.21 ± 0.03); similar results are obtained for k (1.34 ± 0.08).

Such results indicate, then, that the action potential would be expected to disappear at a higher resting potential in low calcium solutions than in high potassium solutions; or, stated somewhat differently, that the change in action potential per unit change in resting potential is greater in low external calcium than in high external potassium.

DISCUSSION

Inasmuch as the relationship between action potential and resting potential here described was somewhat unexpected, and had not been previously reported, the question immediately arose as to whether such a relationship would be predicted by the Hodgkin-Huxley equations (Hodgkin and Huxley (1952)) for the non-propagated action potential of the squid giant axon. We are extremely grateful to Dr. Richard FitzHugh of the Laboratory of Biophysics, National Institute of Neurological Diseases and Blindness, National Institutes of Health, for permitting us to make some preliminary computations on the Berkeley EASE analog computer, already programmed for the Hodgkin-Huxley equations.

Such preliminary computations indicated that for the theoretical equations, the magnitude of the action potential is a linear function of the log of the potassium potential (V_K) minus a constant, if certain complicating factors are taken into account. In the computations, V_K was varied by introducing an instantaneous step decrease of V_K at the moment of the stimulus, which is not comparable to the experimental situation. For lack of information on the resting state (which is in fact unstable, resulting in repetitive activity) following such a change in V_K, the solutions were begun from the same initial condi-

tions for the conductance variables m, n, and h as in the normal resting state, with an instantaneous current shock as stimulus. As a result of these initial conditions, the first action potential has an amplitude actually somewhat greater than normal, but by the time the second action potential occurs, the effect of these unrealistic initial conditions has disappeared. Therefore, in such a repetitive system, the second action potential is computed under the conditions of a new steady state for V_K, and in this case the height of the second spike is a linear function of the log $(V_K - C)$. Such an unstable system, however, affords no measure of the resting potential. The unstable system in reduced V_R may be made stable by increasing the potassium conductance (g_K). In this case, for each change of V_R, a new (stable) resting potential was computed, and from this new resting state, an instantaneous current pulse was applied. For these computations, with g_K sufficiently increased to inhibit repetitive activity throughout the entire range of action potential amplitude ($\bar{g}_K$ at 5 times its normal value), the magnitude of the action potential was also found to be a linear function of the log $(V_K - C)$. However, in the equations, the resting potential does not exactly follow V_R, so that the relationship between the action potential and the resting potential, while of the same general form as found experimentally, is not as good an approximation to the experimental results as the relationship between the action potential and V_R.

The difference between the theoretical results for the squid and the experimental results for the lobster might be explained as follows: In the squid, the resting potential does not exactly follow V_K (for the theoretical equations), but the action potential momentarily approaches the value of V_R at the end of the spike, resulting in a transient hyperpolarization (an "undershoot"). Such undershooting does not occur in the lobster propagated action potential, and it might be inferred that the resting potential more nearly approximates the potassium potential in the lobster than in the squid. Thus, if in actuality the magnitude of the action potential is related to the potassium potential rather than to the resting potential directly, a better fit would be expected (plotting V_{AP} *vs.* V_{RP}) in the lobster than in the squid. Unfortunately, comparable experimental results for the squid axon are not available. Even so, the agreement between the computed relationship between resting potential and action potential for the squid and the experimental results for the lobster is surprisingly good.

These studies were originally begun in order to test the hypothesis that calcium ion has a dual role in the production and maintenance of transmembrane potentials. The problem is complicated by the fact that the magnitude of the action potential is, at least to some extent, dependent on the magnitude of the resting potential. The extent of this dependence is apparently not readily susceptible to experimentation. Ideally, one would like to measure the effect of some substance known to exert a direct effect on the resting potential only,

then measure corresponding changes in action potential. Potassium ion appears to be the substance which would most likely fit this qualification, but one cannot state with certainty that this is so. However, the computed data for the theoretical equations indicate that a simple change of the potassium potential (in the direction which would be expected to occur in high external potassium solutions) would be adequate to explain a progressive reduction of the action potential to zero for a relatively small change in resting potential. It would seem, though, that even if a simple change of V_K were adequate to explain the high potassium results, this would still be insufficient to explain the results obtained with low calcium.

The flow chart shown in Fig. 4 will illustrate this point. For high potassium solutions, there is the well known reduction in resting potential. Whether the simultaneously occurring reduction in action potential is solely through the

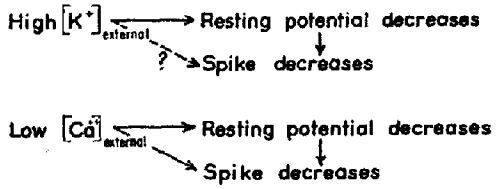

FIGURE 4. Flow chart showing the effects of high external potassium and low external calcium. For explanation, see text.

pathway *via* the resting potential is not clear at this point, although the indications are that it might be, and that this arrow represents a change in V_K. However, in the case of low external calcium, the reduction in action potential per unit change in resting potential is greater than in high potassium. Thus the scheme of a possible direct action on the resting potential and an indirect (or resting potential–mediated) action on the action potential proposed for high potassium solutions is insufficient to explain the increased reduction in action potential which occurs in low calcium solutions. By this sort of reasoning, then, the alternate pathway, a direct action on the action potential is also indicated for low calcium solutions; that is, low external calcium, to some extent, affects resting potential and action potential independently. (It should be pointed out that the pathway between resting potential and action potential is apparently a one-way path. Changes in action potential would not be expected to produce changes in resting potential. This assumption is supported by the fact that the action potential can be completely abolished in low sodium solutions without the occurrence of an appreciable change in resting potential.)

This evidence may indicate a dual role for calcium in the production and maintenance of transmembrane potentials, one of these roles being associated

in some way with the resting potential and the other with the action potential. However, it also appears to be possible that the physical role of calcium in the nerve membrane (if such a role exists) might be so arranged that a reduction in external calcium, although producing only one change in the membrane, might independently affect the resting potential and the action potential. Some of these possibilities will be discussed in the following paper (Adelman and Dalton (1960)).

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