The Effects of Various Ions on Resting and Spike Potentials of Barnacle Muscle Fibers

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ABSTRACT Effects of monovalent cations and some anions on the electrical properties of the barnacle muscle fiber membrane were studied when the intra- or extracellular concentrations of those ions were altered by longitudinal intracellular injection. The resting potential of the normal fiber decreases linearly with increase of logarithm of $[K^+]_{out}$ and the decrement for a tenfold increase in $[K^+]_{out}$ is 58 mv when the product, $[K^+]_{out} \cdot [Cl^-]_{out}$, is kept constant. It also decreases with decreasing $[K^+]_{in}$ but is always less than expected theoretically. The deviation becomes larger as $[K^+]_{in}$ increases and the resting potential finally starts to decrease with increasing $[K^+]_{in}$ for $[K^+]_{in} > 250$ mM. When the internal K^+ concentration is decreased the overshoot of the spike potential increases and the time course of the spike potential becomes more prolonged. In substituting for the internal K^+, Na^+ and sucrose affect the resting and spike potentials similarly. Some organic cations (guanidine, choline, tris, and TMA) behave like sucrose while some other organic cations (TEA, TPA, and TBA) have a specific effect and prolong the spike potential if they are applied intracellularly or extracellularly. In all cases the active membrane potential increases linearly with the logarithm of $[Ca^{++}]_{out}/[K^+]_{in}$ and the increment is about 29 mv for tenfold increase in this ratio. The fiber membrane is permeable to Cl^- and other smaller anions (Br^- and I^-) but not to $acetate^-$ and larger anions ($citrate^{---}$, $sulfate^{--}$, and $methanesulfonate^-$).

INTRODUCTION

With the recent development of intracellular perfusion techniques for the squid giant axon, considerable knowledge has been obtained of the resting and spike potentials of the membrane following various changes in the intracellular ionic composition (Oikawa, Spyropoulos, Tasaki, and Teorell, 1961; Baker, Hodgkin, and Shaw, 1962 *a*, *b*; Tasaki, Watanabe, and Takenaka, 1962; Tasaki and Shimamura, 1962; Tasaki and Takenaka, 1963; Narahashi, 1963).

The major difficulty in extending these experiments to other tissues has been the small size of most other cells or fibers (Davies, 1961). Recently giant muscle fibers with diameters of 0.5 to 2.5 mm have been described in the barnacle, *Balanus nubilus* by Hoyle and Smyth (1963 *a*, *b*). Because of the large size the internal ionic composition can be altered by intracellular injection. Although a spike potential is not normally produced in the barnacle muscle fiber the fiber always becomes capable of producing spike potentials when a small amount of Ca^{++}-binding agent is added to the injected solution (Hagiwara and Naka, 1964). The present work was planned to investigate effects of alteration of the internal ionic composition on the resting and spike potentials. The results are concerned mainly with the effects of various monovalent cations (K^+, Na^+, Rb^+, Cs^+, and monovalent organic cations) and some anions (Cl^-, SO_4^{--}, methanesulfonate$^-$, etc.) on the resting and spike potentials when applied intracellularly or extracellularly. A preliminary note has been published (Hagiwara, Naka, and Chichibu, 1964).

MATERIAL AND METHODS

Large specimens of *Balanus nubilus*, *B. tintinabulum*, and *B. aquilla* were used. The experimental techniques were similar to those described in the preceding paper (Hagiwara and Naka, 1964). The internal solutions were injected into the fiber until the diameter became 1.5 to 2.0 times the original size. The compositions of the internal solutions used in the present experiments are listed in Tables I and II. The normal barnacle saline had the following composition (Hoyle and Smyth, 1963 *a*): NaCl, 466 mM; KCl, 8 mM; $CaCl_2$, 20 mM; $MgCl_2$, 12 mM; $NaHCO_3$, 10 mM. The composition of the Cl-free saline was Na-methanesulfonate, 466 mM; K-methanesulfonate, 8 mM; Ca-gluconate, 20 mM; Mg-methanesulfonate, 12 mM; $NaHCO_3$, 10 mM. All experiments were carried out at room temperature (20–22°C).

RESULTS

1. *Resting Potential and External Ionic Concentrations*

The resting potential of the barnacle muscle fiber ranged between 70 and 80 mv when observed in the normal barnacle saline containing 8 mM K^+. The resting potential decreased when the K^+ concentration in the external saline was increased by replacing a part of the NaCl in the saline with equimolar KCl. The relationship between the resting potential and the logarithm of $[K^+]_{out}$ gave a straight line which had a slope of about 49 mv for a tenfold increase in concentration (Fig. 1A). The decrease of $[K^+]_{out}$ below the normal value (8 mM) resulted in an increase of resting potential but the increase was smaller than that expected from the straight line relationship obtained at higher concentrations. The complete removal of K^+ from the saline caused an increase of resting potential by 10 to 15 mv.

The above results show that the major factor in determining the resting

potential of the barnacle muscle fiber was the K^+ concentration. However, the following evidence indicates that other ions, mainly Na^+ and Cl^-, also contribute in establishing the resting potential in the normal saline. When the Na ions in the normal saline were replaced with organic cations such as tris (tris (hydroxymethyl) aminomethane), TMA (tetramethylammonium), choline, or guanidine, the resting potential always increased by about 10 mv. The

TABLE I

INTERNAL SOLUTIONS OF K-SALTS

	Major salt	K salt of EDTA	KH_2PO_4	Sucrose	Major cation
		mM	mM	mM	
K_2SO_4, solution A	321 mM K_2SO_4	—	50	—	717 mM K
K_2SO_4, solution B	301 mM K_2SO_4	20	50	—	717 mM K
K_2SO_4, solution C	185 mM K_2SO_4	20	50	349	485 mM K
KCl solution	400 mM KCl	20	50	—	—

pH adjusted to 6.9 by adding KOH; KBr, Kl, and K methanesulfonate solutions are made by replacing the KCl in the KCl solution with an equimolar amount of one of the respective salts.

TABLE II

INTERNAL SOLUTIONS OF OTHER SALTS

	Major salt	Na salt of EDTA	NaH_2PO_4	Sucrose	Major cation
		mM	mM	mM	
Na_2SO_4, solution	185 mM Na_2SO_4	20	50	349	485 mM Na
Sucrose, solution	—	20	50	904	115 mM Na
$Tris_2SO_4$, solution	301 mM $tris_2SO_4$	20	50	—	602 mM tris
Choline Cl, solution	502 mM choline Cl	20	50	—	502 mM choline

pH adjusted to 6.9 by adding NaOH; Rb_2SO_4 and Cs_2SO_4 solutions were obtained by replacing the Na_2SO_4 in the Na_2SO_4 solutions with the respective salt; guanidine acetate solution, by replacing the $tris_2SO_4$ in the $tris_2SO_4$ solution; TMA Cl and TEA Cl solutions, by replacing the choline Cl in the choline Cl solution with TMA Cl or TEA Cl.

replacement of Cl in the normal saline with organic anions such as methanesulfonate resulted in a decrease of 8 to 10 mv. These findings indicate that the resting membrane is partly permeable to Na^+ or Cl^-. The removal of Na from the external saline did not much affect the relationship between the resting potential and the K^+ concentration in the range of high $[K^+]_{out}$. However, the relationship did show a change when the external Cl ions were replaced with methanesulfonate ions (Fig. 1B). The relationship was a straight line in the range of higher concentrations but the slope became 56 mv for a tenfold increase in concentration and it intersected the X-axis at about 200 mM which

was smaller than that found with Cl media. When the K^+ concentration was increased in such a manner that the product of $[K^+]_{out}$ and $[Cl^-]_{out}$ was kept constant the slope of the straight line relationship became approximately −58 mv for a tenfold increase in concentration and this agrees with the theoretical value which is expected in a K electrode.

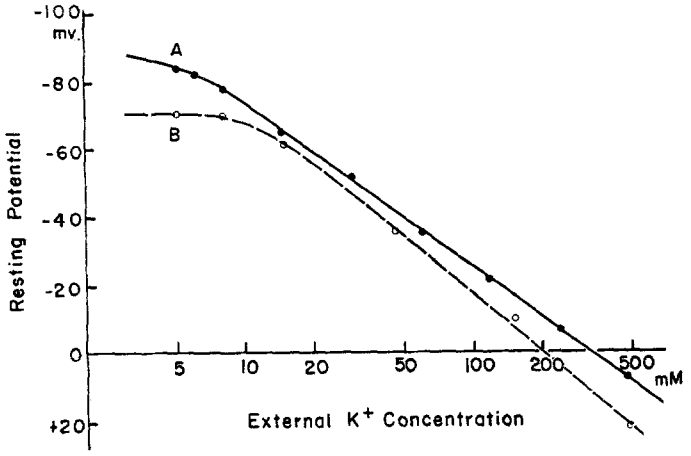

FIGURE 1. Relationships between the resting potential and the K^+ concentration in the external saline. K^+ concentration was altered in the normal saline (A) and in the Cl-free saline in which the Cl^- was replaced with methanesulfonate (B). The effect of K^+ concentration was observed with three single fibers in Cl media and with another three fibers in Cl-free media. Each circle in the figure represents a mean value obtained with these three different fibers.

TABLE III

CONCENTRATION OF K, NA, AND CL

	Internal		
	Millimoles ± standard deviation per kg wet weight of fiber	Millimoles per liter of fiber water	External (normal barnacle saline)
			mM
K	121±10	157	8
Na	16±2	21	476
Cl	25±6	32	539

2. *Internal Ionic Composition of the Normal Fiber*

The average concentrations of the internal K and Na as determined by a flame photometer in ten different single muscle fibers and the Cl^- concentration inside the fiber are shown in Table III. The figures in the first column give concentrations per kilo wet weight while those in the second column show concentrations per liter of internal water estimated from the observed water content of the fiber (76.7 ± 0.5 per cent of the wet weight). These values of the

internal K^+ concentration are smaller than those found for the squid giant axon (Steinbach and Spiegelman, 1943; Keynes and Lewis, 1951) but show a good agreement with those found for some marine crustacean muscle fibers (Shaw, 1955). Since the internal Na concentration is small the major internal cations are probably organic as in the case of crab muscle fiber (Shaw, 1955). The internal Cl^- concentration was examined by the mercuric nitrate method (Smith, 1956). The result shows that the product $[K^+] \cdot [Cl^-]$ does not differ much between the internal and external media.

The resting potential became zero at about 200 mM $[K^+]_{out}$ (curve B, Fig. 1). If the resting potential represents the concentration potential of K^+ across the fiber membrane, the internal K^+ concentration should therefore be about 200 mM. Although the observed value of $[K^+]_{in}$ was somewhat smaller than 200 mM the discrepancy was not too great.

3. *Alteration of the Internal K^+ Concentration*

As described in the preceding paper (Hagiwara and Naka, 1964), the intracellular injection of a Ca^{++}-binding agent such as EDTA renders the fiber capable of producing an all-or-none spike potential even though most normal barnacle muscle fibers do not produce proper spikes. The effects of alteration of the internal K^+ concentration on the resting and spike potential were studied by injecting solutions of different K^+ concentrations. These solutions were obtained by mixing the internal K_2SO_4 solution (K_2SO_4, solution B or C) and K-free internal sucrose solution in various proportions. The internal K concentration of each fiber was examined by a flame photometer after measuring the electrical properties. Each solution was injected uniformly along the fiber until the fiber diameter increased by about 50 per cent. The results obobtained after injecting solutions containing 485, 48, and 0 mM K^+ are shown in Fig. 2A, B, and C respectively. Three major changes were found following decrease of internal K^+ concentration; a decrease of the resting potential, an increase of the spike overshoot, and a prolongation of the spike potential.

Fig. 3 shows the relationship between the internal K^+ concentration (millimoles per kilo wet weight) and the resting potential observed in the normal barnacle saline containing 8 mM K^+. Although considerable variation is seen, the result indicates that the resting potential decreased with decreasing internal K^+ concentration. The resting potential of the normal fiber containing 110 to 120 mM K^+ ranged between 70 and 80 mv (illustrated by a filled circle in Fig. 3). If the membrane behaves as a K electrode the theoretical relation should be given by a straight line which has a slope of 58 mv for a tenfold decrease in $[K^+]_{in}$ and passes through the filled circle in Fig. 3 (this is illustrated by a broken line). The observed relationship deviated from the theoretical curve in the downward direction and the deviation became more marked as the $[K^+]_{in}$ increased. Furthermore the resting potential started to decrease with an increasing $[K^+]_{in}$ when the $[K^+]_{in}$ was raised above about 250 mM.

The injection probably has some secondary effect which contributes to the observed change in the resting potential. Some decrease in the resting potential was always observed following injection even when the K^+ concentration

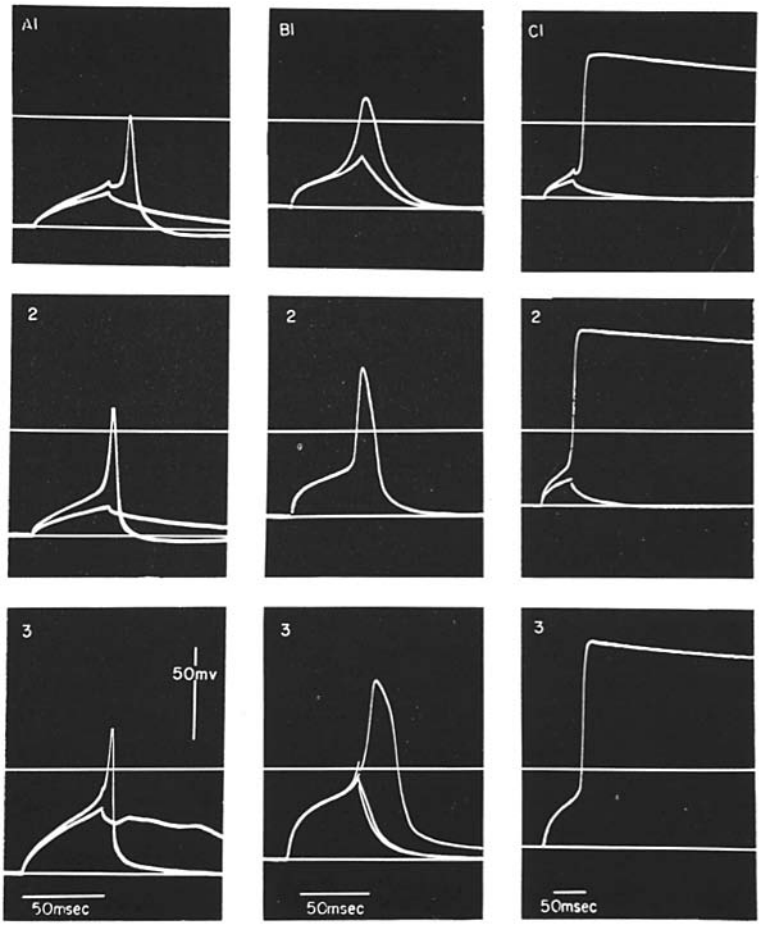

FIGURE 2. Effects of $[K^+]_{in}$ and $[Ca^{++}]_{out}$ on the spike potential A, B, and C were obtained from three different fibers injected with solutions containing 485, 48, and 0 mM K^+ respectively. $[Ca^{++}]_{out}$ was 20, 85, and 338 mM in records 1, 2, and 3 in each series. A trace in the upper part of each record indicates the reference potential level.

of the injecting solution was similar to that of the internal medium of the normal fiber; the discrepancy between the observed and theoretical relations could be explained if this effect became larger as the K^+ concentration of the injecting solution increased.

The overshoot of the spike increased with decreasing internal K^+ concentration when observed at a given $[Ca^{++}]_{out}$ (Fig. 2). As described in the preceding paper (Hagiwara and Naka, 1964) the spike overshoot increased in increasing

$[Ca^{++}]_{out}$. Fig. 2 shows the effects of external Ca^{++} concentrations of 20, 85, and 338 mM in rows 1, 2, and 3 respectively. Fig. 4 shows the relations between the spike overshoot and the internal K^+ concentration obtained at three differ-

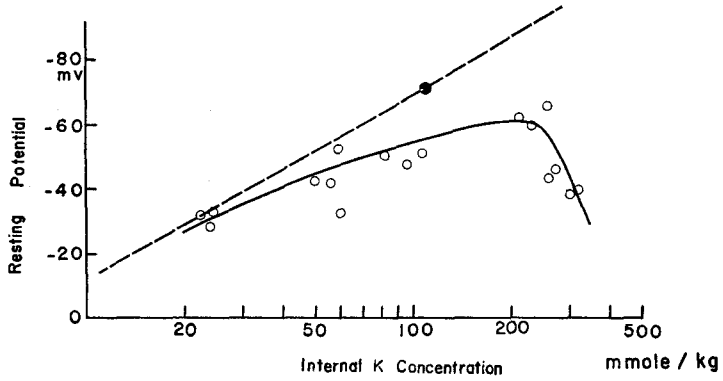

FIGURE 3. Relationship between the resting potential and the internal K concentration (millimoles per kilo wet weight) obtained in the normal saline containing 8 mM K^+. The filled circle indicates the K concentration of the normal uninjected fiber and its resting potential in the normal saline. The broken straight line passing the filled circle has a slope of 58 mv for a tenfold increase in the concentration.

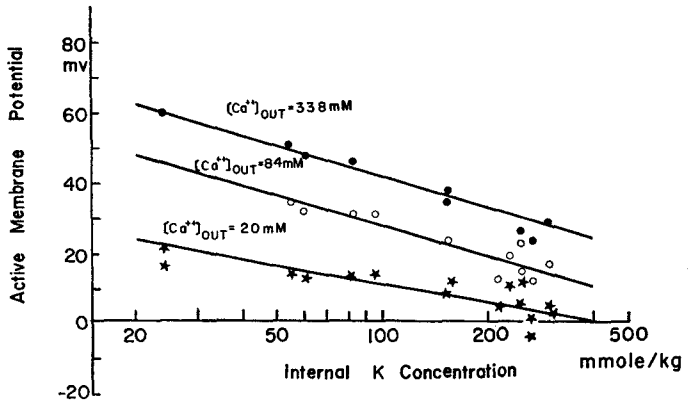

FIGURE 4. Relationships between the active membrane potential and the internal K concentration (millimoles per kilo wet weight). Three sets of relationships illustrated by stars, open circles, and filled circles were obtained when $[Ca^{++}]_{out}$ was 20, 85, and 338 mM respectively.

ent concentrations of external Ca^{++} (20, 84, and 338 mM). The relationships are approximately linear in all three cases except possibly in a small range of high $[K^+]_{in}$ (above 200 millimoles per kilo wet weight). The slope of the straight line relation increased as the $[Ca^{++}]_{out}$ increased. At 20 mM $[Ca^{++}]_{out}$ it was 15 to 20 mv for a tenfold decrease in $[K^+]_{in}$ and it became 26 to 28 mv at 84 and 338 mM $[Ca^{++}]_{out}$. The results indicate that the permeability of the

active membrane to K^+ is an important factor in determining the membrane potential at thepeak of the spike.

The third effect of decreasing the internal K^+ concentration was on the time course of the spike. As the $[K^+]_{in}$ decreased the decline of the spike became slower (Fig. 2B) and finally the spike showed a plateau-type potential that lasted more than several seconds (Fig. 2C). As described in the previous paper (Hagiwara and Naka, 1964) the spike potential has associated with it two kinds of conductance changes of the membrane, the initial rapid increase in Ca conductance related to the rising phase of the spike, followed by the conductance increase to K^+, related to the decline of the spike. The slower decline of

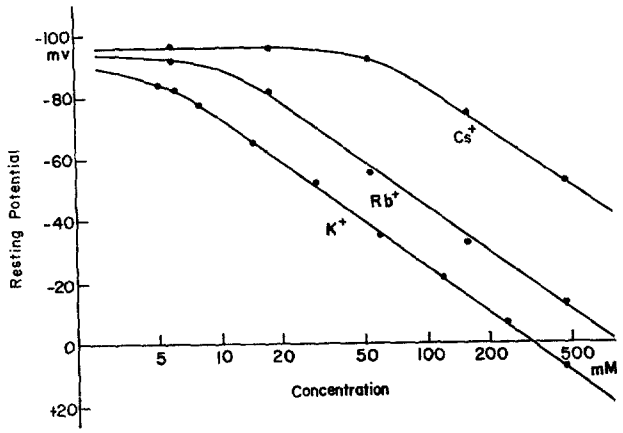

FIGURE 5. Resting potential and K^+, Rb^+, and Cs^+ concentrations. The concentration of one of these ions was altered in Cl saline.

the spike found at lower internal K^+ concentrations is presumably due to a smaller increase of the K conductance in the active membrane.

4. *Effects of Rb^+ and Cs^+*

The effects of Rb^+ and Cs^+ on the resting as well as active barnacle muscle fiber membrane were essentially similar to those found for K^+. As described already, the resting potential decreased with increasing $[K^+]_{out}$ when the NaCl in the normal barnacle saline was replaced with equimolar KCl (Fig. 1). Similar measurements were performed with RbCl and CsCl and the relations between the resting potential and the logarithm of $[Rb^+]_{out}$ or $[Cs^+]_{out}$ are shown in Fig. 5 together with that obtained with KCl. They are linear for the range of high concentrations and show a common slope of about 49 mv for a tenfold increase in the concentration. The concentration at which the resting potential should become zero was estimated for each species of cations from the extrapolated intercept on the X-axis. These concentrations were 350, 770, and 6400 mM for K^+, Rb^+, and Cs^+ respectively. The result suggests that K^+ is

the most permeable ion; that is followed by Rb^+, and Cs^+ is much less permeable than either of the others.

Similar differences of effectiveness were found among K^+, Rb^+, and Cs^+ in the active membrane. Records A and B in Fig. 6 were obtained after injecting Rb_2SO_4 or Cs_2SO_4 solutions with Na salt of EDTA (see Table II). The external Ca^{++} concentration was 180 mM in both cases. The prolongation of the spike potential was seen. The spike potential obtained in the Cs_2SO_4-treated muscle fiber resembled those obtained following injection of K^+-free sucrose solution while the spike in the Rb_2SO_4-treated fiber was similar to those obtained with low K internal solution. A relatively large decrease of the resting potential was observed with the injection of Rb_2SO_4 solution and a marked positive after-potential or undershoot was seen. Some of the decrease of the resting potentia

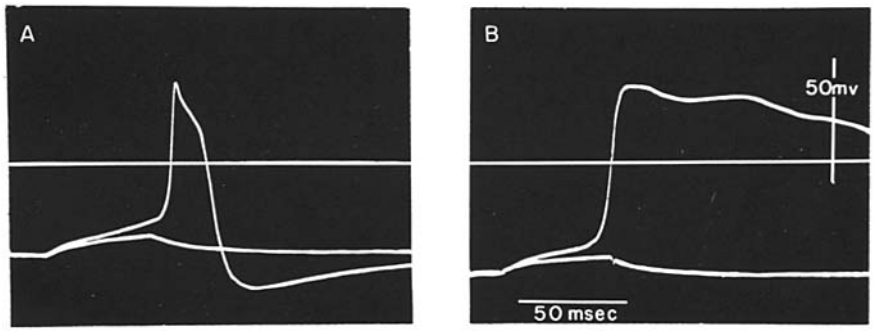

FIGURE 6. Spike potentials obtained in fibers injected with Rb_2SO_4 solution (A) and Cs_2SO_4 solution (B). Na salt of EDTA was added. $[Ca^{++}]_{out}$ was 180 mM.

found for the Rb_2SO_4 injection is presumably due to impurity of the Rb_2SO_4 used (95 per cent purity) especially to contamination by RbCl, since the increase of internal Cl concentration decreases the resting potential as will be described later.

5. *Effects of Na^+ Injection*

As mentioned already the removal of Na from the external bathing solution increased the resting potential by about 10 mv. This indicates that Na ions contribute only slightly to the resting potential at least when the $[K^+]_{out}$ is small. The removal of the Na from the external medium did not alter the spike potential if the $[Ca^{++}]_{out}$ was unaltered (Hagiwara and Naka, 1964). These results show that Na^+ is relatively inert for the resting as well as for the active membrane of the barnacle muscle fiber. This was confirmed by injection experiments for the internal application of Na_2SO_4 solution with EDTA. The result was similar to that obtained with K-free sucrose solution. The major effect was due to the decrease of the internal K^+ concentration and was not

specific for Na^+. The results obtained by the injection were, therefore, a decrease of the resting potential, an increase of spike overshoot, and a prolongation of the spike potential. In the case of the Na_2SO_4 solution, however, the decrease of the resting potential was usually more marked than was seen for the sucrose injection. In fact, because of a low resting potential the membrane was often incapable of producing an all-or-none spike at a low $[Ca^{++}]_{out}$, such as at 20 mM, and the response was a graded potential change (Fig. 7A). When the membrane was hyperpolarized by applying an inward current, the excitability was restored. The records in Fig. 7B were obtained from the same fiber at different levels of hyperpolarization. As the hyperpolarization increased the

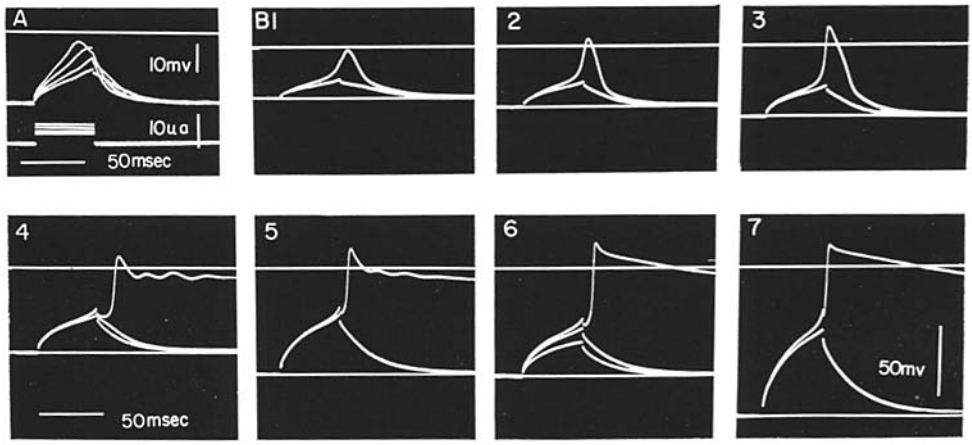

FIGURE 7. Spike potentials in the fiber injected with Na_2SO_4 solution. Obtained in normal saline. Records B1 to 7 were obtained when the intensity of the hyperpolarizing current was gradually increased. Record A, obtained without hyperpolarization.

spike potential showed characteristic changes in the overshoot, in the time course, and in the firing membrane potential. With a modest amount of hyperpolarization, the rates of rise and fall of the spike were usual. At a certain level of hyperpolarization an oscillatory potential change appeared after the spike, followed by a plateau-type potential change (B4 and 5). As the hyperpolarization increased, the oscillatory potential change became less marked, and finally the plateau immediately followed the peak of the spike (B6 and 7). In parallel with the change of spike duration, the overshoot increased and the membrane potential at the firing level became more negative with increasing levels of hyperpolarization up to maximum values.

These changes in spike potentials found at different levels of hyperpolarization however, were not specific to the Na^+ injection. Similar changes were obtained following injection of sucrose or organic cations when the resting potential became too small to permit an all-or-none spike potential.

The critical membrane potential below which an all-or-none spike potential was no longer produced shifted towards zero membrane potential as the external Ca^{++} concentration increased. Therefore, an all-or-none spike could be obtained at a high external Ca^{++} concentration even when the resting potential was too small to obtain a spike at lower Ca^{++} concentrations.

6. *Organic Cations*

The injection of guanidine acetate, choline Cl, and tetramethylammonium Cl solutions with the Na salt of EDTA prolonged the spike markedly with a plateau-type potential (Fig. 8A, B, and C). Since Cl salts were used in some of these solutions the reduction of the resting potential was partly due to the excess internal Cl^-, as will be described later, and partly due to the reduction

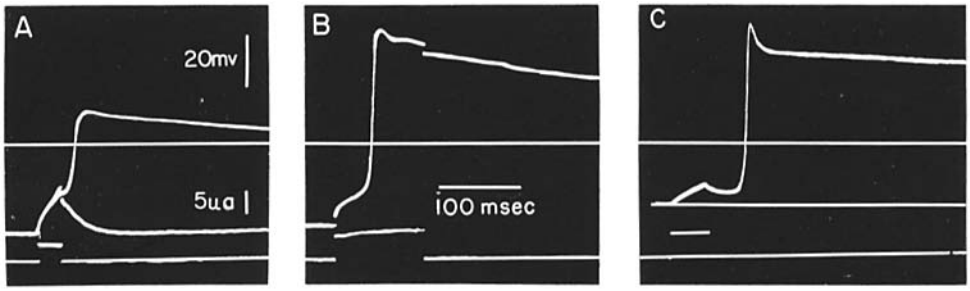

FIGURE 8. Spike potentials obtained from the fibers injected with guanidine acetate solution (A), choline chloride solution (B), and TMA Cl solution (C). $[Ca^{++}]_{out}$ was 180 mM.

of $[K^+]_{in}$. The major changes in the spike potential were, however, exclusively due to the reduction of $[K^+]_{in}$ just as was found for the injection of the Na_2SO_4 solution. As described in the preceding paper these organic cations show no effect on the spike potential when applied externally; *i.e.*, the overshoot, the time course, and the firing membrane potential for the spike potential of the K_2SO_4- and EDTA-treated fiber do not change following the replacement of the NaCl in the external saline with an equimolar amount of guanidine Cl, choline Cl, or tris Cl.

Tetraethylammonium (TEA) was different from the organic cations mentioned above for the substitution of TEA for Na ions in the external medium resulted in a prolongation of the spike potential in fibers treated with high K^+ solutions. Record A1 of Fig. 9 was obtained with a fiber injected with the K_2SO_4 solution at 180 mM external Ca^{++}. The other major cation in the external medium was Na. Substitution of external Na^+ ions with tetraethylammonium (TEA) ions prolonged the spike potential (record A2). The injection of the TEA Cl solution with the Na salt of EDTA also produced a prolonged

spike as shown by record C. Record B shows the whole time course of the prolonged spike after internal (B2) or external (B1) application of TEA. The effect, therefore, was apparently similar to that of the previous organic cations when applied internally. However, in the case of TEA the injection of a small amount was enough to produce a prolonged spike, whereas for the other organic cations it was necessary to inject an amount of solution sufficient to dilute the internal K^+. Therefore, the prolongation of the spike potential by TEA observed for the intracellular injection seems to be due to a specific effect of TEA. In contrast to this, the effect of other organic ions such as tris seems to be mainly due to the dilution of the internal K^+.

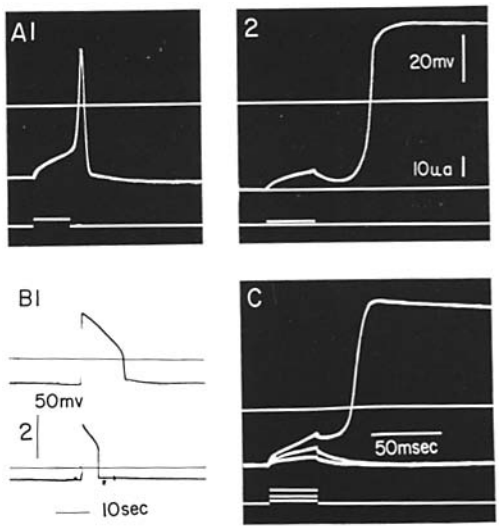

FIGURE 9. Effect of TEA on the spike potential. A1 and 2 were obtained from the K_2SO_4, solution B-treated fiber before and after the total replacement of NaCl in the saline with TEA Cl. $[Ca^{++}]_{out}$ was 180 mM. C, spike potential of the fiber injected with TEA Cl solution. $[Ca^{++}]_{out}$ was 180 mM and the other major cation was Na. B shows full time course of spike prolonged by external (B1) or internal (B2) application of TEA.

Crustacean muscle fibers have been shown to produce prolonged spike potentials in TEA media even though no all-or-none spike was found in the same fiber in Na media (Fatt and Katz, 1953; Fatt and Ginsborg, 1959; Werman and Grundfest, 1961). Fatt and Ginsborg (1959) found that the amplitude of the TEA spike is related to the external Ca^{++} concentration and, therefore, no spike is initiated in TEA media if the $[Ca^{++}]_{out}$ is too small. Similar results were obtained with barnacle muscle fibers. The present results show that the effect of TEA does not depend on whether it is applied internally or externally. A similar effect was also observed with tetrapropylammonium and tetrabutylammonium ions in these experiments.

7. *Effects of Anions*

When a KCl solution was used for the injection instead of K_2SO_4 solutions, a considerable decrease in the resting potential was always found. This did not seem to be due to the difference in ionic strength since no such decrease was

observed following injection of K methanesulfonate solution. In Fig. 10 records A and B were obtained in 180 mM Ca^{++} external medium following injection of K methanesulfonate and KCl solutions respectively. The K salt of EDTA was added to each solution and the K^+ concentration was 400 mM in both solutions, with the osmotic pressure maintained by adding sucrose. The resting potential was decreased to about −30 mv by the injection of KCl while the resting potential was about −60 mv for K methanesulfonate.

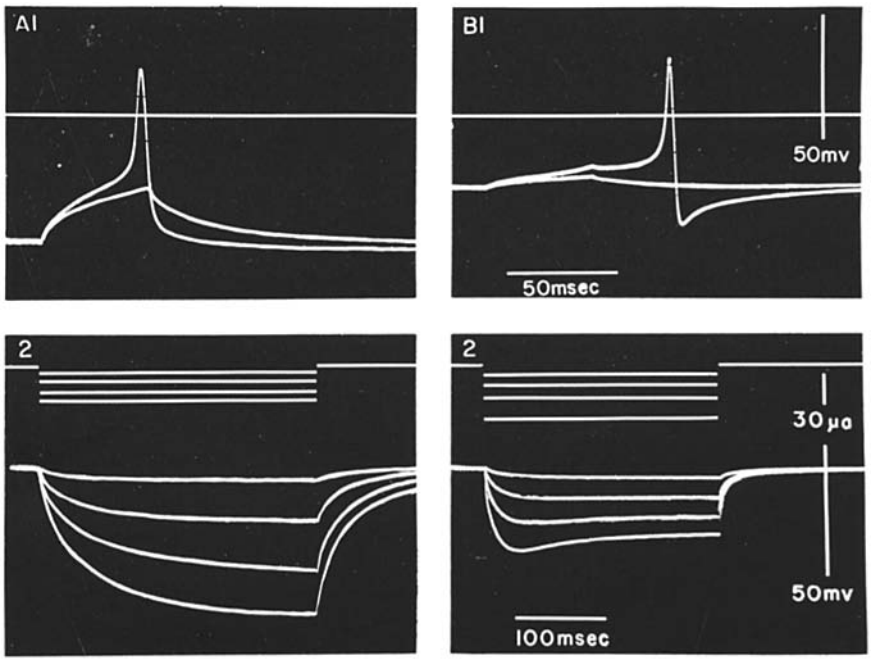

FIGURE 10. Effects of internal Cl^-. Internal solution was K methanesulfonate solution in A and KCl solution in B. $[Ca^{++}]_{out}$ was 180 mM.

Although the resting potential was smaller in the KCl-treated fiber than in the K methanesulfonate fiber, the overshoot of the spike was similar in the two cases. A marked positive after-potential or undershoot was always found in KCl-treated fibers. This is probably due to the fact that the resting potential level is much less negative than the K equilibrium potential which is related to the potential level at the peak of the undershoot.

When inward current pulses of increasing intensity were applied, the amplitude of hyperpolarization increased linearly with the current intensity in K methanesulfonate-treated fibers (Fig. 10 A2). However, in KCl-treated fibers, as shown in Fig. 10 B2 the hyperpolarization showed a secondary decrease when the intensity of current exceeded a certain limit. At the steady state of hyperpolarization the amplitude became smaller than expected from

the resting membrane resistance. By superimposing a small test pulse on a hyperpolarizing current the membrane resistance at the steady state was examined, and it was found that a decrease of the membrane resistance was always associated with the secondary decrease of the hyperpolarization. The rectification found in KCl-treated muscle fibers is probably due to an increase in the Cl^-conductance of the membrane. A similar result has been obtained for crayfish muscle fibers by Grundfest (1962).

Among anions examined, the results obtained with citrate^{---}, sulfate^{--}, and acetate$^-$ are similar to those found with methanesulfonate, whereas the depolarization effect was common for Cl^-, Br^-, and I^-. The fiber membrane seems to be permeable to Cl^- and to other smaller ions, but not to acetate$^-$ or to other larger anions.

DISCUSSION

The resting potential decreased with increasing $[K^+]_{out}$ just as expected for a K electrode. The decrease of the $[K^+]_{in}$ also resulted in a decrease of the resting potential. Thus the major factor in determining the resting potential is the transmembrane ratio of the K^+ concentrations. However, the relationship between the resting potential and $[K^+]_{in}$ showed a significant deviation from the theoretical curve. The deviation becomes more marked as $[K^+]_{in}$ increased. Furthermore the resting potential showed a decrease with increasing $[K^+]_{in}$ when the $[K^+]_{in}$ exceeded 200 mM and this is just opposite to what is expected in a K electrode. A similar phenomenon has also been observed during intracellular perfusion of the squid axon by Baker, Hodgkin, and Shaw (1962 *b*). They have explained this discrepancy by the leakage though small cut branches of the axon. At high internal concentrations of K^+ the leakage through branches is large because of the high conductivity of the internal solution and the observable resting potential becomes much smaller than the actual one. As the internal K^+ concentration is decreased by replacing K salt with sucrose the conductivity of the internal solution becomes smaller and therefore, the leakage bcomes smaller. This effect may overcome the decrease of the resting potential caused by a decreasing internal K^+ concentration and thus, the observed resting potential may show an increase with decreasing internal K^+ concentration in a certain range of concentrations. A similar explanation may be applicable to the present case. Although no branches are found in the barnacle muscle fiber, corresponding leakages may exist.

As described in the preceding paper the membrane of the barnacle muscle fiber becomes capable of initiating an all-or-none spike when the internal Ca^{++} concentration is reduced by injecting Ca^{++}-binding agents. The magnitude of the overshoot of the spike increases with increasing external Ca^{++} concentration and the experimental results show that the spike potential is obtained as a result of a permeability increase of the membrane to Ca^{++}. Follow-

ing the injection, however, the Ca^{++} concentration inside the fiber is practically zero, and therefore the active membrane potential does not seem to represent a simple concentration potential of Ca^{++} across the membrane. The major cation inside the fiber is potassium. The overshoot of the spike potential increases with decreasing internal K^+ concentration. In other words $[K^+]_{in}$ also determines the active membrane potential. The concentration of K^+ in the external medium was kept at 8 mM thoughout the measurement of the active membrane potential. The removal of this small amount of external K^+ does not cause any appreciable change in the active membrane potential. If the external K^+ is neglected the active membrane potential can be considered as a biionic potential between a Ca salt on one side of the membrane and a K salt on the other side.

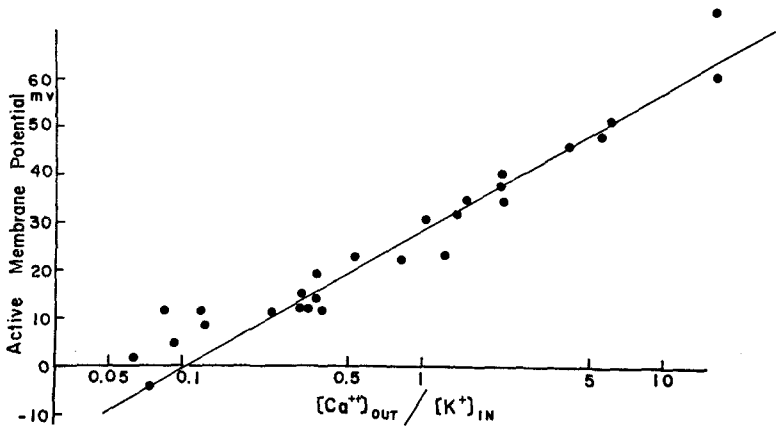

FIGURE 11. Relationship between the active membrane potential and $[Ca^{++}]_{out}/[K^+]_{in}$.

In Fig. 11 the overshoot of the spike is plotted against logarithm of $[Ca^{++}]_{out}/[K^+]_{in}$. The plot approximates a straight line which has a slope of 29 mv for a tenfold increase in $[Ca^{++}]_{out}/[K^+]_{in}$. In other words the active membrane potential is determined simply by $[Ca^{++}]_{out}/[K^+]_{in}$. This is significant since biionic potentials are complicated phenomena especially when the valences of the two ion species are different. Under some conditions the biionic potential is expected to be a function of $[Ca^{++}]_{out}/[K^+]^2_{in}$ instead of $[Ca^{++}]_{out}/[K^+]_{in}$ (Helfferich, 1962). Therefore the result at least shows that the present muscle membrane is not under such a condition. More information about the muscle membrane is necessary for further theoretical considerations.

By studying crayfish muscle fibers in Ba or Sr media, Fatt and Ginsborg (1959) concluded that the spike potential of these muscle fibers occurs as a result of a permeability increase of the membrane to divalent cations. They have assumed that the active membrane potential represents a biionic membrane potential of external Ca^{++} and internal K^+, and proposed an explanation based on the constant field hypothesis.

The increase of spike overshoot has been found in the squid giant axon when the perfusing K salt solution is diluted with sucrose solution (Baker, Hodgkin, and Shaw, 1962 *b*; Tasaki, Watanabe, and Takenaka, 1962; Narahashi, 1963). The results are, therefore, similar in these two cases. However, the important external ions are Ca ions in the barnacle muscle fiber while they are Na ions in the squid giant axon.

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REFERENCES

BAKER, P. F., HODGKIN, A. L., and SHAW, T. I., 1962 *a*, Replacement of the axoplasm of giant nerve fibres with artificial solutions, *J. Physiol.*, **164,** 330.

BAKER, P. F., HODGKIN, A. L., and SHAW, T. I., 1962 *b*, The effects of changes in internal ionic concentrations on the electrical properties of perfused giant axons, *J. Physiol.*, **164,** 355.

DAVIES, P. W., 1961, A method for measuring membrane potential of intracellularly perfused single skeletal muscle fibers, *Fed. Proc.*, **20,** 142.

FATT, P., and GINSBORG, B. L., 1959, The ionic requirements for the production of action potentials in crustacean muscle fibres, *J. Physiol.*, **142,** 516.

FATT, P., and KATZ, B., 1953, The electrical properties of crustacean muscle fibres, *J. Physiol.*, **120,** 171.

GRUNDFEST, H., 1962, Ionic transport across neural and non-neural membranes, in Properties of Membranes and Diseases of the Nervous System, (M. D. Yahr, editor) New York, Springer Publishing Company, 71.

HAGIWARA, S., and NAKA, K., 1964, The initiation of spike potential in barnacle muscle fibers under low intracellular Ca^{++}, *J. Gen. Physiol.*, **48,** 141.

HAGIWARA, S., NAKA, K., and CHICHIBU, S., 1964, Membrane properties of barnacle muscle fiber, *Science*, **143,** 1446.

HELFFERICH, F., 1962, Ion Exchange, New York, McGraw-Hill Book Co., Inc., 378.

HOYLE, G., and SMYTH, T., JR., 1963 *a*, Giant muscle fibers in a barnacle *Balanus nubilus* Darwin, *Science*, **139,** 49.

HOYLE, G., and SMYTH, T., JR., 1963 *b*, Neuromuscular physiology of giant muscle fibers of a barnacle, *Balanus nubilus* Darwin, *Comp. Biochem. and Physiol.*, **10,** 291.

KEYNES, R. D., and LEWIS, P. R., 1951, The sodium and potassium content of cephalopod nerve fibres, *J. Physiol.*, **114,** 151.

NARAHASHI, T., 1963, Dependence of resting and action potentials on internal potassium in perfused squid giant axons, *J. Physiol.*, **169,** 91.

OIKAWA, T., SPYROPOULOS, C. S., TASAKI, I., and TEORELL, T., 1961, Methods for perfusing the giant axon of *Loligo pealii*, *Acta Physiol. Scand.*, **52,** 195.

SHAW, J., 1955, Ionic regulation in the muscle fibres of *Carcinus maenas*. I. The electrolyte composition of single fibres, *J. Exp. Biol.*, **32,** 383.

SMITH, H. W. 1956, Principles of Renal Physiology, Oxford University Press, 214.

STEINBACH, H. B., and SPIEGELMAN, S., 1943, The sodium and potassium balance in squid nerve axoplasm, *J. Cell. and Comp. Physiol.*, **22,** 187.

TASAKI, I., and SHIMAMURA, M., 1962, Further observations on resting and action potential of intracellularly perfused squid axon, *Proc. Nat. Acad. Sc.*, **48,** 1571.

TASAKI, I., and TAKENAKA, T., 1963, Resting and action potential of squid giant axons intracellularly perfused with sodium-rich solutions, *Proc. Nat. Acad. Sc.*, **50,** 619.

TASAKI, I., WATANABE, A., and TAKENAKA, T., 1962, Resting and action potential of intracellularly perfused squid giant axon, *Proc. Nat. Acad. Sc.*, **48,** 1177.

WERMAN, R., and GRUNDFEST, H., 1961, Graded and all-or-none electrogenesis in arthropod muscle. II. The effects of alkali-earth and onium ions on lobster muscle fibers, *J. Gen. Physiol.*, **44,** 997.