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EFFECTS OF ANIONS AND CATIONS ON THE RESTING MEMBRANE POTENTIAL OF INTERNALLY PERFUSED BARNACLE MUSCLE FIBRES

BY N. LAKSHMINARAYANAIAH AND E. ROJAS

From the Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, 19174, U.S.A. and from the Laboratory of Cellular Physiology, University of Chile, Santiago, Chile

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SUMMARY

1. Single barnacle muscle fibres from *Megabalanus psittacus* (Darwin) were internally perfused with a number of K salt solutions (200 mM) which were made isotonic to the barnacle saline with sucrose.

2. 200 mM-K acetate solution, in general, was found to be more effective than other solutions of K salts in generating and maintaining stable resting membrane potential of $-56{\cdot}0 \pm 0{\cdot}7$ mV (all potentials are referred to the external solutions as ground). The various K salts, on the basis of the magnitude of the resting potential they generated in the muscle fibres, followed the sequence, acetate > isethionate > aspartate > glutamate > fluoride > monohydrogen phosphate > succinate > citrate > sulphate > oxalate > iodobenzoate > ferrocyanide > chlorate > nitrate > chloride > thiocyanate > iodide > bromide > cyanide.

3. The resting potential in muscle fibres perfused with solutions of acetate, aspartate and glutamate increased linearly with the logarithm of the K concentration (slope = 30·4 mV for K acetate and 27·4 for K aspartate and glutamate) when the ionic strength of the solutions was progressively increased from 50 to 650 mM. On the other hand, similar increase of ionic strength beyond 200 mM of solutions of K isethionate, fluoride, monohydrogen phosphate, succinate and citrate depolarized the muscle fibres.

4. Perfusion of acetate solutions of other alkali metal ions gave low values for the resting potential and followed the sequence K > Na > Rb > Li > Cs. Also NH_4 and Tris ions gave low values for the resting potential which underwent oscillations associated with the twitching of the fibre and occasionally became positive in value (action potential).

5. Addition of tetraethyl ammonium chloride (TEA-Cl), 20–100 mM, to K acetate solutions (200 mM) depolarized the fibre membrane and the consequent reduction of resting potential varied linearly with the logarithm of TEA concentration.

6. Replacement of chloride ion by acetate or isethionate in the external solution did not change significantly the resting potential although the values were consistently lower by about 2 mV.

7. Complete elimination of K in the external solution and reduction of its ionic strength using sucrose depolarized the muscle fibres by about 27 mV when Na was changed from 475 to 1 mM. Under these conditions, external solutions completely in acetate form gave resting potentials which were more positive than those observed in completely chloride solutions by 6–8 mV.

8. Replacement of Na by Li, Tris, choline, tetramethyl or tetraethyl ammonium ion in the external solution made the values of the resting potential more positive (depolarization). Similarly increasing the concentration of K (or Cs or Rb in place of K) by correspondingly decreasing the concentration of Na in the outside solution depolarized the fibres and the resting potential became zero at a concentration of 280 mM (or 308 or 1500 mM for Rb or Cs, respectively) on extrapolation.

INTRODUCTION

With the introduction of methods for perfusing giant axons (Baker, Hodgkin & Shaw, 1961; Oikawa, Spyropoulos, Tasaki & Teorell, 1961) a considerable body of knowledge concerning resting and spike potentials of the membrane under a variety of intra- and extracellular conditions has been acquired (see Lakshminarayanaiah, 1969 for a brief account). Extension of these methods to other cells or fibres was prevented by their small size (Davies, 1961). However, with the availability of giant barnacle muscle fibres (Hoyle & Smyth, 1963*a*, *b*) the techniques of intracellular injection (Hagiwara & Naka, 1964; Hagiwara, Chichibu & Naka, 1964; Hagiwara, Naka & Chichibu, 1964; Brinley, 1968), dialysis (Brinley & Mullins, 1967; Mullins & Brinley, 1967; DiPolo & Latorre, 1972; DiPolo, 1972) and perfusion (Keynes, Rojas, Taylor & Vergara, 1973) have been applied to study their behaviour in various environments. Keynes *et al.* (1973) have described a method for internally perfusing the single barnacle muscle fibre from *Megabalanus psittacus* (Darwin). Using this method which enabled them to control the internal environment of the muscle cell, they were able to study the behaviour of the K and Ca carrying systems existing in the barnacle muscle fibre under both resting and voltage-clamp conditions. Their technique of intracellular perfusion has

been used in the present study to explore further the behaviour of the single fibre at rest and subject to various intra- and extracellular conditions.

METHODS

The barnacle muscle fibres from *Megabalanus psittacus* (Darwin) readily available in the coast of Chile were used in this study. The experimental procedures were similar to those described by Keynes *et al.* (1973).

Liquid junction potentials. There are two liquid junctions, one existing between the tip of the internal capillary electrode filled with 0·5 M-KCl (Ag-AgCl) and the internal perfusing solution and the other existing between the tip of a similar capillary electrode and the external saline solution. The net potential due to these two junction potentials was measured by connecting the two half-cells with an agar-KCl (3·0 M) bridge (Baker, Hodgkin & Meves, 1964). These measurements using various perfusing solutions showed that the net junction potentials were small (< 2 mV) and therefore these corrections for the measured values of resting potential have been ignored.

TABLE 1. Composition of external solutions (concentration in m-mole/l. solution, pH = 7·5)

Solution	K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	$Tris^+$	Ac^-	Ise^-
Standard saline*	10	430	20	40	565	5	—	—
K-free saline	—	440	20	40	565	5	—	—
Ca-free saline	10	430	—	60	565	5	—	—
Acetate(Ac) saline(1)†	10	430	20	40	120	5	445	—
Acetate(Ac) saline(2)‡	10	430	20	40	—	5	565	—
Isethionate saline (Ise)	10	430	20	40	135	5	—	430

* Tris(trihydroxymethyl)amino methane saline, choline saline, tetramethyl and tetraethyl ammonium salines were prepared by complete substitution of the respective cation for Na. Similarly high K solutions were made by substituting the required quantity of K for Na.

† High K or in its place Rb or Cs acetate saline solution (1) was made by substituting the required quantity of K (or Rb or Cs) for Na.

‡ LiAc saline (2) was prepared by substituting Li for Na.

Solutions. The composition of the artificial sea water is given in Table 1. High K, chloride and acetate saline solutions were prepared by substitution of K for Na. External solutions (chloride or acetate) which were K-free and in which the ionic strength was varied contained the following salts: $CaCl_2$ or $Ca(CH_3COO)_2$ 35 mM and Tris(hydroxymethyl)amino methane-Cl or Tris-acetate 5 mM. The solutions of highest ionic strength contained 475 mM of either NaCl or Na acetate. The solutions of low ionic strength contained 300, 200, 100, 50 and 1 mM of the salt and were made osmotically equivalent with sucrose using osmolality tables (Weast, 1969).

Osmolarity of the internal solutions were made up using sucrose. Analar grade salts were used. In the case of those salts of K which were not readily available, for example, K acetate, aspartate, glutamate, etc., a stock solution (1·0 M) was prepared by neutralizing the exact quantity of KOH to pH 7·5 using the acid required. 1·0 M solutions of Rb and Cs acetates were made by neutralizing to pH 7·5 exactly known weights of Rb and Cs carbonates with 6 M solution of acetic acid.

K phosphate solutions which contained Tris ion were prepared using the salt KH_2PO_4. A solution containing a known quantity of the salt was neutralized to pH 7·5 with Tris base and so the exact concentration of Tris was not known.

5 mM Tris-Cl was used to maintain the pH of the internal solution at 7·5. All experiments were carried out at room temperature (18–20° C).

RESULTS

Effects of various anions in the internal solution on the resting membrane potential

The values given in Table 2 illustrate the effects on the resting membrane potential when solutions of various K salts are perfused through the muscle fibres lying in the standard saline. They show that solutions of K acetate, isethionate, aspartate and glutamate allow the fibres to give values for the resting potential which are close to those reported by Keynes *et al.* (1973), by DiPolo (1972) and DiPolo & Latorre (1972). The values obtained when other solutions were used are low. If the generation and maintenance of steady resting potential and its magnitude are taken as the criteria for choosing a solution for internal perfusion, the anions of potassium may be arranged in the descending order, acetate > isethionate > aspartate > glutamate > fluoride > monohydrogen phosphate > succinate > citrate > sulphate > oxalate > iodobenzoate > ferrocyanide > chlorate > nitrate > chloride > thiocyanate > iodide > bromide > cyanide.

In the case of KCN, although one could anticipate its destructive effect on the cell at the high concentration used (200 mM), it should be stated that the instantaneous value of the resting potential was about -40 mV and in about 10 min the fibre became opaque and the resting potential was completely abolished.

The preference of an anion for internal perfusion indicated above do not seem to follow any set pattern. The anions cannot be arranged according to their valence or size or polarizability; neither can they be arranged according to the lyotropic series as Tasaki (1968) has done for the effects of various anions in suppressing the action potential of the giant axon of the squid. However, monovalent organic anions (at pH 7·5 one carboxyl group in aspartic and glutamic acids is ionized) seem to be well tolerated by the barnacle muscle fibre. This may be due to the fact that the total ionic composition of the muscle cell is only about 220 mM (Keynes *et al.* 1973; Brinley, 1968; Hagiwara, Chichibu & Naka, 1964), the rest about 800 mM being made up of amphoteric biopolymers. However, there seems to be about 100 mM of inorganic phosphate present in these fibres (V. Nassar & E. Rojas, unpublished). This may be the reason why monohydrogen phosphate is also tolerated by the muscle cell. Nevertheless

the study of the behaviour of the muscle cells when internally perfused with solutions of high ionic strength described below indicate that only acetate, aspartate and glutamate solutions are well tolerated by the fibres in the range of ionic strength 50–650 mM.

Effects of ionic strength of the internal solution on the resting membrane potential

The various salts of K are arranged in Table 2 in descending order of the magnitude of the resting potential generated by them. The salts listed below citrate generate low values and therefore have not been used to study the effects of their ionic strength on the resting potential. The results obtained with the solutions of other salts of different ionic strength are summarized in Table 3. K acetate, aspartate and glutamate solutions do

TABLE 2. Resting membrane potential observed in the barnacle muscle fibre when perfused internally with a solution of K (200 mM with respect to K ion) associated with various anions, pH = 7·5

Salt	Symbol	Number of fibres (n)	Resting potential (mV)
Acetate	KAc	15	$-56{\cdot}0 \pm 0{\cdot}7$
Isethionate	KIse	4	$-55{\cdot}0 \pm 0{\cdot}4$
Aspartate	KAsp	10	$-50{\cdot}7 \pm 1{\cdot}0$
Glutamate	KGlu	4	$-50{\cdot}2 \pm 0{\cdot}5$
Fluoride	KF	12	$-48{\cdot}9 \pm 0{\cdot}9$
Monohydrogen phosphate	K_2HPO_4	14	$-48{\cdot}7 \pm 0{\cdot}8$
Succinate	KSuc	5	$-48{\cdot}2 \pm 0{\cdot}7$
Citrate	KCit	5	$-44{\cdot}4 \pm 1{\cdot}2$
Sulphate	K_2SO_4	8	$-41{\cdot}1 \pm 1{\cdot}9$
Oxalate	KOxa	3	$-39{\cdot}7 \pm 0{\cdot}3$
Iodobenzoate	KIdb	5	$-37{\cdot}4 \pm 2{\cdot}0$
Ferrocyanide	K_4FeCy_6	4	$-35{\cdot}9 \pm 1{\cdot}2$
Chlorate	$KClO_3$	4	$-31{\cdot}0 \pm 1{\cdot}3$
Nitrate	KNO_3	3	$-29{\cdot}7 \pm 1{\cdot}2$
Chloride	KCl	7	$-28{\cdot}7 \pm 0{\cdot}8$
Thiocyanate	KCNS	5	$-27{\cdot}1 \pm 1{\cdot}0$
Iodide	KI	5	$-25{\cdot}7 \pm 1{\cdot}3$
Bromide	KBr	4	$-19{\cdot}6 \pm 0{\cdot}2$
Cyanide	KCN	4	Destroyed

Values for mV given as ± 1 S.E. of the mean.

not depolarize the membrane when solutions of high ionic strength are perfused whereas the other solutions of potassium monohydrogen phosphate, fluoride, isethionate, succinate and citrate do. The results obtained in this study with K aspartate solutions of high ionic strength are not in agreement with those reported earlier for the same barnacle species by Keynes *et al.* (1973) who observed the fibres to depolarize when the

TABLE 3. Effect of ionic strength on the resting potential (mV) in the barnacle muscle fibre internally perfused with various solutions, pH = 7·5

Solution conc. (m-equiv/l.)	KAc	KAsp	KGlu	K_2HPO_4	KF	KIse	KSuc	KCit
20	−33·0 ± 2·0 (4)	−32·8 ± 2·4 (3)	−39·0 ± 1·2 (3)	−28·5 ± 0·9 (3)	−34·0 ± 1·2 (3)	—	—	—
50	—	−36·6 ± 1·2 (9)	—	—	—	—	—	—
100	−45·8 ± 1·7 (4)	−40·6 ± 0·9 (11)	−43·0 ± 1·7 (3)	−32·9 ± 1·0 (7)	−47·2 ± 0·6 (3)	—	—	—
150	−54·4 ± 0·5 (4)	−45·4 ± 0·5 (9)	—	—	−52·2 ± 0·8 (5)	—	—	—
200	−56·0 ± 0·7 (15)	−50·7 ± 1·0 (10)	−50·2 ± 0·5 (4)	−48·7 ± 0·8 (14)	−48·9 ± 0·9 (12)	−55·0 ± 0·4 (4)	−48·2 ± 0·7 (5)	−44·4 ± 1·2 (5)
300	−60·2 ± 1·5 (5)	−52·1 ± 0·6 (6)	—	−52·2 ± 1·0 (7)	−37·9 ± 2·7 (5)	−59·0 ± 0·9 (4)	—	−41·8 ± 1·2 (5)
350	—	—	—	—	—	—	−61·9 ± 0·7 (3)	—
400	−67·0 ± 2·3 (5)	−61·1 ± 1·6 (5)	−61·3 ± 0·7 (3)	−56·8 ± 1·3 (6)	—	−49·7 ± 0·9 (3)	—	—
450	—	—	—	—	—	—	−61·0 ± 2·1 (6)	—
500	−67·1 ± 1·6 (5)	−62·1 ± 1·6 (6)	—	−39·2 ± 0·4 (3)	—	—	—	—
550	—	—	—	—	—	−48·7 ± 1·8 (3)	—	—
600	—	−62·2 ± 1·0 (5)	—	—	—	—	—	—
650	−71·1 ± 1·1 (4)	−66·4 ± 1·0 (5)	−64·1 ± 1·2 (3)	−37·3 ± 1·4 (3)	—	—	−34·0 ± 2·3 (3)	—

The numbers in brackets indicate *n*, the number of fibres.

internal K concentration was increased from 180 to 550 mM. Whether this discrepancy is due to a seasonal effect or not requires further investigation. The experiments, the results of which are reported herein, were carried out during the month of August 1972, which year in Chile was quite unusual in that there were heavy and continuous rains and the weather was very cold.

Muscle fibres perfused with solutions of high ionic strength took about 30 min to give a steady value for the resting potential. So it is reasonable to assume that the fibre attained equilibrium with the perfusing solution and that the concentration of K in the fibre was the same as that in the perfusing solution.

When solutions of low ionic strength (below 100 mM) were used, it seemed as though considerable time was required before a steady value for the resting potential was obtained. The values given in Table 3 for concentrations of K salt less than 100 mM were those that were observed after about 45 min. It was difficult to decide whether they were the steady values or not since they drifted to lower values with passage of time. However in some cases they were steady. For example in one experiment an internal solution of 20 mM-K monohydrogen phosphate generated a resting potential of -30 mV at the start of the experiment. At the end of an hour, it was -27 mV which at the end of another 2 hr stood at -20 mV. In another experiment extending for a long period of time, the initial value was -36 mV and at the end of an hour was -35 mV which changed to -20 mV after a period of another 5 hr.

The results of the variation of the resting potential with the concentration of K in the internal perfusing solution for acetate, aspartate and glutamate are given in Fig. 1. In all these cases, the resting potential E_m is approximately related linearly to log $[K]_i$ where $[K]_i$ is the concentration of K in the perfusing solution giving a slope of 30·4 mV for K acetate solution and of 27·4 mV for aspartate and glutamate solutions. These slopes were evaluated by the method of least squares. The points corresponding to the low ionic strength solutions, i.e. 50 and 20 mM, do not fall on the regression line drawn through all the points corresponding to solutions of higher ionic strength. The deviations are more negative by about 8–10 mV for the acetate and aspartate solutions and about 14 mV for the glutamate solution. This arises from the fact that the perfusion of the solution of low ionic strength over a period of time removes besides other materials some K from the fibres which under the stress of unphysiological environment deteriorate with time. Attainment of equilibrium by the fibre with its environment, a condition necessary for the attainment of a steady value for the resting potential, is not possible when the fibre itself is deteriorating. In an otherwise tolerable situation, equilibration of the fibre with the internal perfusing solution is determined by (1) the state of existence of K

in the fibre, (2) concentration of K in the perfusing solution and (3) rate of perfusion. With solutions of ionic strength containing about 100 mM-K or more, a steady value for the resting potential was realized in 20–30 min. The K concentration of the fresh barnacle muscle fibre is about 170 mM/l. fibre water. All of this is considered to exist in the ionized form and in Donnan equilibrium following the relation $(a_K)_o(a_{Cl}^-)_o = (a_K)_i(a_{Cl})_i$ where a_K and a_{Cl} are the activities of K and Cl respectively and o and i stand for the external and internal phases of the muscle fibre (Hinke & Gayton, 1971). Chloride concentration of the fibre is about 30 mM. With the perfusing solution of 100 mM-K, a steady value for the resting potential can

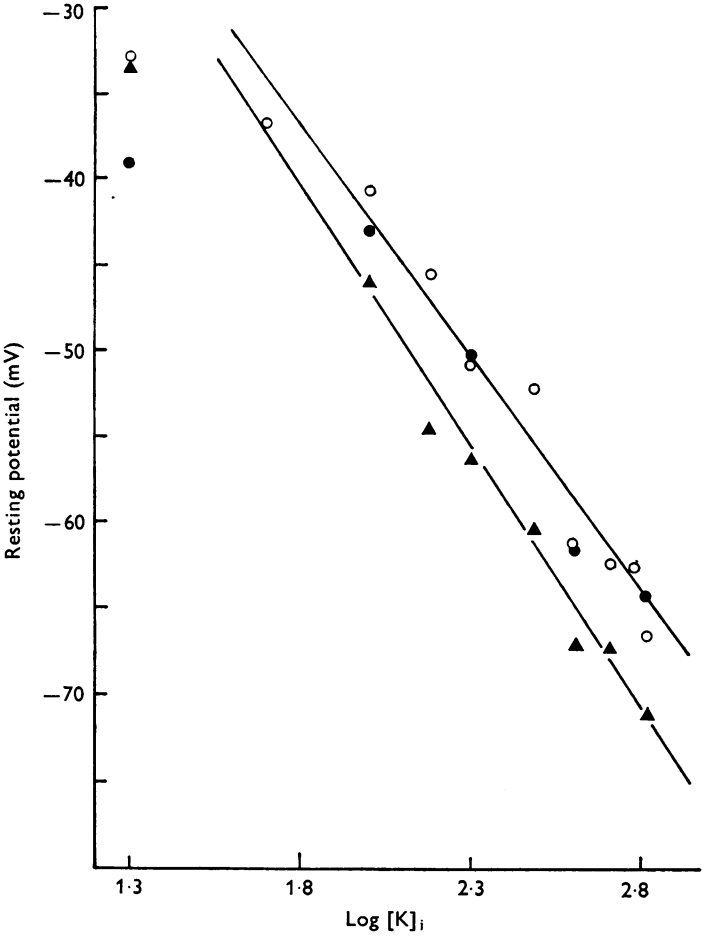

Fig. 1. Relation between resting potential and log of internal concentration (mM) of K acetate (▲), K aspartate (○) and K glutamate (●) for barnacle muscle fibres immersed in normal saline. Isotonicity of internal solutions was maintained with sucrose.

be realized in about 30 min. Perfusion of this solution involves removal of some fibre K probably associated with 30 mM-Cl and some other small and mobile anion, as, for example, phosphate so that the final concentration of K in the fibre corresponds to that in the perfusing solution. But with solutions of low ionic strength (< 100 mM), the K that is associated with mobile ions in the fibre is easily removed and the rest, probably associated with the anionic sites in the fibre membrane is not easily removed so that the concentration of K in the fibre will remain high compared to that in the perfusing solution. This results in a higher value for E_m and thus lead to the deviation noted in Fig. 1.

An attempt therefore was made to see how much of K came out of the fibre in the first 60 min or so of perfusion. In this experiment, the external barnacle saline contained no K or Ca but only 60 mM-$MgCl_2$ and 440 Tris-Cl (pH = 7·5). The solution used for the perfusion of the fibre was isotonic sucrose only whose pH was maintained at 7·5 using Tris-Cl. At a perfusion rate of 0·02 ml./min the amount of K that came out in the perfusate in 30 min in one fibre corresponded to 37 mM-K per kg wet fibre. In another fibre, the amount of K that came out in 45 min corresponded to 53 mM-K per kg wet fibre. The resting potential during this period changed from about −40 mV at the beginning to about −28 mV at the end of the perfusion period. Thus when very low ionic strength solutions are used, the inability of the fibre to reach equilibrium with the perfusing solution is responsible for the deviation seen in Fig. 1.

Effects of various cations in the internal solution on the resting membrane potential

Considerable difficulty was experienced in performing these experiments. This was due mainly to breakage of the fibres following contractions when they were perfused with various solutions. Solutions of Na acetate and Rb acetate were tolerated by the fibres while with the other solutions the fibres reacted differently (see Table 4).

If one considers the behaviour of the barnacle muscle fibre subject to internal perfusion with different cationic solutions and the magnitude of the resting potential observed, the various cations can be arranged in the descending order K > Na > Rb > Cs ⩾ NH_4 ⩾ Tris ⩾ Li > TEA. In order to establish clearly this sequence, measurements of resting membrane potential were made using Ca-free saline in the bath. This has been shown to eliminate development of tension in the fibre (Keynes *et al.* 1973; I. Atwater, E. Rojas & J. Vergara, unpublished). This procedure eliminated contractions of the muscle fibre and thus led to reliable measurements of resting potential whose values are given in Table 5. These results show that the magnitude of the resting potential generated by various

cations follow the sequence K > Na > Rb > Tris > Li > NH_4 > Cs > TEA.

Tris and TEA ions have been used in many electrophysiological studies where Tris is used as a substitute for various cations (Adelman & Senft, 1968) and TEA to study its effects on the resting potential in different muscle preparations (Fatt & Katz, 1953; Fatt & Ginsborg, 1958; Werman & Grundfest, 1961) and to depress or block K currents in voltage clamp experiments (Tasaki & Hagiwara, 1957; Armstrong & Binstock, 1965;

TABLE 4. Resting potential (RP) observed in the barnacle muscle fibre perfused internally with 200 mM solutions of different cations, pH = 7·5

LiAc	RP started at about −45 mV and in 15–20 min depolarized to about −20 mV (3)
NaAc	−41·0 ± 0·7 (4)
RbAc	−38·3 ± 1·2 (4)
CsAc	RP started at about −43 mV and slowly dropped; contractures were observed. Potential slowly changed and overshot zero to about +8 mV and came back to about −20 mV (5)
NH_4Ac	Contractures were observed and in a number of cases the fibres broke. In fibres which did not break, the RP was about −20 mV. Generally twitching and oscillation of potential occasionally overshooting zero to about +5 mV and coming back to about −20 mV were observed (6)
Tris Ac	Behaviour was similar to that observed with NH_4Ac (3)
TEA Cl	Very difficult to perfuse and the fibres broke always (6)

The numbers in brackets indicate *n*.

TABLE 5. Resting potential observed in the barnacle muscle fibre in Ca-free saline and perfused internally with 200 mM solution of different cation, pH = 7·5

Salt	*n*	Resting potential (mV) ± S.E.	$\frac{P_M}{P_K}$
LiAc	7	−29·7 ± 2·2	0·48
NaAc	4	−37·6 ± 0·9	0·65
KAc	7	−48·4 ± 1·0	1
RbAc	4	−35·5 ± 0·6	0·60
CsAc	4	−23·6 ± 0·4	0·37
NH_4Ac	4	−24·0 ± 0·7	0·38
Tris Ac	4	−31·5 ± 1·2	0·51
TEA Cl	4	−21·0 ± 1·1	0·34

Stanfield, 1970*a*, *b*; Keynes *et al.* 1973). So their actions on the resting potential of the barnacle muscle fibres were further explored and some of the results are presented in Table 6.

TEA ion in general exerts a depolarizing action on the resting potential, this action increasing as the logarithm of increasing concentration of TEA

in the range 20–100 mM (see Fig. 2). When its concentration is increased to 150 mM, greater depolarization than that indicated by the exponential data of Fig. 2 is observed. On the other hand extrapolation of the straight line (Fig. 2) towards zero concentration of TEA cuts the −56 mV line (i.e. the resting potential observed in 200 mM-K acetate solution) at a point corresponding to a TEA concentration of 2·4 mM. Obviously this concentration of TEA will not depolarize the muscle membrane and this was found to be so in a number of experiments.

Tris ion seems to exert both a depolarizing and a hyperpolarizing action on the resting potential depending on the concentration of the K ion with which it is used. When approximately 100 mM-Tris is used with 100 mM-K,

TABLE 6. Effects of TEA and Tris ions on the resting potential in the barnacle muscle fibre in normal saline when perfused with various solutions, pH = 7·5

Solution (mM)	n	Resting potential (mV) ± S.E.
100 KAc	From Table 3	−45·8 ± 1·7
100 KAc + 100 TEA Cl	4	−34·3 ± 1·9
200 KAc	From Table 3	−56·0 ± 0·7
200 KAc + 20 TEA Cl	4	−44·0 ± 0·9
200 KAc + 50 TEA Cl	4	−39·3 ± 1·7
200 KAc + 100 TEA Cl*	4	−35·1 ± 0·2
200 KAc + 150 TEA Cl*	3	−23·7 ± 0·7
100 K and HPO_4	From Table 3	−32·9 ± 1·0
100 K + 100 Tris and HPO_4	6	−36·0 ± 2·5
200 K and HPO_4	From Table 3	−48·7 ± 0·8
200 K + 200 Tris and HPO_4†	4	−33·0 ± 3·3
324 K + 324 Tris and HPO_4†	2	−27·0

* Slight mechanical disturbance like changing external solution gave change in potential which overshot zero (action potential).

† Solutions flowed into the fibre which was initially being perfused with 200 m-equiv/l. solution of K_2HPO_4. Contractures and change of potential which became positive were observed. Recovery in 200 mM-K and HPO_4 solution was poor.

the membrane is hyperpolarized raising the resting potential from −32·9 to −36·0 mV; whereas 200 mM (approx.) Tris in presence of 200 mM-K depolarized changing the resting potential from −48·7 to −33·0 mV. Higher concentration of Tris is not well tolerated by the fibre either in presence or complete absence of K. This seems to indicate that the depolarization of the fibre membrane is brought about probably by the saturation of the anionic sites on the membrane with Tris counterions.

Effects of external ionic concentrations on the resting membrane potential

The resting membrane potential of the barnacle muscle fibre subject to internal perfusion with an isotonic solution containing 200 mM-K acetate

ranged from −48 to −60 mV when observed in standard saline which contained 10 mM-KCl. The resting potential decreased (more positive) when the concentration of K in the saline was increased by substituting it for Na (Fig. 3). The relationship between E_m and the logarithm of $[K]_o$ was a straight line for $[K]_o$ > 100 mM with a slope of about 48 mV and deviated from the straight line at lower concentrations. In Fig. 4 are given the results of a similar relationship between E_m and logarithm of $[K]_o$ when the fibre was in acetate saline solution (1). These results although

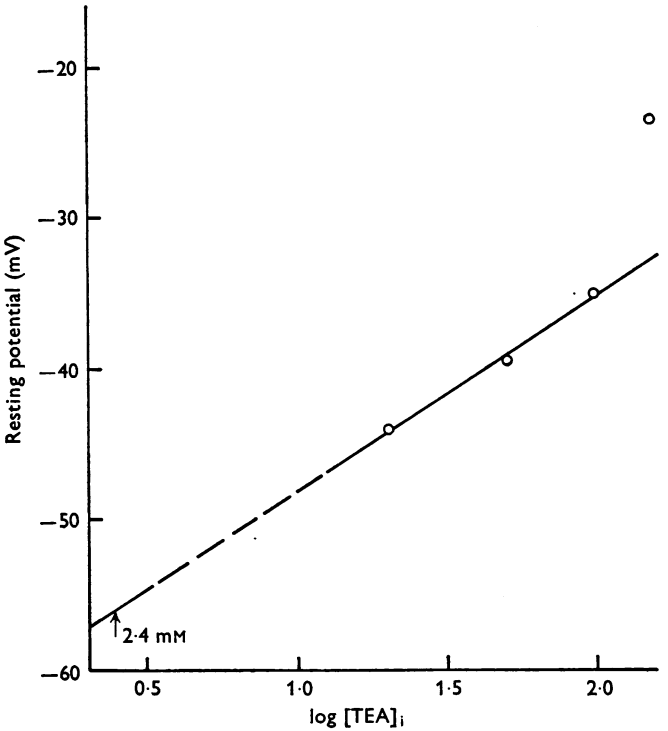

Fig. 2. Relation between resting potential and log of internal concentration (mM) of TEA-Cl in the perfusing solution which contained 200 mM-K acetate. Isotonicity of the internal solutions was maintained with sucrose. 2·4 mM is the concentration of TEA-Cl which had no effect on the resting potential.

a little more positive are not significantly different from those observed in the standard saline. The points corresponding to $[K]_o$ below 100 mM deviated from the straight line relationship which had a slope of 46 mV. In either case the resting potential was −60 mV when K was completely removed from the saline solutions.

In Fig. 4 are given also the results which show the effects of Rb and Cs

ions on the resting potential when they were used in place of K in the external saline solution (1). These results are similar to those obtained with K except that the slopes of the straight lines in the high concentration range are about 43 mV for Rb and about 37 mV for Cs.

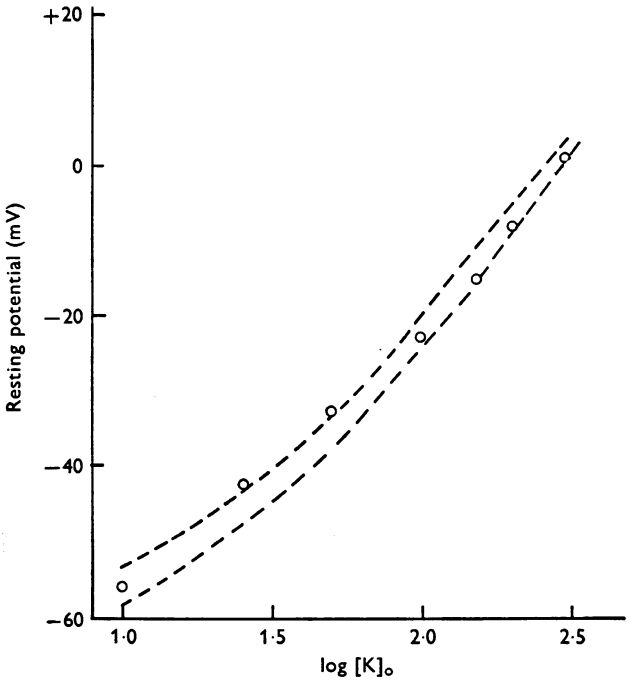

Fig. 3. Effect of increasing the concentration (mM) of external KCl on the resting potential of the barnacle muscle fibres which were internally perfused with a solution of K acetate (200 mM) made isotonic with sucrose; (○) indicate the average resting potential measured for four fibres. The dashed lines indicate the calculated values of resting potential when $P_K:P_{Na}:P_{Cl} = 1:0{\cdot}05:0{\cdot}1$ (upper curve) and $P_K:P_{Na}:P_{Cl} = 1:0{\cdot}05:0{\cdot}2$ (lower curve).

The concentration at which the resting potential should become zero may be estimated for each species of cations by extrapolation of the straight line to cut the zero-potential tie line. These concentrations were estimated to be 280, 380 and 1500 mM for K, Rb and Cs respectively. These results suggest that K ion is more permeable, followed by Rb, and Cs ion is very much less permeable than the other two ions.

In order to test the relative effects of other cations on the resting potential, chlorides of Tris, choline, TMA (tetramethyl ammonium) and TEA were used in the external saline in the place of NaCl. The results obtained with each of these saline solutions are given in Table 7. In the case of all

these ions, the value of the resting potential are lower (depolarization) than the control value of −54 mV. These results are not in agreement with the observations of Hagiwara *et al.* (1964) who state that Tirs, TMA and choline increased (hyperpolarization) the resting potential by about 10 mV. This discrepancy is difficult to understand; it may be ascribed to some effect on the membrane produced by the different ways in which the fibre membrane has been treated. The depolarization recorded in this study may arise from either an increase in the permeability of the membrane

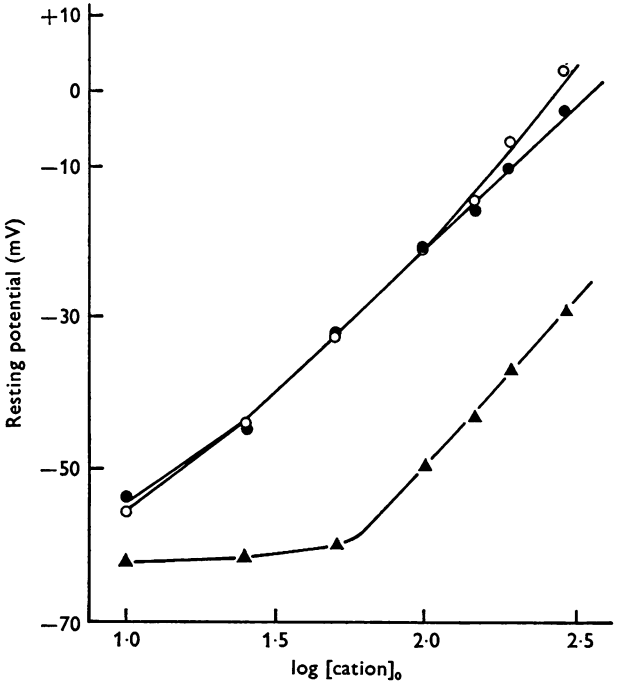

Fig. 4. Relationship between resting potential and log of concentration (mM) of K (○), Rb (●) and Cs (▲) in the external saline which had chloride partially replaced with acetate. The values given are the averages of values derived for four fibres.

to the ions concerned (Tris, choline, TMA, TEA) existing in the external saline or some other cause like flow of Ca ions into the fibre or their release from some sources inside the fibre itself leading to a membrane depolarization. As internal perfusion of Tris and TEA (Tables 4 and 5) also lead to a depolarization of the membrane, the former possibility seems to be unlikely. To say specifically that the latter is responsible requires further investigation.

Also given in Table 7 are some other results which describe the actions

of another cation Li and anions acetate and isethionate. The action of Li ion is similar to the depolarizing actions of other cations described above. When isethionate or acetate ion is partially substituted for the chloride ion in the external saline, the resting potential is lowered slightly, about 2 mV more positive than the control value, in the case of isethionate and remains practically unchanged in the case of the acetate ion. Even when the chloride ion is completely eliminated by the acetate ion, i.e. use of acetate saline solution (2), the resting potential became a little more positive, about 1·5 mV, compared to the control value of −54 mV.

TABLE 7. Effects of substitution of cations in place of Na and of anions in place of Cl in the external solution on the resting potential in the barnacle muscle fibre, pH = 7·5

External solution	*n*	Resting potential (mV) ± S.E.
Perfusion solution: 200 mM-KAc		
Normal saline (control)	8	−53·8 ± 0·7
Isethionate saline	6	−51·7 ± 1·0
Tris chloride saline	4	−44·5 ± 1·0
Choline chloride saline	7	−43·3 ± 0·9
TMA chloride saline	5	−42·2 ± 1·0
TEA Cl saline*	4	−29·5 ± 1·3
Normal saline (control)	3	−55·5 ± 2·6
Acetate saline solution (1)	3	−55·7 ± 2·8
Perfusion solution: 200 mM-KIse		
Normal saline (control)	2	−54·0
Acetate saline solution (2)	2	−52·5
Li acetate saline solution (2)	2	−41·0

* Four other fibres broke on contraction.

Absence of a significant change in the resting potential in these cases indicate that these anions are equally permeable or impermeable to the membrane.

To further explore the relative effects of chloride and acetate ions on the resting potential, K was completely eliminated from the external saline solutions in which the NaCl or Na acetate was progressively replaced by sucrose. Such solutions have been used with skeletal muscle fibres by Holtzman (1967) to accentuate effects on the membrane potential following changes in the concentration of various salts. The results obtained in the present study with such solutions containing 475, 300, 200, 100, 50 and 1 mM-NaCl or Na acetate are given in Fig. 5. The resting potential of each fibre was measured in the normal saline which was then changed successively to various test solutions. The fibre was left in each solution until a steady value for the resting potential was observed. After the last

test solution was used, the resting potential of the muscle fibre was again measured in the normal saline. If the initial and the final values observed in the normal saline did not agree within about 5 mV, the results were discarded. The results given in Fig. 5 are the averages of measurements made in each case on five fibres which satisfied this viability test. In 1 mM-NaCl solution (80 mM total extracellular chloride) there was a

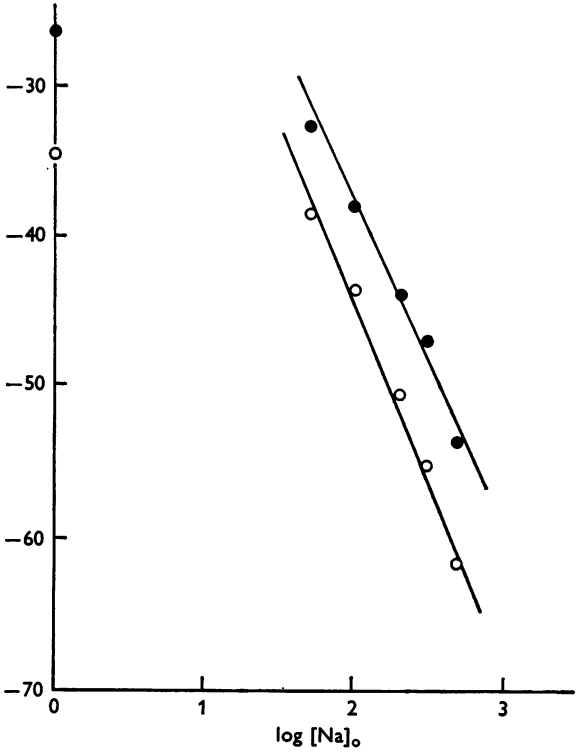

Fig. 5. Relationship between resting potential and log of concentration (mM) of NaCl (○) or Na acetate (●) in the external saline of different ionic strength. Each point represents the results of measurements on five fibres which were internally perfused with a solution of K acetate (200 mM) made isotonic with sucrose. The saline solutions were K-free and contained 35 mM-$CaCl_2$ or Ca acetate. They were made isotonic using sucrose.

depolarization of 27 mV. Similarly in 1 mM-Na acetate solution, there was a similar depolarization which in each case is logarithmically related to $[Na]_o$ with a slope of −23 mV from 475–50 mM. In the case of the acetate saline, the values of the resting potential were 6–8 mV more positive over the whole range of ionic strength. This may perhaps be ascribed to the low permeability of the membrane to the acetate ion in relation to that of the chloride ion.

TABLE 8. Estimates of relative permeabilities to Na and K
$[K]_o$ = 10 mM; $[Na]_o$ = 430 mM; $[Cl]_o$ = 565 mM

Internal solution	KAc		KAsp		KGlu	
$[K]_o$ mM	E_m (mV)	(P_{Na}/P_K)	E_m (mV)	(P_{Na}/P_K)	E_m (mV)	(P_{Na}/P_K)
20	−33·0	—	−32·8	—	−39·0	—
50	—	—	−36·6	0·004	—	—
100	−45·8	0·015	−40·6	0·023	−43·0	0·019
150	−54·4	0·017	−45·4	0·034	—	—
200	−56·0	0·027	−50·7	0·039	−50·2	0·048
300	−60·2	0·041	−52·1	0·065	—	—
400	−67·0	0·042	−61·1	0·059	−61·3	0·059
500	−67·1	0·058	−62·1	0·076	—	—
600	—	—	−62·2	0·095	—	—
650	−71·1	0·067	−66·4	0·086	−64·1	0·084

TABLE 9. Estimates of relative permeabilities to Na, Cl and K
$[K]_i$ = 200 mM; $[Cl]_o$ = 565 mM; and (P_{Na}/P_K) = 0·038

$[K]_o$ mM	$[Na]_o$ mM	E_m mV	(P_{Cl}/P_K)
10	430	−56·0	0·075
25	415	−42·2	0·029
50	390	−32·3	0·059
100	340	−22·7	0·138
150	290	−15·1	0·165
200	240	−8·1	0·156
300	140	+1·0	0·165

DISCUSSION

The main conclusion of this study is that the membrane potential in the barnacle muscle fibre depends upon the internal and external concentrations of K. This in general is in accordance with the behaviour of the resting potential observed in other excitable cells. Only some anions of K, for example, acetate, aspartate and glutamate are tolerated by the barnacle muscle fibre over the whole range of ionic strength (up to 650 mM) when it is internally perfused with those solutions. Other anions like monohydrogen phosphate, succinate, etc. (Table 3) are tolerated only when the ionic strength is moderate. The other anions (Table 2) used in this study gave very low values for the resting potential even when solutions of normal ionic strength are used internally. The factors causing or controlling this behaviour remain unknown at the present time and more experiments are needed to explain it.

The resting potentials observed in the barnacle muscle preparation when the fibres are in normal or Ca-free saline solution and internally

perfused with various alkali metal ions follow the sequence K > Na > Rb > Li > Cs (Table 5). This sequence is the same as the selectivity sequence VII-a established by Eisenman (1962, 1969) who, using a simplified model in which an anion exchange site on the membrane was homopolar, derived the equilibrium selectivity isotherms by calculating the free energy changes involved in the interactions of various alkali metal ions with water and the anionic site. It was shown also that as the water was removed, the anionic field strength increased thereby affecting only the magnitude of selectivity without changing its pattern. Accordingly the factor which would determine the selectivity sequence noted above was the magnitude of the coulombic energy associated with the interactions of various cations with the membrane site. This simple theory ignores the ionic mobility factor involved in the determination of the sequence. On the other hand, the constant field equation (Goldman, 1943; Hodgkin & Katz, 1949) takes into account both the equilibrium parameter, i.e. distribution coefficient K_j for the distribution of j between the membrane and the aqueous phases, and the mobility factor u_j, i.e. $P_j = (RT/F)\, u_j (K_j/d)$ where P_j is the permeability coefficient of j and d is the membrane thickness, and relates them to the membrane potential E_m. On the assumption that the chloride ion is distributed passively in accordance with E_m (Hinke & Gayton, 1971; DiPolo, 1972; DiPolo & Latorre, 1972) and that the acetate ion is impermeable (see p. 628), the constant field equation simplifies to

$$E_m = \frac{RT}{F} \ln \frac{[K]_o + (P_{Na}/P_K)\,[Na]_o}{[K]_i + (P_{Na}/P_K)\,[Na]_i}. \qquad (1)$$

This equation, modified to suit the experimental conditions under which the data given in Table 5 are derived, could be used to derive values for the permeability ratio (P_M/P_K) where P_M stands for any other cation. These values which establish the selectivity of the barnacle muscle fibre to various cations are given in Table 5.

Further if P_{Na} is small in relation to P_K, eqn. (1) reduces to the Nernst equation for a K electrode. In this case, plot of E_m against log $[K]_i$ would give a straight line with a slope of 58 mV at room temperature. The data given in Fig. 1 show that the slopes are nearly half of what they should be for a K electrode. In skeletal muscle fibre, deviations from the theoretical slope (58 mV) have been attributed to increased permeability of Na (Adrian, 1956; Hodgkin & Horowicz, 1959). Accordingly eqn. (1) for the condition $[Na]_i = 0$ for the muscle fibre under internal perfusion, together with the data given in Fig. 1, have been used to estimate the relative permeabilities of the muscle membrane to Na and K ions. The results of these calculations given in Table 8 show that (P_{Na}/P_K) varied between

0·02 and 0·08. Incidentally this corresponds to the same order of magnitude as that noted for the perfused giant axon of the squid (Baker *et al.* 1964). At $[K]_i$ = 20 mM, (P_{Na}/P_K) value becomes negative. As discussed already, this is due to the fact that the measured value of E_m (for example −33·0 mV in the case of K acetate solution) does not correspond to $[K]_i$ = 20 mM but to a value somewhat higher than that. If $[K]_i$ = 20 mM is used to calculate the factor $[K]_i\, e^{E_m F/RT}$, the value of the factor which is 5·4 becomes less than the value of $[K]_o$ which is 10 mM. In order to get a positive value for (P_{Na}/P_K), the above factor must be greater than 10. A minimum value of 37·1 mM for $[K]_i$ makes the factor positive. If a value of 40 mM which is still underestimated for $[K]_i$ is used, a value of 0·002 is obtained for (P_{Na}/P_K).

This type of analysis can also be applied to the data given in Fig. 3 using the appropriate form of the constant field equation to suit the conditions of internal perfusion, i.e. $[Na]_i = 0$ and $[Cl]_i = 0$. In this analysis an average value of 0·038 for (P_{Na}/P_K) estimated for the fibre ($[K]_i$ = 200 mM) in normal saline (see Table 8) was taken as a constant. As can be seen from the results given in Table 9, (P_{Cl}/P_K) changed little and had an average value of 0·156 when $[K]_o$ was changed from 100 to 300 mM. Below 100 mM, the value was a little less. The results given in Fig. 3 show that the values of E_m observed with KCl concentrations less than 100 mM were well fitted by $P_K : P_{Na} : P_{Cl} = 1 : 0{\cdot}05 : 0{\cdot}1$. With higher external concentrations of KCl, the resting potential was more than that calculated indicating either a better discrimination between K and other ions or a higher permeability of chloride contributing to the observed potential. In this region, $P_K : P_{Na} : P_{Cl} = 1 : 0{\cdot}05 : 0{\cdot}2$ gave a reasonable fit. This indicates that the chloride ion in the region of high KCl concentrations has a higher permeability and contributes to the potential. In this connexion it is interesting to note that in contrast to what is observed in the barnacle muscle fibre (i.e. $(P_{Cl}/P_K) \approx 0{\cdot}2$), the ratio (P_{Cl}/P_K) or rather (g_{Cl}/g_K) where g_j is the conductance of j, is about 2 or higher for the frog skeletal muscle (Hodgkin & Horowicz, 1959; Hutter & Noble, 1960).

TEA ion produces a number of effects in various muscle and nerve preparations both in resting and voltage-clamp conditions. The resting potential in the crab muscle fibres was reduced by TEA (Fatt & Katz, 1953), while it had no effect on the resting potential in crayfish muscle (Fatt & Ginsborg, 1958). On the other hand, Werman & Grundfest (1961) found TEA to hyperpolarize the lobster muscle. In skeletal muscle the results of Stanfield (1970*a*, *b*) indicate that TEA reduced the resting potential. However, Kao & Stanfield (1970) found this effect to be negligible. The results of this study concerned with the barnacle muscle show that TEA

whether applied internally or externally consistently reduced the resting potential. Depression or elimination of late K currents in the nerve under voltage-clamp conditions by TEA has been well documented (Armstrong & Binstock, 1965; Hille, 1967). Similarly in skeletal muscle both the delayed rectifier current (delayed K outward current) and the anomalously rectifying (inward) K current have been depressed or abolished by TEA (Stanfield, 1970*a*, *b*; Kao & Stanfield, 1970). Adrian (1960) has suggested that in skeletal muscle there are two types of K channel with opposite rectifier properties, in parallel with each other. In addition, there exists the third type of K channel controlling the resting membrane potential or the resting K conductance. The delayed rectifier K channel and the anomalously rectifying K channel are both affected by TEA, while the K channel controlling the K conductance at rest is not very much affected by TEA. Recently it has been shown (Keynes *et al.* 1973) that TEA depressed the outward K currents in the barnacle muscle fibre. In view of this similar depressive action of TEA on both the resting and active membrane conductance to K, it is possible, as opposed to the situation considered to prevail in the skeletal muscle, that one and the same K channel in the membrane controlled both the resting and the active K conductance in the barnacle muscle.

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