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Effect of Inorganic Phosphate on Membrane Potential and Excitability of an Isolated Frog Muscle Fibre

By

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(With 1 Text-figure)

In general, living organisms tend to take up certain substances but to exclude some others. Chemical analysis of intracellular and extracellular electrolytic ions was made by Fenn ('36), and Boyle and Conway ('41). Inside the muscle fibre the concentration of potassium ions is comparatively much higher than that of anions. The amount of chloride inside the muscle fibre is either nil or exceedingly small. Although the intracellular phosphate ions are most abundant among the anions inside muscle fibre, the total sum of cations exceeds the sum of all anions, and the anion deficit seems to be made up by proteins and amino acids.

According to Kamada and Kinoshita ('43), the diminution of intracellular free phosphate causes the muscle fibre to contract, and it will be considered that the role of phosphate ions inside the muscle fibre is more or less different from the biochemical significance (in ATP-actomyosin system) as reported by various authors, e.g. Szent Györgyi ('51).

In order to ascertain the role of phosphate ions in the muscle an electrophysiological investigation was undertaken. The experiments described here deal with membrane potentials and responses to electrical stimuli in single muscle fibres treated with various phosphate or chloride solutions. From the results obtained the role of environmental free inorganic phosphate ions in relation to the muscle fibres will also be discussed.

Material and methods

Single muscle fibres were isolated free of injury from semitendinosus muscle of the frog, *Rana nigromaculata*. A muscle fibre was attached to a piece of tendon at each end, in which a hole was bored and it was stretched horizontally in a Ringer's solution¹⁾ or various test solutions by means of a pair of glass hooks through the hole in the tendon.

The membrane potentials were measured by using intracellular glass microelectrodes (tip diameter within 1μ , electrical resistance of about 10-20 M ohms) filled with 3M-KCl solution and connected to a cathode follower (Matsuda 6AK5) with precautions to reduce grid currents working into a direct coupled push-pull amplifier (Matsuda 6SN7-GT) and

1) 0.12 M NaCl, 0.002 M KCl, 0.0012 M CaCl_2 , pH 7.1-7.2 by Na-phosphate with p=6 mgm %.

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a galvanometer (10^{-7} amp.) as the potentiometric null instrument. The P.D. measured was accurate to 0.5 mV. Electrical stimulation of the single muscle fibre was made with two liquid external electrodes under the condition that the interpolar length was 5 mm, and the interpolar region of the fibre was electrically insulated in moistened air (Tamasige '50). The non-polarizable electrodes used were of Ag-AgCl type.

Results

A fresh preparation of single muscle fibre (the rheobase: 50 mV., Tamasige '53) was placed at first in Ringer's solution and the membrane potential was measured observing the penetration of the microelectrode tip across membrane under a microscope. Determinations of the excitability and the membrane potential were then made in test solutions (phosphate or chloride solutions) for the same fibre and at last again in Ringer's solution. The room temperature was 28°–31°C.

In order to check the result of the above determinations a series of measurements was made with varying hydrogen ion concentrations of the isotonic (M/8) Na-phosphate mixture or of the isotonic K-phosphate mixture. The muscle fibre twitched or produced contracture on application of these solutions but soon it recovered its resting state. Potentials obtained were always lower than those found in the same muscle fibre in Ringer's solution. In K-phosphate solutions potentials were lower than those in Na-phosphate solutions. A comparison was made of potentials at four different hydrogen ion concentrations of Na- or K-phosphate solutions (Table 1). It is shown in this table that the membrane potential depends rather on the kind of cations contained in the phosphate solution than on the value of pH.

Table 1. Effects of inorganic phosphate solutions upon the membrane potential of frog muscle at 30°C. (1) is pure M/8 NaH_2PO_4 or M/8 KH_2PO_4 ; (2) and (3) are mixtures of M/8 NaH_2PO_4 and M/8 Na_2HPO_4 or of M/8 KH_2PO_4 and M/8 K_2HPO_4 ; and (4) pure M/8 Na_2HPO_4 or M/8 K_2HPO_4 . The effect in (1) is significantly different from that in the others, (2), (3) and (4).

	pH	Membrane potential (-mV.) inside negative	
		in Na-phosphate	in K-phosphate
(1)	4.6	51±7	12±2
(2)	5.9	39±3	7.5±1.5
(3)	7.2	40.5±2.5	3±1
(4)	10.0	41±5	4±1

Another series of measurements was made in order to compare the membrane potential in the single muscle fibre in the phosphate solution (M/8 Na- or M/8 K-phosphate) at pH 5.9 with the membrane potential in the chloride solution (M/8 NaCl or M/8 KCl) containing common cations at the same pH. Moreover

the reversibility of the value of potential on return to the Ringer's solution was examined. Fig. 1 shows the results obtained for each two specimens treated with various test solutions. Potentials both in phosphate solutions, as is mentioned above, and in chloride solutions are lower than the normal one. In K-phosphate solution the potential is lower than the potentials in other solutions; the value is below 10 mV.; in KCl solution it is about 24 mV.; both in NaCl and in Na-phosphate it is between 36 and 43 mV.

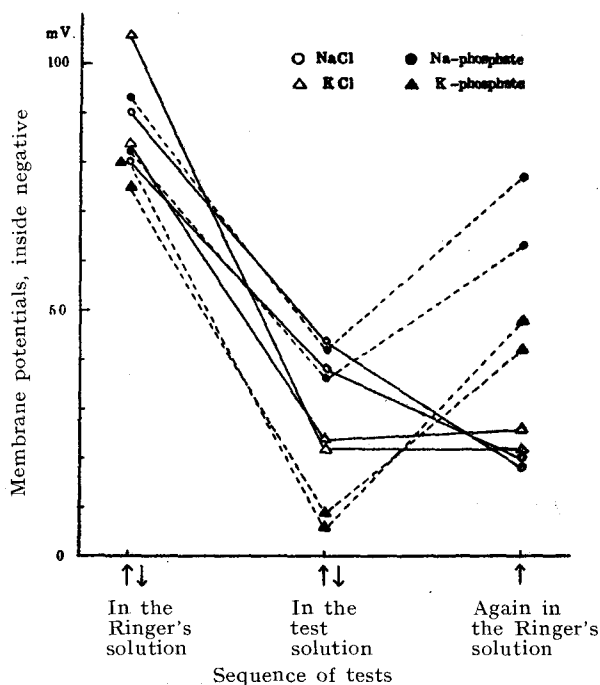


Fig. 1. The reversibility of the value of membrane potential of the single muscle fibre on return to Ringer's solution at 28°-31° C.

Stimulus was applied to the single muscle fibre treated with various test solutions. In the isotonic KCl solution the responses of fibre to electrical stimuli were a cathodic shortening as already reported by Tamasige ('53), and also in the other solutions the responses took the form of a local contraction. In the Na-phosphate solution the threshold was the lowest, the values being 2V, $0.5\mu\text{A}$, 0.5 sec.; in K-phosphate solution it was 65V, $2\mu\text{A}$, 0.5 sec.; in NaCl solution, 65V, $3\mu\text{A}$, 0.5 sec.; in KCl solution, 65V, $20\mu\text{A}$, 0.5 sec.; and in normal Ringer's solution, 0.05V, $1 \times 10^{-9}\text{A}$, 0.5 sec.

After stimulation in various test solutions the single fibre was returned to

Ringer's solution and the excitability was examined. The recovery of excitability was nearly complete in the case of phosphates and even a propagative contraction was observable, but no recovery occurred at all in the case of chlorides. In fibres treated with isotonic Na-or K-phosphate solutions the threshold value recovered to 0.2V, 0.5 μ A, 0.5 sec. or to 0.7V, 0.5 μ A, 0.5 sec. respectively, while in fibres treated with isotonic NaCl or KCl no recovery of the excitability was observed.

Discussion

Membrane potentials of the muscle cell in phosphate or chloride solutions were always lower than those in Ringer's solution. However, the degree of potential depression varies amongst them. In K-phosphate solution the potential is lower than those in Na-phosphate solution. The difference is probably in part due to an effect of K-depolarization on the membrane. In K-phosphate solution (125 mM.) the potential is within 10 mV., and the value is similar to the one reported by Harris and Martins-Ferreira ('55) for the muscle of the frog *Leptodactylus ocelatus*. In the experiments in which chloride solution was used in place of phosphate solution the same difference of potential between NaCl and KCl was obtained. Accordingly, it is clear that the remarkable depressions in membrane potential are attributable to K-ions both in phosphate solution and in chloride solution.

On the other hand, attempts were made to replace the anions Cl^- by phosphate at a given concentration of the common cations K^+ . Such procedure led to the same lower membrane potential with a value similar to the results obtained by Harris and Martins-Ferreira. They thought that the phenomena were understandable since the contribution of Cl^- ions to the potential is of slight importance in the phosphate solution. However, in use of phosphate ions in place of Cl^- ions at a given Na-concentration no significant difference of membrane potential was found between the two kinds of anions.

From these facts it is considered that the application of K-ions in place of Na-ions certainly causes a great change in membrane potential, whereas the application of phosphate ions in place of Cl^- ions does not do so.

However, when muscle fibres are transferred again into the Ringer's solution after various treatments the reversibility is high in the order of Na-phosphate > K-phosphate > KCl = NaCl. In the muscle fibres treated with chloride solution the membrane potentials are not recovered on return to Ringer's solution. The difference between phosphate and chloride solutions may be due to differences in ability of the ions to penetrate the membranes since the rate of penetration of phosphate ions through membrane is lower than that of Cl^- ions. Harris ('53) has shown that phosphate can not penetrate muscle.

It is worth remarking that environmental free phosphate ions do not directly affect the muscle fibre through membrane. It is certain, therefore, that phosphate ions have an important role to play inside the muscle fibre.

Summary

1. The membrane potential and the excitability in single muscle fibres of the frog, *Rana nigromaculata*, treated with isotonic phosphate or chloride solutions were described.

2. In Na- and K-phosphate solutions the membrane potential may increase with the decrease in pH.

3. In K-phosphate the potential is the lowest, the value is below 10 mV; in KCl it is about 24 mV; both in NaCl and in Na-phosphate it is between 36 and 43 mV. The difference between K- and Na-phosphate and that between KCl and NaCl seems to be due to an effect of K-depolarization on the membrane.

4. Upon application of phosphate ions in place of Cl-ions at a given Na- (common cations) concentration, no significant change in the membrane potential is observed.

5. Mechanical responses to electrical stimuli are a cathodic shortening or a local contraction in all test solutions used in the present experiments.

6. The recovery of membrane potential and excitability on return to Ringer's solution is nearly complete from the phosphate solution, but there is no recovery at all from the chloride solution. The difference of the reversibility between the phosphate and the chloride may be attributed to the difference of the ability of different ions to permeate the membrane since the rate of penetration of phosphate ions is lower than that of Cl ions.

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