ACTIVITY OF POTASSIUM AND SODIUM IONS IN THE CYTOPLASM OF THE STRIPED MUSCLE FIBERS OF THE FROG

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The object of the present investigation was to measure the activity of K^+ and Na^+ in the cytoplasm of the striped muscle fibers of the frog using cation-selective microelectrodes having a tip 0.5-1.0 μ in diameter.

METHOD

Experiments were carried out on the sartorius muscles of frogs of the species Rana temporaria and R. ridibunda. The basic solution was Ringer's phosphate solution of the following composition: Na^+) 116.25 mM; K^+) 2.5 mM; Ca^{++}) 1.8 mM; Cl^-) 117.1 mM; HPO_4^-) 2.5 mM, and $H_2PO_4^-$) 0.75 mM. The activity of K^+ and Na^+ was determined by means of microelectrodes made of cation-selective glass by the method described previously [1, 3], and the total concentration of K^+ and Na^+ in the muscles was obtained by flame photometry. The intracellular concentration of K^+ and Na^+ in mM/kg water of the fiber ($C_{w.fiber}$) was calculated from the formula:

$$C_{w.fiber} = \frac{C_m - 0,12\ C_{n.R.s.}}{0,66},$$

where C_m is the concentration of the ion in the whole muscle, $C_{n.R.s.}$ the concentration in the medium, 0.12 is the water in the intercellular spaces. Resting potentials (RP) were recorded with open microelectrodes made of Pyrex glass, filled with 3M KCl solution.

RESULTS

The results of a series of experiments carried out on nonisolated muscles of summer frogs, with a normal blood and nerve supply, are shown in Tables 1A and 2A. From the values of the total concentrations of electrolytes and the coefficients of activity of KCl and NaCl, the activity of K^+ (α_K) and of Na^+ (α_{Na}) in aqueous solutions of these salts were calculated. The values of γ_K and γ_{Na} were taken as being equal to the mean coefficient of activity of the electrolyte $\pm\gamma$. It is clear from the results given that the values of α_K and α_{Na} in nonisolated muscles are fairly stable and vary only within narrow limits.

Results of a large number of measurements on isolated muscles of summer and autumn frogs are given in the same tables (B). The muscles were kept in Ringer's solution for 2-3 h. Measurements began 1 h after isolation and immersion of the muscle in the solution. The results given demonstrate that isolation of the muscle leads to a very small decrease in the intracellular concentration (C_K) and activity (α_K) of potassium. The mean value of γ_K remained substantially unchanged. The Na^+ concentration in the muscles, on the other hand, rose. Changes of the same character in the concentrations of electrolytes in isolated muscles have repeatedly been described in the literature [2, 5, 8]. With an increase in the total concentration of Na^+, there was an associated increase of α_{Na}; the value of γ_{Na} remained unchanged.

The scatter of the values of α_K and α_{Na} in the isolated muscles was rather greater than in the nonisolated muscles, but the differences between the corresponding mean values were significant ($P < 0.001$).

TABLE 1. Mean Values of Activity of K^+, Measured with Selective Microelectrodes, and Calculated Values of its Activity within Fibers of Sartorius Muscles of the Frog

A. Nonisolated muscles

Date of experiment	No. of meas.	Resting potential (in mV)	α_K	γ_K	Conc. of K^+ (in mM/kg water of fiber)	α_K (calc.)	γ_K (calc.)
23/VIII	6	85,2	0,095	0,71	135,5	0,101	0,752
23/VIII	6	85,1	0,095	0,71	135,5	0,101	0,752
23/VIII	6	86,1	0,098	0,72	135,5	0,101	0,752
23/VIII	7	87,9	0,095	0,71	135,5	0,101	0,752
23/VIII	7	87,6	0,098	0,72	135,5	0,101	0,752
23/VIII	8	84,8	0,097	0,71	135,5	0,101	0,752
23/VIII	8	85,2	0,095	0,71	135,5	0,101	0,752
Mean	48	85,9	0,096±0,0002	0,71	135,5±0,21		

B. Isolated muscles

15/VIII	41	86,1	0,099	0,73	132,8	0,102	0,754
15/VIII	22	82,7	0,091	0,68	132,8	0,102	0,754
15/VIII	13	85,3	0,095	0,70	132,8	0,102	0,754
16/VIII	28	81,1	0,095	0,70	132,8	0,102	0,754
21/VIII	20	81,1	0,093	0,69	132,8	0,102	0,754
21/VIII	21	75,6	0,090	0,67	132,8	0,102	0,754
21/VIII	21	86,5	0,099	0,73	132,8	0,102	0,754
21/VIII	21	80,6	0,091	0,68	132,8	0,102	0,754
22/VIII	19	84,0	0,095	0,70	132,8	0,102	0,754
22/VIII	23	88,1	0,099	0,73	132,8	0,102	0,754
22/VIII	20	85,1	0,097	0,72	132,8	0,102	0,754
9/IX	20	84,8	0,093	0,69	132,8	0,102	0,754
11/IX	22	82,9	0,095	0,70	132,8	0,102	0,754
Mean	291	83,3±0,14	0,094±0,00017	0,70	132,8±0,26		

TABLE 2. Mean Values of Activity of Na^+, Measured with Selective Microelectrodes, and Calculated Values of its Activity within Fibers of Sartorius Muscles of the Frog

A. Nonisolated muscles

Date of experiment	No. of meas.	Resting potential (in mV)	α_{Na}	γ_{Na}	Conc. of Na^+ (in mM/kg water of fiber)	α_{Na} (calc.)	γ_{Na} (calc.)
21/X	10	84,9	0,012	0,63	18,8	0,016	0,875
21/X	8	85,4	0,011	0,57	18,8	0,016	0,875
21/X	11	86,9	0,010	0,52	18,8	0,016	0,875
21/X	8	87,0	0,010	0,52	18,8	0,016	0,875
21/X	3	85,5	0,011	0,57	18,8	0,016	0,875
Mean		85,9	0,0108±0,00024	0,56	18,8±0,21		

B. Isolated muscles

30/VIII	21	82,6	0,015	0,62	23,8	0,020	0,869
30/VIII	20	84,1	0,013	0,54	23,8	0,020	0,869
31/VIII	20	83,9	0,014	0,58	23,8	0,020	0,869
31/VIII	20	83,1	0,014	0,58	23,8	0,020	0,869
17/X	19	86,4	0,012	0,50	23·8	0,020	0,869
24/X	12	85,3	0,013	0,54	23,8	0,020	0,869
28/X	20	81,7	0,015	0,62	23,8	0,020	0,869
Mean		83,8±0,12	0,013±0,0007	0,56	23,8±0,17		

In this series of experiments it was not always possible to discover a strict relationship between the value of the RP and the intracellular K^+ activity. As may be seen in Table 2 and from results obtained by other workers [4, 6, 7], this discrepancy may be explained by the fact that in the isolated muscles the permeability of the membrane to Na^+ ions is increased, so that these ions accumulate inside the fibers. For this reason, in the tissues not in a state of equilibrium, the RP is a function of the activity gradients of all the penetrating ions and of their relative permeabilities [10]:

$$E = -\frac{RT}{F}\log\frac{P_K\,(a_{K\cdot int}) + P_{Na}\,(a_{Na\cdot int}) + P_{Cl}\,(a_{Cl\cdot ext})}{P_K\,(a_{K^+ ext}) + P_{Na}\,(a_{Na^+ ext}) + P_{Cl}\,(a_{Cl^- int})},$$

where α_K, α_{Na}, and α_{Cl} are the activities of the ions and P_K, P_{Na}, and P_{Cl} their permeability constants. According to this formula, the RP must be the smaller the higher the value of α_{Na} in the muscle fiber; the results given in Table 2 confirm that this actually happened.

It is possible to calculate from these results the concentrations of free and bound potassium and sodium ions within the muscle fibers. The calculations were made by comparing the experimental and calculated values of the activities. For K^+ the difference between the experimental and calculated values of α_K was 5-7%. This difference may be attributed to the following errors.

1. The potentials of the selective microelectrodes were measured with an accuracy of up to 0.25 mV. This degree of accuracy is not close enough for solutions with high ionic activities.

2. The diffusion potential of the ordinary microelectrode changes when it is moved from one calibration solution to another, and it is not the same in Ringer's solution and inside the cell. Its value in each concrete case may be calculated from Henderson's formula:

$$E = \frac{RT}{F}\cdot\frac{(U_I - V_I) - (U_{II} - V_{II})}{\left(U_I^1 - V_I^1\right) - \left(U_{II}^1 - V_{II}^1\right)}\ln\frac{\left(U_I^1 + V_I^1\right)}{\left(U_{II}^1 + V_{II}^1\right)},$$

where $U_1 = \Sigma m_1 U_1$, $V_1 = \Sigma m_1 V_1$, $U_1^1 = \Sigma z_1 m_I U_I$, $V_1^1 = \Sigma z_1 m_1 V_1$ for the first solution, and the same sums and indices (II) for the second solution (U and V—mobilities of cations and anions, m—their concentration, and z—their valency). Calculations showed that the diffusion potential of the microelectrode varies within the range from +4 to −2 mV (provided that the mobilities of the anions and cations in the cytoplasm are equal to their mobilities in the corresponding aqueous solutions). Changes in the diffusion potential may reduce the gradient of the calibration curves (amounting to -52.2 ± 1.05 mV/log α_K for the K^--selective microelectrodes and to -53.0 ± 1.03 mV/log α_{Na} for the Na^--selective microelectrode) and introduce an error into the determination of the activity of the ions. When the microelectrode was transferred from Ringer's solution into the muscle fiber its diffusion potential changed by −1.87 mV, corresponding to an error of 4-5% in the determination of α_K and α_{Na}.

3. The accuracy of the method of flame photometry is 5-10%. To increase the accuracy of the analysis, a large number of mice was investigated (25 animals in each series of measurements); the results were then analyzed by statistical methods.

Hence, the error of the method of determination of the activities must be regarded as being not more than 5-10%. It may therefore be concluded that potassium ions inside the muscle fibers of the frog are electrochemically completely free. For sodium ions the difference between the experimental and calculated values of α_{Na} was approximately 35%. This shows that part of the intracellular sodium is in a bound state.

Results showing convincingly that a large proportion (up to 90%) of the K^+ inside the cells is in a free state were obtained by Hinke [9], who measured α_K and α_{Na} inside the giant axon of the squid. Assuming that $\gamma_K = \gamma_{Na}$, Hinke concludes that about 76% of the Na^+ in the axon is free. The value of α_{Na} in his experiments was very low—only half that which we measured.

The experimental results described above show that the K^+ and Na^+ in the cytoplasm of the striped muscle fibers of the frog differ in their activity. The cytoplasm is known to be an heterogeneous structure. Much of it is occupied by macromolecular complexes, such as nucleoproteins, glycoproteins, lipoproteins, and so on, which are typical polyelectrolytes. Some polyelectrolytes possess marked selectivity in relation to certain ions. In chemistry,

however, no macromolecular compound is known which can react selectively with either K^+ alone or Na^+ alone. For this reason, the difference which was found between the experimental values of α_K and α_{Na} in the cytoplasm of the muscle fibers was quite unexpected.

A simple explanation may be suggested for these results if the existence of a nonspecific cell region is accepted. This suggestion was first put forward by Carey and Conway [6], who consider that in this so-called special region there is about 80% of the whole intracellular Na^+ but only a very small amount (about 10%) of the K^+. Somewhat later a three-component hypothesis was suggested [13], according to which the muscle fiber contains, besides extracellular spaces, two regions, one of which is accessible for all ions, but the other only to K^+. The two- or the three-component hypothesis readily explains the kinetics of the exchange of radioactive isotopes, which are known to be exchanged in accordance with 2-3 exponents and not with a simple logarithmic equation [3, 5, 8], and also the character of the changes in the ionic composition of tissue cells when placed in a medium with an altered ionic composition [12, 14]. It may also be regarded as having obtained experimental confirmation from the fact that large quantities of intracellular Na^+ are concentrated in the nucleus and in the organoids of the cell [11, 15].

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