HYDROGEN ION ROLE IN ACTIVE TRANSPORT OF POTASSIUM AND SODIUM THROUGH NEURON MEMBRANES IN THE SNAIL HELIX POMATIA

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The Na and K flux along the concentration gradients and the active transport of these ions through the neuron membrane of the snail Helix pomatia were investigated as functions of pH of the medium by Conway's method (1, 2) of loading cells with Na ions in the cold followed by restoration of the ionic composition by incubation at room temperature. The pH-maximum of the ion migration rates along concentration gradients was in the acid region for Na flux and in the neutral or weakly alkaline region for K. Hydrogen has an inhibitory effect on active ion transport. Analysis of the results by the Lineweaver–Burk method showed that the inhibition of the K influx is competitive, and that of the Na efflux noncompetitive.

INTRODUCTION

The dependence of ionic diffusion fluxes and active transport on pH were studied by determining the degree of dissociation of their ionogenic groups and modifying the activity of associated enzymes. Fluxes of K and Na along the concentration gradients and the active transport of these ions through the neuron membrane of Helix pomatia were investigated by Conway's method (1, 2) of loading cells with Na ions in the cold and subsequent restoration of the ionic composition by incubation at room temperature.

METHOD

The isolated CNS of Helix pomatia was used. After dissection the ganglia were kept at room temperature (18–20°C) for 1 hr in the animals' hemolymph, and then transferred to the test solution. The basic solution comprised (mM): NaCl 70.9, NaHCO₃ 7.5, CaCl₂ 10.1 and MgCl₂ 16.0. To load the neurons with Na ions and remove K ions from them the ganglia were placed for from 1 to 7 hr in potassium-free solution at 0.5°C. Solutions for restoring the ionic composition of the neurons contained 0–35 mM K, and had a pH of 5.4–8.0. Sufficient hydroxymethylaminomethane was added to make the solutions alkaline to pH 8.0; pH in the acid zone was adjusted by the addition of HCl; the pH of the solutions was monitored every 30–60 min.

After incubation, K and Na ion concentrations expressed in millimoles/liter cell water were determined with a flame photometer. In the calculations the extracellular space was taken as 37.37% (3). The experiments were carried out from March to May. Results were analyzed statistically by dispersion analysis.
A previous investigation showed that, like cells of other tissues, the isolated ganglia of snails in potassium-free solution at 0.5°C lose K and gain Na ions. However, transferring the ganglia to a medium with high K concentration at room temperature leads to loss of Na from the cells and an intake of K ions into them against a considerable concentration gradient.

Fig. 1. Semilogarithmic graph of electrolyte composition of neurons during incubation in potassium-free solution at 0.5°C. K—loss of K⁺ from neurons, Na—intake of Na⁺ into neurons, mM—molar concentration in cell water.

Changes in the intracellular concentrations of K and Na ions during incubation of the ganglia for various times in potassium-free solution at 0.5°C are shown in Fig. 1. Accumulation of Na and, correspondingly, loss of K are exponential in character as a first approximation. Deviations from a straight line are observed only during the first 90 min of the experiment. These results suggest that at the beginning of incubation migration of ions along concentration gradients takes place very rapidly, probably due to the complexity of organization of the extracellular space of the snail ganglia. An earlier histological investigation showed that the extracellular space contains a large connective tissue membrane of nonhomogeneous composition (3). Potassium ions leaving the cells evidently give rise initially to a redistribution of ions in the structural elements of the ganglionic connective tissue and to the appearance of fast components of ionic migration.

After incubation for 90 min at 0.5°C the decrease in intracellular potassium can be described by the equation

\[ C_i = C_{t=0} \cdot \exp \left( -\frac{t}{\tau} \right), \]

where \( C_t \) is the K concentration in the neurons at time \( t \), \( C_{t=0} \) is the initial concentration of the ion, and \( \tau \) the time constant of its outflow. The inflow of Na takes place in accordance with the equation

\[ C_i = C_{t=\omega} \left[ 1 - \exp \left( -\frac{t}{\tau} \right) \right], \]

where \( C_t = \omega \) is the Na concentration in the neurons after loading in a potassium-free solution.

The time constants can be approximated graphically (Fig. 1). The tangents of the angles formed by the straight lines with the abscissa are equal to the velocity constants of the change in the intracellular ionic concentrations (k). Since \( k = \frac{1}{\tau} \), the mean values of the time constants of K outflow and Na inflow can be calculated easily as 20.58 and 15.02 hr respectively.

From these results the flux velocities of K and Na ions (\( M_K \) and \( M_{Na} \)) can be calculated. The values of these fluxes express resultant velocities at which ions penetrate into the cells or leave them. It was assumed that in potassium-free solutions at 0.5°C the processes of active transport are blocked, and that the increase in Na and decrease in K in the cells are the result of migration of ions along the concentration gradients (\( M_K \) and \( M_{Na} \)). The diffusion component of these fluxes was disregarded, for according to the constant field theory, to calculate it, values would be required for the transmembrane potential difference and coefficients of permeability for the particular ion.