88. Prolongation of the Action Potential of Squid Giant Axons in Viscous Solutions

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(Communicated by Yasuji Katsuki, M. J. A., June 8, 1976)

Keynes\(^1\) and many other investigators considered that the rate-limiting process of the membrane excitation was probably the conformation change of the membrane molecules rather than the diffusion of ions across the membrane. The purpose of the present paper is to examine to what extent the diffusion process contributes the rate of the electrophysiological change of the membrane. The viscosity of the external and the internal solutions of the intracellularly perfused squid giant axon was changed by adding non-electrolytes to solutions. As reported previously, the resultant increase in the osmotic pressure did not cause any damage to the axon when the osmolarities of the external and the internal solutions were equal.\(^2\)

It was found that the time course of the action potential was prolonged in proportion to the increase of the viscosity of solutions.

A hindmost stellar giant axon of squid (Doryteuthis bleekeri) was used. The intracellular perfusion technique was the same as that previously reported.\(^3\) A whole system was kept at 10–15°C. Current stimuli were supplied by a platinized platinum wire electrode and the membrane potentials were recorded by a capillary electrode filled with 0.6 M KCl-agar. The viscosity of solutions was raised by increasing the concentration of neutral solutes, i.e., glycerol, glucose and sucrose. Molarities of electrolytes were kept constant. External solutions contained 450 mM NaCl and 100 mM CaCl\(_2\) and internal solutions contained 100 mM KF. The resistivity of solutions was measured with a usual conductivity bridge.

Fig. 1 illustrates typical space clamped action potentials in viscous glucose solutions. The time course of the action potentials was prolonged as the viscosity of solutions was increased by raising the concentration of glucose. We used the resistivity instead of the bulk viscosity as the measure of the interaction between ions and viscous solvents, since the microscopic viscosity which is important to the ionic diffusion is different from the bulk viscosity.\(^4\) For the

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present, we use the sum of the resistivities of external and internal solutions as the measure of the solution viscosity. The sum of resistivities (at 10°C) of the external and the internal solutions are shown above the potential records. External solutions contained 450 mM NaCl and 100 mM CrCl₂ and the internal solutions contained 100 mM KF.

Fig. 1. Action potentials and the maximum rate of rise in glucose solutions at various resistivities (10°C). Sum of resistivities of the external and the internal solutions is shown above the potential records. Exterional solutions contained 450 mM NaCl and 100 mM CrCl₂ and the internal solutions contained 100 mM KF.
The relationship between the time course and the resistivity of solutions of the axon shown in Fig. 1 is represented in Fig. 2. The reciprocal of the maximum rate of rise multiplied by the amplitude of the action potential, \( Vdt/dV \), was used for a kinetic parameter of the rising phase of the action potential. The value \( \tau_{1/2} \) was used for a kinetic parameter of the rising and the falling phases. These quantities were roughly proportional to the sum of the resistivities of the external and the internal solutions. A 100 \( \Omega \text{cm} \) increase in resistivity increased \( Vdt/dV \) by 0.3 msec and \( \tau_{1/2} \) by 1.6 msec. The membrane resistance measured by a hyperpolarizing pulse was linearly related to the resistivity of solutions. A 100 \( \Omega \text{cm} \) increase of the resistivity corresponds to a 100\% increase of the membrane resistance. Errors by a series resistance were estimated and found to be very small.

The experimental results demonstrated that when the viscosity of solutions was increased, all the rate constants were reduced in a manner similar to the effect of lowering the temperature. When the viscosity of solutions is raised, the diffusion of ions is slowed down but probably the rates of the conformation change of macromolecules cannot be affected. Therefore the slowing down of the rate constants.
of the membrane excitation was due to the suppression of the diffusion of ions. The increase of the resting membrane resistance can be explained also. We cannot exclude the effect of the change of the dielectric constant of solvents on the membrane macromolecules but the change of the ionic environments (e.g., the enhancement of the activities of electrolytes in mixed solvents) cannot explain our results. For example, raising the concentration of external sodium ions increases the maximum rate of rise of the action potential and raising the concentration of internal potassium ions decreases $\tau_{1/2}$. Spyropoulos and Conti reported analogous results when $H_2O$ was replaced with $D_2O$. They thought that the prolongation of the action potential was due to chemical effects of $D_2O$ on membrane macromolecules. The reason was that the increase of the time constants by a factor 1.4 was not explained by the increase of the solution resistivity by a factor 1.2. Our results showed that $5 \Omega cm$ increase (i.e., 20% increase of the resistivity of sea water) of the solution resistivity of external and internal solutions corresponds to 0.03 msec increase of $Vdt/dV$ and 0.16 msec increase of $\tau_{1/2}$. $D_2O$ effects can be also explained by the increase of the solution viscosity. The increases of the solution viscosity by different ways give the same results. This suggests that the solution viscosity is important to the kinetic properties of the membrane excitation.

Our results suggest that the diffusion of ions across the membrane is one of the rate-limiting processes of the membrane excitation. A more precise analysis of the viscosity effects, especially a comparison with the effect of lowering the temperature, will further be reported.

We thank Professor A. Watanabe and the members of the Ine Fishery Co-operation for their kind help to conduct this work.

References