16. **Excitability of Squid Giant Axons in Hypertonic and Hypotonic Solutions**

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Membranes of excitable cells are known to be highly permeable to water and less permeable to various ions and larger neutral molecules i.e. glycerol, glucose, or sucrose (Villegas and Villegas 1960; Villegas and Barnola 1961). That is, membranes of excitable cells are approximately semi-permeable to physiological solutions like other biological membranes. In most of animal cells, a difference of osmotic pressure across the membrane does not exist and therefore a net water flow does not occur. When the media other than isotonic solutions are used, the volume of a cell changes due to an inflow or an outflow of water (Lucké and MacCutcheon 1932). Then cells are frequently destructed by a rapid volume change. When solutions of low concentrations of electrolytes are used, many investigators have used glucose, sucrose, and glycerol in order to maintain isoosmolarity without any effect on nerve activity (Baker, Hodgkin, and Shaw 1962; Tasaki and Takenaka 1963).

We report here that the intracellularly perfused squid giant axon was not only destructed but also maintained the nerve activity in hypertonic and hypotonic solutions when osmolarities of extracellular and intracellular solutions are equal.

**Methods.** Live squid (*Doryteuthis bleekeri*) were obtained at Ine, Kyoto, Japan. Giant axons which were 40–50 mm in length and 500–600 μm in diameter were dissected from a mantle of decapitated squid. The axon was mounted on a Lucite chamber horizontally. The technique of the intracellular perfusion was the same as that reported previously (Takenaka and Yamagishi 1969). Both ends of the axon were cut and an inlet and an outlet glass cannulae were inserted to the axon through openings. The middle part of the axon, which was 7–8 mm in length, was intracellularly perfused. The entire perfusion system was kept at 8–12°C.

0.6 M NaCl, 0.4 M CaCl₂, and 12 vol% glycerol, which were osmotically balanced with the intact intracellular solution across the

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membrane (in this case, a reflection coefficient of glycerol was 0.75, if that of ions were assumed to be 1.0.) were used as isotonic stock solutions for external solutions. For the same reason, 0.6 M KF and 12 vol% glycerol were used for internal solutions. Isotonic solutions of desired ion concentrations were made by mixing the stock solutions. In an experiment in hypertonic and hypotonic solutions, concentrations of electrolytes were kept constant and only osmolarities were changed by raising and lowering the concentration of glycerol. The pH was adjusted to 7.2 for internal solutions and 8.0 for external solutions by adding a small amount of Tris-HCl buffer.

The recording electrode was a fine glass pipette filled with 0.6 M KCl-agar connected to an Ag-AgCl wire electrode. The current electrode was a piece of platinized platinum wire.

Results. Fig. 1 illustrates a typical result of the change in the excitation of the squid giant axon when the isotonic solutions in the

![Fig. 1. Time course of hypertonic effect on the membrane potential and the membrane resistance. Isotonic solutions in both sides of the membrane were respectively changed to hypertonic solutions, the osmolarity of which was four times as many as that of the isotonic solution. RP: Resting potential. Peak: The peak of the action potential.](image)
inside and the outside of the membrane were changed respectively to the hypertonic solutions, the osmolarity of which was four times as many as that of the isotonic solution. In this case, the isotonic external solution contained 450 mM NaCl and 100 mM CaCl₂, and the isotonic internal solution contained 100 mM KF and 10 vol% glycerol. The hypertonic external solution contained 450 mM NaCl, 100 mM CaCl₂, and 36 vol% glycerol, and the hypertonic internal solution contained 100 mM KF and 46 vol% glycerol. The resting and action potentials in the isotonic solution were -43 mV and 107 mV respectively. When only the internal solution was changed to the hypertonic solution, the amplitude of the action potential decreased rapidly. Soon after the external solution was also changed to the hypertonic solution of the same osmolarity, the action potential was restored to 95 mV and was maintained for more than 20 minutes. It was about 20 mV larger than that in the presence of the osmotic pressure difference across the membrane. The resting potential, once decreased, was restored to -37 mV. The duration of the action potential was prolonged about three times. The membrane resistance increased by about 50%.

When the osmolarity was raised to the level two times as many as the isotonic level, almost the same results were obtained. But the axons were more tolerable to the shock of the change of solutions to solutions of different osmolarities. The amplitude of the action potential was much larger.

The diameter of the giant axon was not changed, although connective tissues and fine nerves around it shrank and became transparent in the hypertonic medium.

Fig. 2 illustrates a typical result of the experiment in the hypotonic solution, the osmolarity of which was one-fourth of that of the isotonic solution. In this case, the isotonic external solution contained 112 mM NaCl, 25 mM CaCl₂, and 9 vol% glycerol, and the isotonic internal solution contained 25 mM KF and 11.5 vol% glycerol. The hypotonic external solution contained 112 mM NaCl and 25 mM CaCl₂, and hypotonic internal solution contained 25 mM KF and 2.5 vol% glycerol. The resting and action potentials of the axon bathed in the isotonic solutions were -28 mV and 110 mV respectively. When the external and internal solutions were changed to the hypotonic solutions simultaneously, the resting potential depolarized considerably and reached to -15 mV. The amplitude of the action potential decreased to 70 mV. However, the excitability was maintained for a long time (more than 40 minutes). The membrane resistance decreased by about 30% in the hypotonic solution. When
only the external or internal solution was changed to the hypotonic solution of this osmolarity, the axons lost their excitability very frequently and were not recovered any more, presumably by the destruction of some spots of the membrane.

When the osmolarity was lowered to a half of the isotonicity, the amplitude of the action potential was larger than in the former case and was 85% of the control. When only the external or the internal solution was changed to the hypotonic solution, the action potential decreased rapidly. Soon after the solutions in both sides of the membrane were changed to the hypotonic solutions of the same osmolarity, the action potential was restored and was maintained for a long time. The membrane resistance decreased also.

The diameter of the axon was not changed, although connective tissues and fine nerved around it swelled and became opaque in the hypotonic medium.

Discussion. The excitability of the squid giant axon was maintained in solutions of non-physiological osmolarity in the range be-
between one-fourth and quadruple of isotonicity. This means that the change of osmolarity around the membrane does not affect the macromolecular structure to which nerve excitation is attributed. If the membrane is thick and has a complex three dimensional structure, the change of the osmolarity around the membrane is able to deform the membrane structure by altering water content of the membrane matrix, and it may change nerve activity drastically. Therefore, our result implies that the membrane is thin and has a simple structure in the direction perpendicular to the membrane layer. Recently it is thought that the membrane is the two dimensional liquid crystal of lipids. Proteins which are important to membrane functions float in it (Singer and Nicolson 1972). The change of water exchange rate through the membrane matrix (perhaps the lipid bilayer) and the water content of it does not affect nerve excitation, which is attributed to the more complex structure of proteins and lipids. The fact that the water permeability of an artificial lipid bilayer membrane is of the comparative order of magnitude as that of the biological membrane in spite of great difference of the membrane resistance also supports our idea indirectly (Henn and Thompson 1969). The membrane resistance increased, as the osmolarity of the solution increased, when concentrations of electrolytes were the same. This implies that the impermeable solute glycerol which is used to adjust the osmolarity lowers ion permeability without significant change in cation selectivity.

When osmolarities of solutions in both sides of the membrane were different, the amplitude of the action potential decreased and also at the time of the change of solutions to solutions of different osmolarity. Perhaps a massive water flow through the membrane gives the damage to the functional structure of the membrane by a transient change of the surface area of the membrane. In hypotonic solution, the condition for the excitation is more severe due to the low concentration of Na⁺.

More precise analysis of the effect of the osmolarity on nerve excitation may elucidate the mechanism of the excitation and the membrane structure.

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References