J. Membrane Biol. 47, 303–325 (1979)

Slowing of the Time Course of the Excitation of Squid Giant Axons in Viscous Solutions

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Received 18 September 1978; revised 12 January 1979; revised again 13 February 1979

Summary. The time course of excitation of intracellularly perfused squid giant axons was slowed as the solution viscosity was raised by adding neutral molecules, i.e., glucose and glycerol. By twofold increase of the solution viscosity, the duration of action potential was prolonged to 2.7-fold and the maximum rate of rise decreased to one-half. At the same time, the membrane resistance at resting state increased by 60%. These effects were reversible. The time course of inward and outward currents was slowed also. When the solution viscosity increased to twofold, the time to peak inward current increased by 80%, and the amplitudes of peak inward and steady outward currents decreased by 60% and by 70%, respectively. These effects were not specific for the sodium or the potassium channel. Effects of solution viscosity occurred in both hypotonic and hypertonic solutions. Q_{10} values of temperature dependence of the time course of the action potential were equal in any viscous solutions. These effects in viscous solutions were explained by the change in solution viscosity but not by the change in solution osmolarities, ionic activities, or solution resistivity.

Kinetic properties of nerve excitation have been examined for many years. Many investigators have reported that the time course of the action potential and kinetic parameters of the membrane excitation were determined by the voltage-dependent nature of membrane proteins and other membrane components (Hodgkin, 1964; Keynes, 1972). They considered that ionic flows across the membrane did not affect the membrane conformation change but were only the result from both the membrane conformation change and ionic concentration gradients across the membrane. But there is no clear evidence that ionic flows by themselves are not related to the mechanism determining the time course of the membrane excitation. Some experimental results showed that the voltage-dependent nature of the membrane components was not sufficient to explain the mechanism of the membrane excitation. One of these is

0022-2631/79/0047-0303 $04.60

a chemical excitation model of nerves (Tasaki, 1968). In this model, the ionic environment near the membrane surface changes time-dependently and causes the membrane conformation change from the resting state to the excited state. The other is a model which includes the storage of ions in the membrane. In this model, a part of kinetic properties, especially the time course of the sodium current is determined by the velocity of loss of stored ions from the membrane (Hoyt & Strieb, 1971; Landowne, 1973; Cohen & Landowne, 1974). In these two models, the ionic flows play an important role in determining kinetic properties. Some modification of ionic flows might result in a change of kinetic properties of nerve excitation. But there has been no experimental evidence for this. The purpose of our experiment is to examine how kinetic properties are affected by slowing ionic flows on increase of solution viscosity.

As reported elsewhere (Kukita & Yamagishi, 1979),[1] experiments on nerves can be performed in hypertonic and viscous solutions when solution osmolarities inside and outside the membrane were balanced. Therefore, effects of solution viscosity on nerve excitation could be examined easily. We report here that the time course of the action potential and the membrane currents was prolonged in viscous solutions and that these effects were closely related to the microscopic viscosity around ions. The preliminary result was reported previously (Kukita & Yamagishi, 1976).

Materials and Methods

Squid (*Doryteuthis bleekeri*) from the sea of Japan was obtained at Ine, Kyoto, Japan. Hindmost stellar giant axons (500 μm in diameter and 6 cm long) were dissected and mounted in a Lucite chamber (28 mm wide) filled with seawater. Intracellular perfusion was performed according to the method of Tasaki and his collaborators (1962). From both ends of the axon in the chamber, an inlet fine glass tube (200 μm outside diameter) and an outlet fine glass tube (about 350 μm) were inserted. Before perfusion with test solutions, most of the axoplasm was removed using protease (Takenaka & Yamagishi, 1969). The length of a perfused section was 14 to 18 mm. External bathing solutions were circulated around the axon in the chamber by way of the thermostat bath, and the temperature was measured near the axon. The membrane potential was recorded at the middle of the perfused section using a fine glass pipette filled with 0.6 M KCl-agar (connected with a piece of Ag-AgCl wire) put into the axon through the outlet glass tube. The electrical resistance of this electrode was less than 3 MΩ. An external reference

[1] Kukita, F., Yamagishi, S. 1979. Excitation of the squid axons in hypotonic and hypertonic solutions. (*in preparation*)

electrode was a 3-M KCl-agar bridge connected with a piece of Ag-AgCl wire. Errors due to a liquid junction potential were corrected by subtracting the difference of the potential of the internal electrode *vs.* the reference calomel electrode in each pair of internal and external solutions. A long wire internal electrode inserted throughout the perfused section and external current electrodes with a pair of guard electrodes were used for current stimuli. This long current electrode was used for the space-clamp throughout the perfused section. In the experiment on the propagated action potential, the long wire electrode was not used and electrical stimuli were supplied just at the end of the perfused section with the internal electrode. Action potentials were measured at the other end. The conduction velocity was calculated from the time delay between the time of the stimulus and that when the membrane potential reached the threshold potential. Action potentials were recorded through a high input impedance preamplifier. The voltage clamp was performed according to the usual method (Hodgkin, Huxley & Katz, 1952). The compensation of the series resistance was not performed. Records of action potentials and membrane currents were displayed on the oscilloscope and recorded with a pen recorder through a transient digital time convertor.

The viscosity of solutions was raised by adding inert molecules, glycerol and glucose, (Baker, Hodgkin & Shaw, 1962, Tasaki & Takenaka, 1964) with the osmolarity of the internal solution and that of the external solution matched (Kukita & Yamagishi, 1979).[2] Molar concentrations of ions were kept constant during one series of experiments. The microscopic viscosity of solutions (Robinson & Stokes, 1970), which is the viscosity around ions, is defined by the Stokes' equation for the solution resistivity and is different from the bulk viscosity of solutions (Stokes & Stokes, 1958). This microscopic viscosity must be used instead of the bulk viscosity when the ion movement is considered. At the same ionic concentrations, the ratio of the resistivity of the solution with neutral molecules to that of the solution without neutral molecules shows the ratio of the microscopic viscosity and hereafter is called a relative viscosity. Since the temperature dependence of the relative viscosity was smaller than that of the microscopic viscosity itself, we used values measured at 10 °C to indicate the solution viscosity. The value of the relative viscosity is roughly equal to that of the microscopic viscosity represented in cP, since the viscosity of water is about 1 cP. The relative viscosity of the internal solution was different from that of the external solution, but their ratio was maintained approximately constant during the experiment. Therefore, we used the arithmetic mean of relative viscosities of the internal and the external solutions as the measure of the solution viscosity. The relative viscosity of solutions which contained neutral molecules was larger than unity. The relative viscosity of isotonic external solutions was unity except for the case of lowering the external ionic concentrations, but that of isotonic internal solutions was always larger than unity. Therefore, the mean of relative viscosities was always larger than unity. The resistivity of solutions was measured by the usual conductivity meter.

The pH was adjusted to 8.0 with Tris-HCl buffer or HEPES buffer for external solutions and 7.4 with potassium phosphate buffer or Tris-HCl buffer for internal solutions.

Results

Effects of Viscous Glucose Solutions on the Action Potential

Typical results on action potentials and their derivatives in viscous glucose solutions are represented in Fig. 1. When the solution viscosity

[2] *Ibid.*

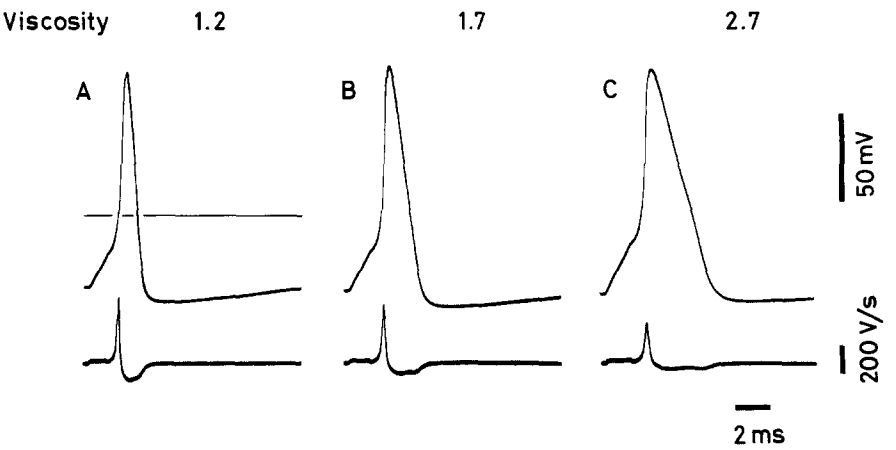

Fig. 1. Action potentials in hypertonic solutions. Action potentials and their derivatives in solutions of various solution viscosities are represented. The relative solution viscosity (*see Materials and Methods*) is shown above the records. Record *A* was measured in isotonic solutions. External solutions contained 440 mM NaCl, 10 mM Na-HEPES buffer, and 100 mM $CaCl_2$ (pH 8.0). Internal solutions contained 80 mM KF and 20 mM K^+ as phosphate (pH 7.4). Glucose concentrations were (in M) 0 (*A*), 0.78 (*B*), and 1.56 (*C*) outside and 0.88 (*A*), 1.59 (*B*), and 2.3 (*C*) inside. The solution temperature was 11 °C. The horizontal bar in record *A* shows the zero membrane potential

was increased by adding glucose, solutions became hypertonic but did not do any damage to the axon. As the viscosity was raised, the duration of action potential was prolonged and the maximum rate of rise decreased, while the amplitude changed little. When the solution viscosity was doubled, the duration increased to 2.3-fold (mean 2.7-fold) and the maximum rate of rise decreased to 65% (mean 50%). The resting potential was around −40 mV and was hyperpolarized a little and the peak of the action potential was around +80 mV and somewhat increased, as the solution viscosity was raised. The threshold potential increased a little. Effects of increasing the solution viscosity were reversible. These effects appeared immediately after the solution change and were maintained for a few hours.

Typical results of the action potential and its derivative in hypotonic solutions are represented in Fig. 2. The solution viscosity was reduced by decreasing the glucose concentration, so the solution became hypotonic. When the solution viscosity was decreased to 80% of the original value, the duration of action potential decreased by 23% (mean 17%) and the maximum rate of rise increased by 9% (mean 23%). The conduction velocity increased by 30% (mean 27%). The resting potential and

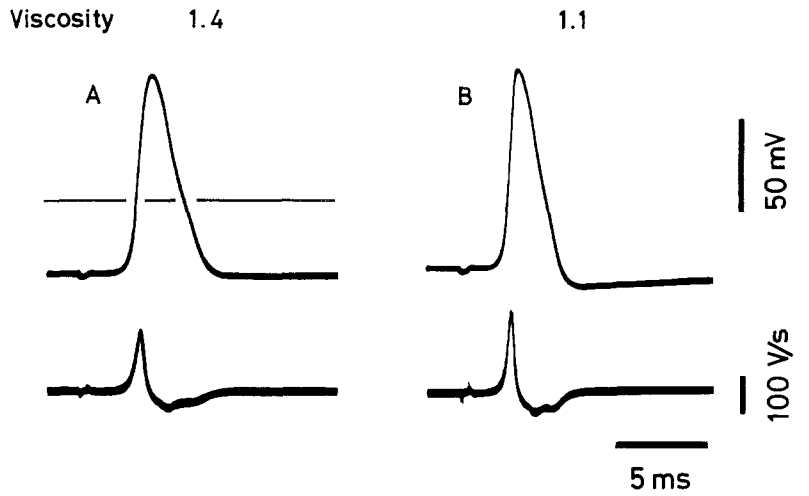

Fig. 2. Action potential in hypotonic solutions. Action potentials and their derivatives in isotonic (record *A*) and hypotonic (record *B*) solutions are represented. The relative solution viscosity is shown above the records. External solutions contained (in mM): 225 NaCl, 50 $CaCl_2$ and 12 Tris-HCl buffer (pH 8.0). Internal solutions contained 100 mM K^+ (as fluoride and phosphate). Glucose concentrations were 600 mM (*A*) and 0 mM (*B*) outside and 1010 mM (*A*) and 370 mM (*B*) inside. The solution temperature was 6.2 °C. The horizontal bar in record *A* shows the zero membrane potential

the peak of the action potential did not change. The relative changes in the duration and the maximum rate of rise with one unit increase of the solution viscosity were approximately the same as those in hypertonic solutions. Effects of the solution viscosity were observed not only in hypertonic solutions but also in hypotonic solutions.

The relationship between the solution viscosity and relative changes in the duration of action potential, the maximum rate of rise, the membrane resistance at resting state, and the conduction velocity are represented in Fig. 3. The duration was measured at the level of half the amplitude. The membrane resistance at resting state was calculated from the slope of the current-voltage relations obtained with hyperpolarizing pulses. Experimental values at different temperatures are normalized to values at 10 °C by using the Q_{10} values shown in Figs. 12 and 13. All points except for values of the conduction velocity, which is the mean of three experimental values, are means of values obtained with ten axons, and the standard deviations mainly due to the difference of preparations are less than 20%. As the solution viscosity increased, relative changes of membrane properties increased almost linearly. When the

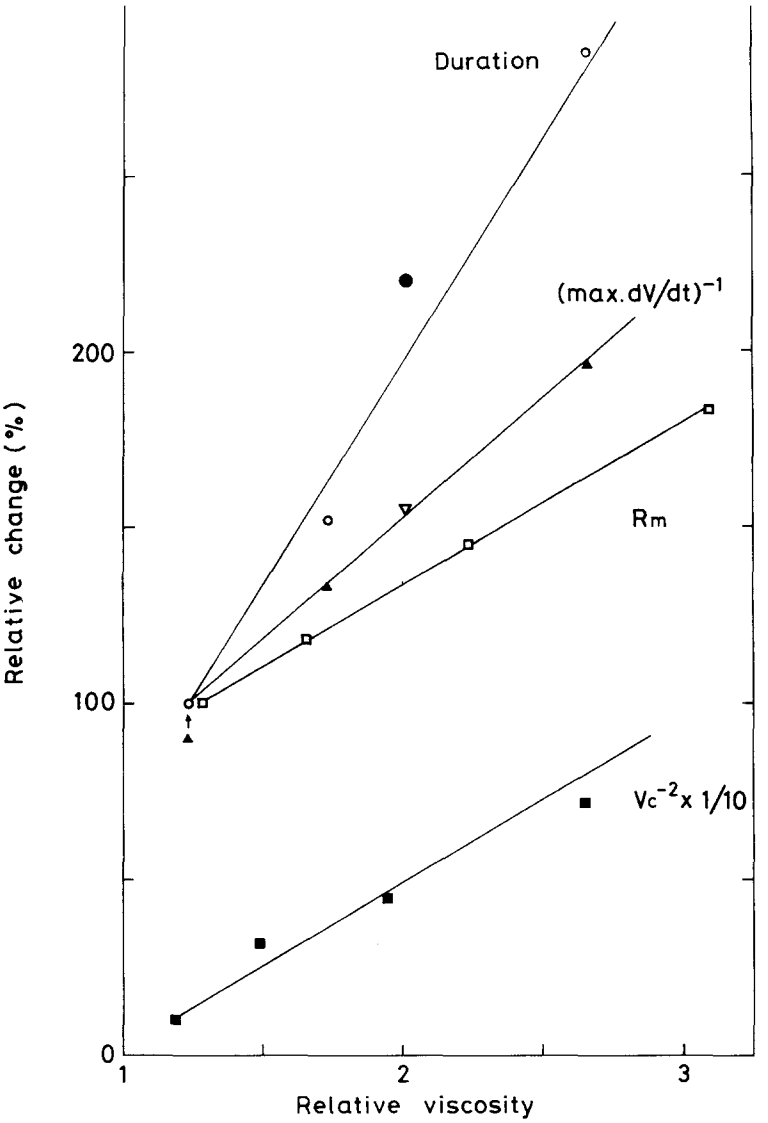

Fig. 3. The relationship between the relative change of the membrane properties and the solution viscosity. The relative change (in %) of the duration of action potential (*Duration*), the reciprocal of the maximum rate of rise (1/max.dV/dt), the membrane resistance at resting state (*Rm*), and the reciprocal of the square of the conduction velocity (Vc^{-2}) on the reduced scale of one-tenth are represented in the ordinate. The relative solution viscosity is represented in the abscissa. The viscosity was raised by adding glucose. External solutions contained 450 mM Na^+ and 100 mM Ca^{++}. Internal solutions contained 100 mM K^+ except for the case of the conduction velocity which was measured in 200 mM K^+. In case of *Rm*, glucose concentrations were (in M) 0, 0.5, 1.0, and 1.5 outside and 0.92, 1.38, 1.84, and 2.3 inside. Solution compositions in case of the duration and the maximum rate of rise were as in Fig. 1 and those in case of the conduction velocity were as in Fig. 11. Intermediate solutions were one to one mixtures of other solutions. The results in glycerol solutions represented in Fig. 7 are plotted also (●, ▽). Solution compositions were shown in Fig. 7. Experimental values were normalized to the values at 10 °C using the Q_{10} values

solution viscosity increased by one unit, the duration increased to 2.3-fold, the membrane resistance at resting state increased to 1.5-fold, the reciprocal of the maximum rate of rise increased to 1.7-fold, and the reciprocal of the square of the conduction velocity increased to fivefold. The durations were larger when the internal potassium concentration was reduced, but they changed in the same manner at all potassium concentrations with the increase in the solution viscosity. The reciprocal of the maximum rate of rise changed in proportion to the solution viscosity at various potassium concentrations but the slope of the change was different (not represented in Fig. 3). The increase of the duration showed the prolongation of the time course of the action potential, since the time course of rising and falling phases of action potential prolonged to the same extent. The decrease of the maximum rate of rise is related to both the decrease of the transient inward current and/or the membrane conductance at excited state and the decrease of the rate constants of rising phase of action potential. These changes are related to each other and are proportional to the increase of the solution viscosity. The square of the conduction velocity is closely related to the membrane resistance, the solution resistivity inside and outside the axon, and the time constants of the action potential (Hodgkin & Huxley, 1952*b*; Huxley, 1959). Other properties are also related together by way of the membrane resistance and the time constants of the membrane excitation.

Viscosity Effects on Membrane Currents

Typical records of the membrane current under the voltage clamp are shown in Fig. 4. The resting potential did not change in this experiment, and the membrane potential was held at resting potential (-45 mV). As the solution viscosity increased, the time course of the membrane current was prolonged like that of the action potential. Both the inward and the outward currents were prolonged to the same extent at all clamping voltages as the solution viscosity increased. The magnitude of peak inward current and steady outward current decreased to the same extent. When solution viscosity was doubled, the magnitude of inward current decreased to 35% and that of steady outward current decreased to 30% at the clamping voltage of 58 mV. The rate of rise and fall of the inward current decreased largely, but the total charge

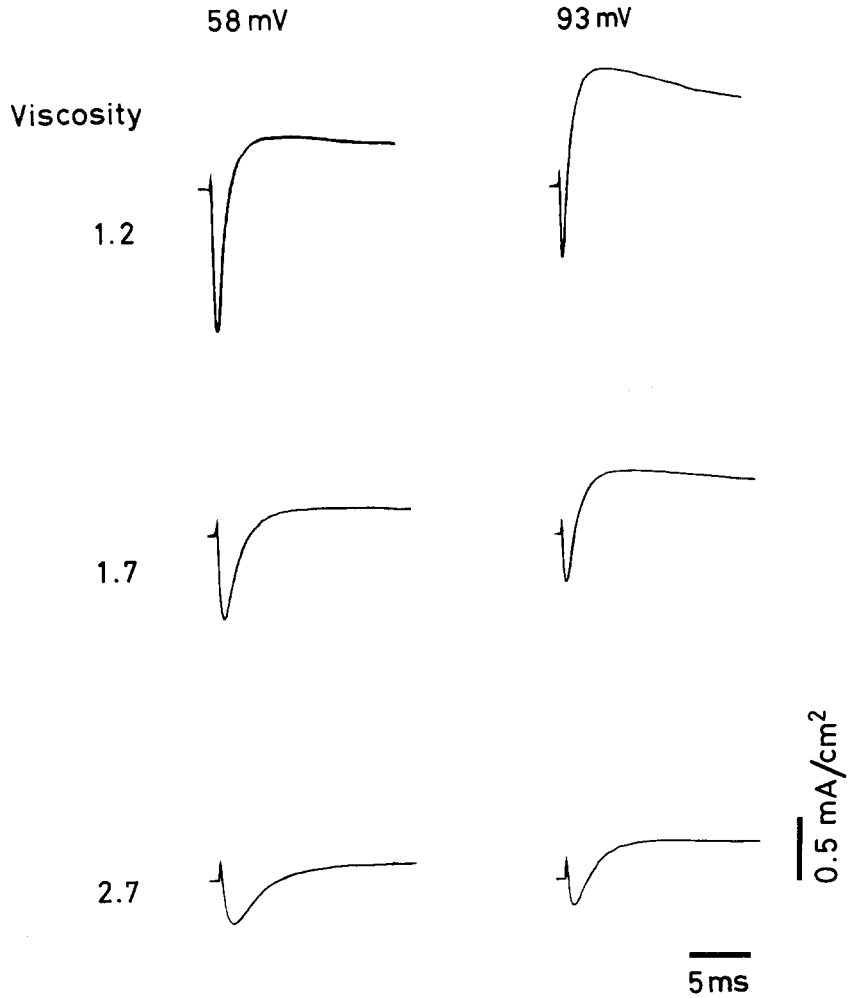

Fig. 4. The membrane currents at various solution viscosities. The membrane current under the voltage clamp at the clamping voltage of 58 and 93 mV from the resting potential. The membrane potential was held at resting potential (−45 mV). The solution viscosity was shown in the left side of each record. Solution compositions were the same as in Fig. 1. The solution temperature was 11.2 °C

carried by the inward current changed little. The effect of the solution viscosity was not specific for the sodium or the potassium channel, as expected from the results of the action potential.

The relationship between peak inward and steady outward currents and clamping voltages from the resting potential in solutions of various viscosity are represented in Fig. 5. Resting and reversal potentials did not change. Both inward and outward currents decreased in the same

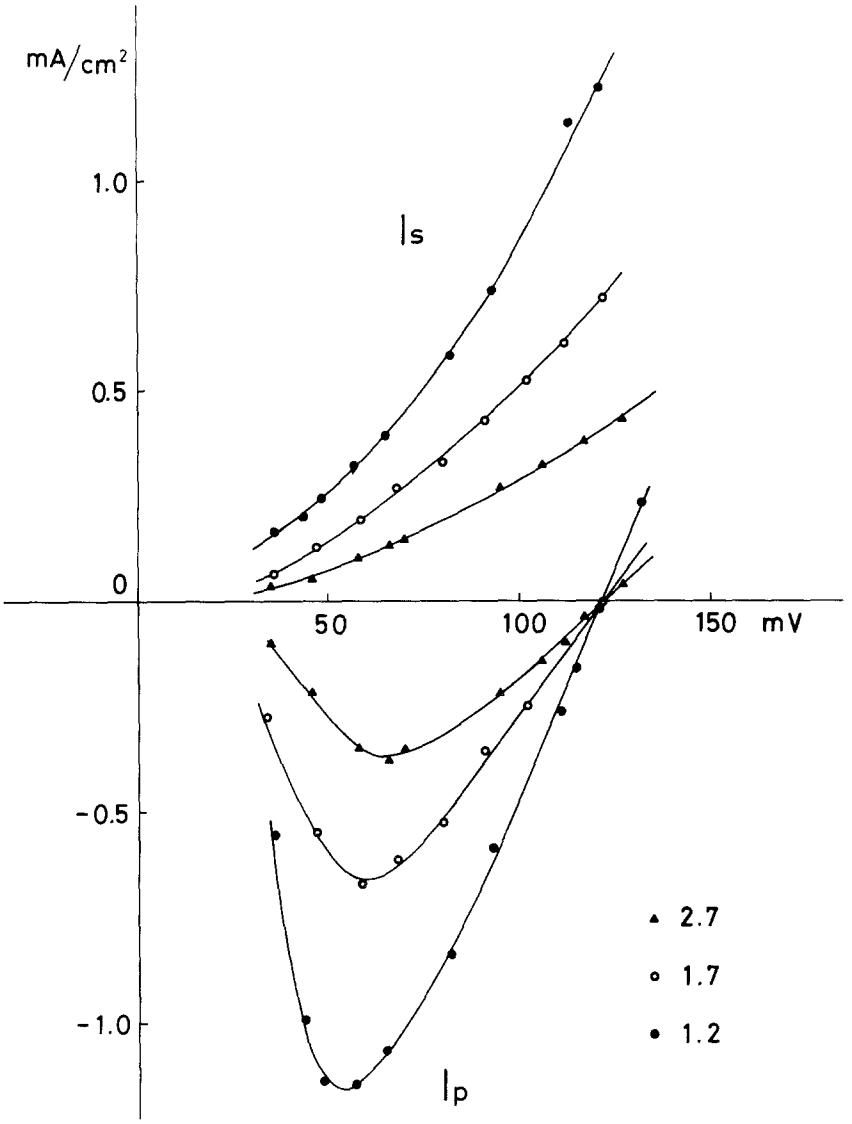

Fig. 5. The current-voltage relationship of records under the voltage clamp. The same results as shown in Fig. 4 are plotted. Membrane currents are plotted against the clamping voltages from the resting potential. The peak inward currents (*Ip*) and the steady outward currents (*Is*) at various solution viscosities are plotted. The solution viscosities were 1.2 (●), 1.7 (○), and 2.7 (▲), respectively

manner at all clamping voltages. The early slope conductance obtained from the linear portion of the peak inward current *vs.* voltage relation curves decreased to 30% when the solution viscosity increased from

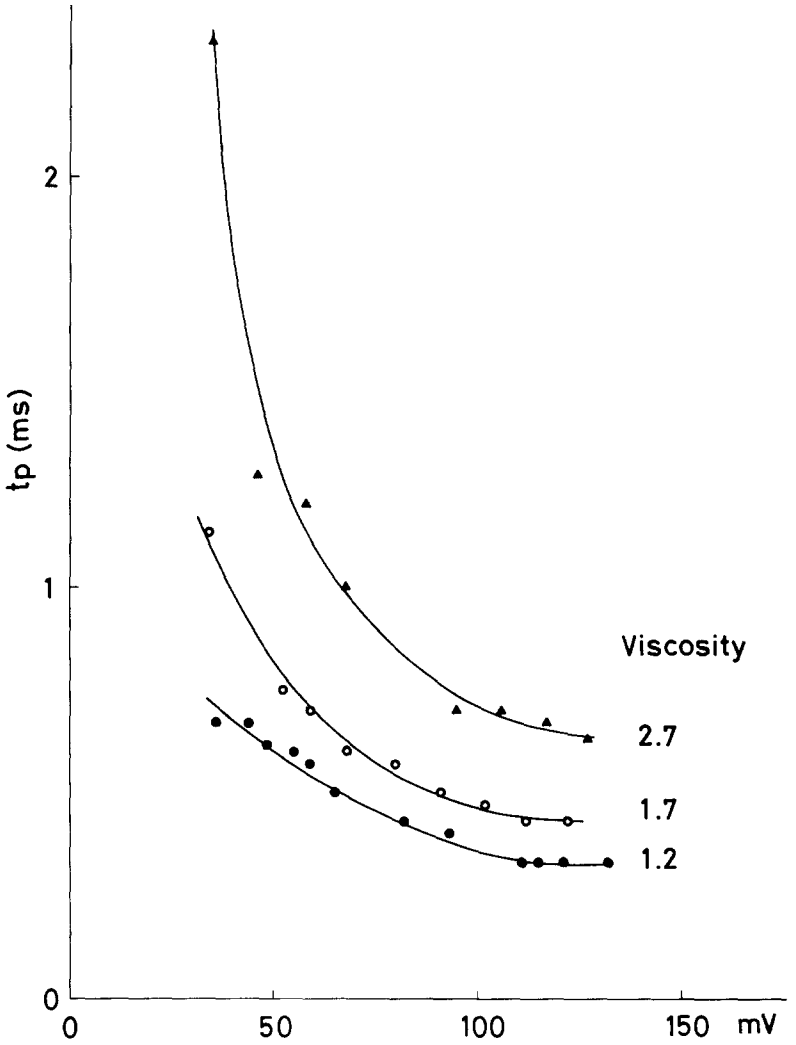

Fig. 6. The relationship between the time to peak inward current (*tp*) and the clamping voltage at various solution viscosities. The same results as shown in Fig. 4 are plotted

1.2 to 2.7. The late slope conductance at the stage of the steady outward current decreased to the same extent.

The time to peak inward current *vs.* clamping voltage relations at various viscosities are represented in Fig. 6. The time to peak inward current increased at all clamping voltages, as the solution viscosity increased. The change was roughly proportional to the increase in solution viscosity. It increased to 1.9-fold at the large clamping voltage when the solution viscosity increased from 1.2 to 2.7. This differed from the

effect of increasing external calcium. When the external calcium concentration increases, the rate constants at small clamping voltages decreased but those at large clamping voltages do not change. The curves of rate constants *vs.* clamping voltage relations at various calcium concentrations can be shifted to coincide with that at the normal calcium concentration (Frankenhaeuser & Hodgkin, 1957). The effect of solution viscosity was different at this point. The increase of external calcium does not affect the time course of the action potential. The viscosity effect was different at this point also.

The increase of solution viscosity reduced not only the early and the late slope conductances but also rates of kinetic properties of channels.

Comparison of Viscosity Effects of Glucose and Glycerol

The viscosity effect in different solutions of the same osmolarity was compared by examining the effect of glucose solutions and glycerol solutions. The duration of action potential in isotonic glucose solutions was somewhat larger than that in isotonic glycerol solutions (Kukita & Yamagishi, 1979).[3] Effects of the solution change from hypertonic glucose solutions to hypertonic glycerol solutions on the action potential and membrane currents under the voltage clamp are shown in Fig. 7. Considering the reflection coefficient, osmolarities of these solutions were balanced across the membrane not to affect the excitation of the axon. Physicochemical osmolarities of glycerol solutions were larger than those of glucose solutions, since glycerol permeates through the membrane to some extent. Physicochemical osmolarities, i.e., molalities of solutes of the internal and the external glucose solutions, were 3.41 and 3.44 osmoles, respectively, and those of glycerol solutions were 5.13 and 4.53 osmoles. The mean of relative viscosities of internal and external glucose solutions was 2.66 and those of glycerol solutions was 2.01. The duration of action potential was 2.5 msec in glucose solutions and then decreased to 1.9 msec in glycerol solutions. The maximum rate of rise was 295 V/sec in glucose solutions and then increased to 410 V/sec in glycerol solutions. These were restored to the original values when glycerol solutions were replaced with glucose solutions again. Values of the duration and the maximum rate of rise in glycerol solutions are plotted in Fig. 3. Relative changes in glycerol solutions were almost equal to those in glucose solu-

[3] *Ibid.*

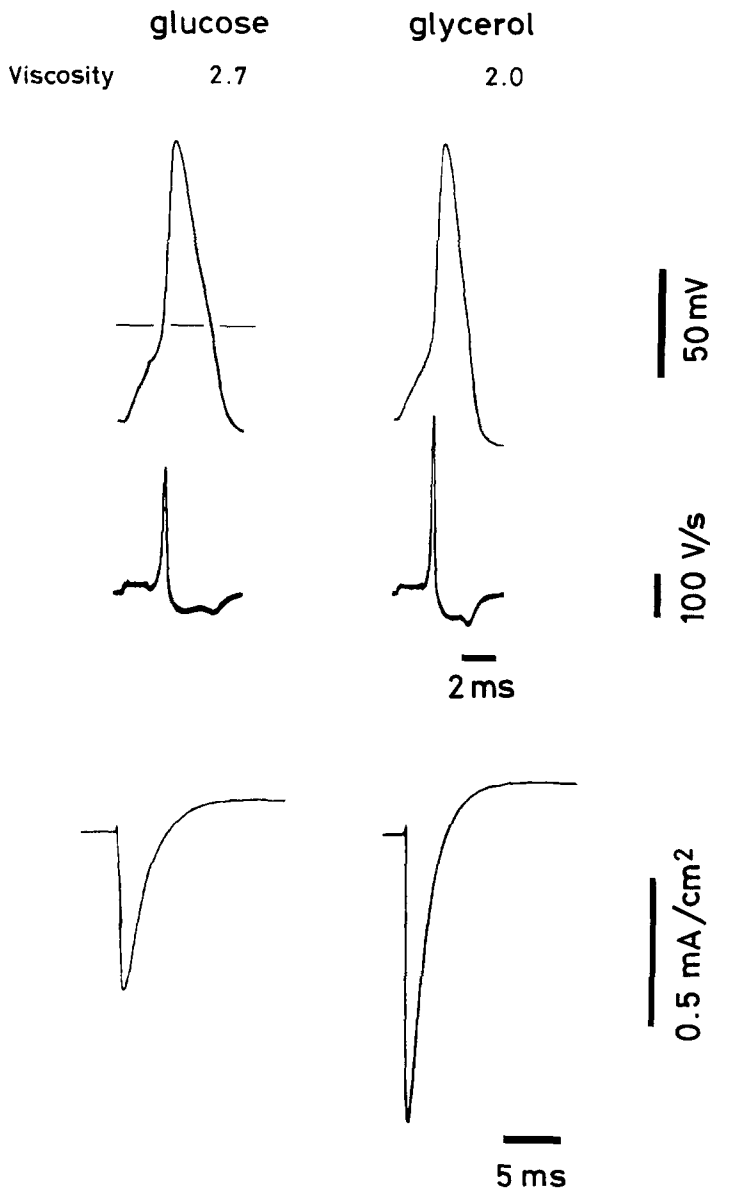

Fig. 7. Comparison of the effects of the glucose and the glycerol solutions. The action potentials, their derivatives, and the membrane currents at the clamping voltage of 60 mV in the glucose and the glycerol solutions of the same osmolarity are represented. External solutions contained 450 mM Na^+ and 100 mM Ca^{++}, and internal solutions contained 100 mM K^+. Glucose concentrations were 1.56 M outside and 2.3 M inside, and glycerol concentrations were 2.43 M outside and 3.58 M inside. The solution temperature was 12 °C

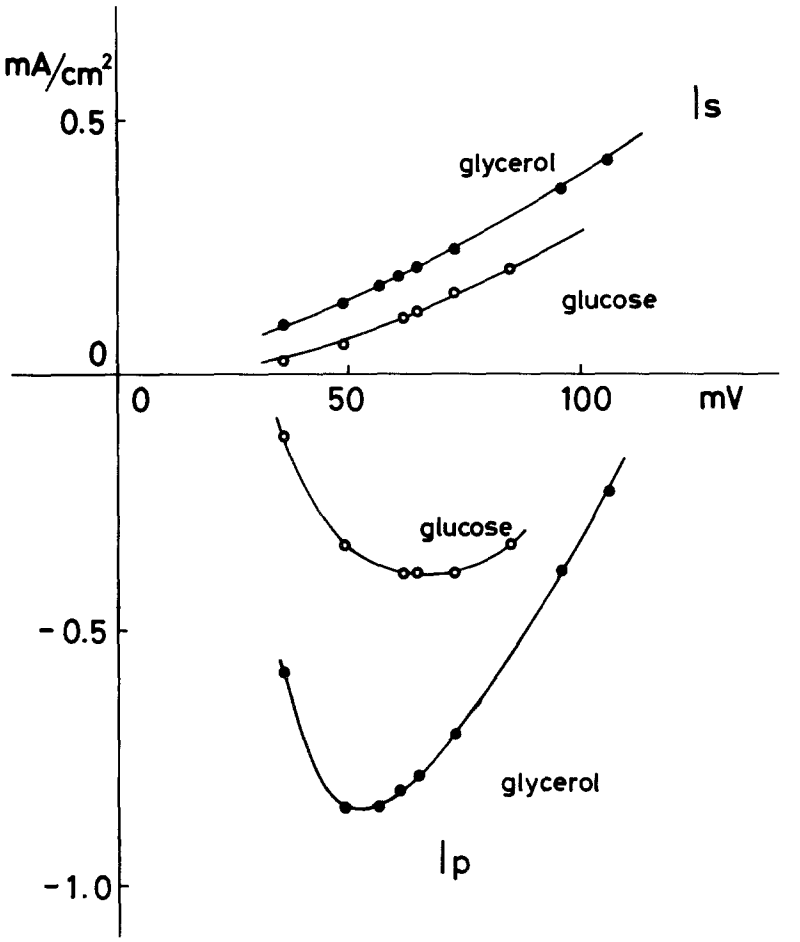

Fig. 8. The current-voltage relationship of the records under the voltage clamp. The resting potential was −50 mV in glucose and glycerol solutions, and the membrane potential was held at resting potential. The peak inward current (*Ip*) and the steady outward current (*Is*) are plotted. The solutions were glycerol (●) and glucose (○) solutions of the same osmolarity. The solution viscosities were 2.0 and 2.7, respectively. Solution compositions were the same as in Fig. 7

tions of the same viscosity. The resting potential was somewhat larger in glucose solutions than in glycerol solutions. The amplitude of the action potential did not change. The inward current at the clamping voltage of 60 mV increased from 0.53 to 0.97 mA/cm^2 and the steady outward current increased from 0.11 to 0.18 mA/cm^2 when glucose solutions were replaced with glycerol solutions. The time course of inward and outward currents became short, and the time to peak inward current shortened from 0.97 to 0.70 msec. These changes were practically reversible.

The relationship between peak inward and steady outward currents and clamping voltages from the resting potential is represented in Fig. 8. The results different from those in Fig. 7 are plotted against the clamping voltages. The resting potential was about −50 mV in glucose and glycerol solutions. The membrane potential was held at resting potential. The peak inward and steady outward currents were smaller in glucose solutions at all clamping voltages. The membrane current increased to 1.8-fold when the solution viscosity decreased from 2.66 to 2.01 and the change was nearly equal to that in hypertonic glucose solutions represented in Fig. 5. The time to peak inward current and clamping voltage relations in glucose and glycerol solutions are compared in Fig. 9. The time to peak inward current was smaller in glycerol solutions at all clamping voltages like the results in Fig. 6. It was smaller by about 25% at the large clamping voltage. Changes of magnitudes of peak inward and steady outward currents and the time to peak inward current in glycerol solu-

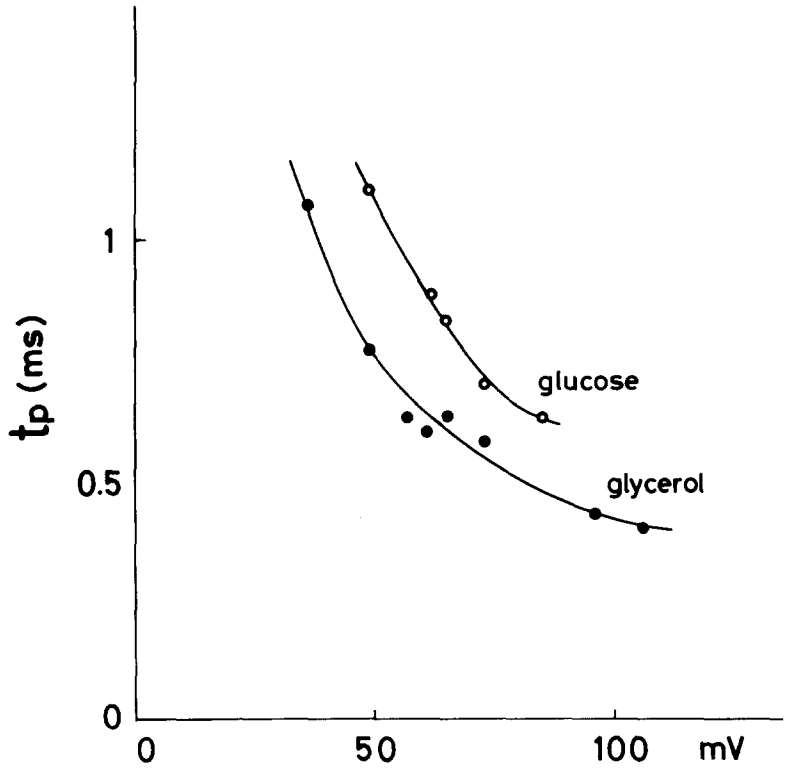

Fig. 9. The relationship between the time to peak inward current (*tp*) and the clamping voltage. The records represented in Fig. 8 are plotted. The solutions were glycerol (●) and glucose (○) solutions

tions were almost equal to those in the glucose solutions of the same viscosity. The change of membrane currents tended to be sometimes larger than that expected from the results obtained with a series of glucose solutions represented in Fig. 5. Anyway, these results showed that the difference of relative changes of membrane properties was due to the difference of solution viscosity but not due to the difference of solution osmolarity.

Effect of the Solution Resistivity

Effects of increasing the solution resistivity on action potentials and their derivatives are shown in Fig. 10. First the action potential and its derivative were recorded in isotonic solutions which contained 440 mM NaCl and 100 mM $CaCl_2$, 10 mM Tris-HCl (pH 8.0) outside and 200 mM K^+ and 810 mM glucose inside the axon (record *A*). Then the half of electrolytes was replaced with glucose keeping the isotonicity (record

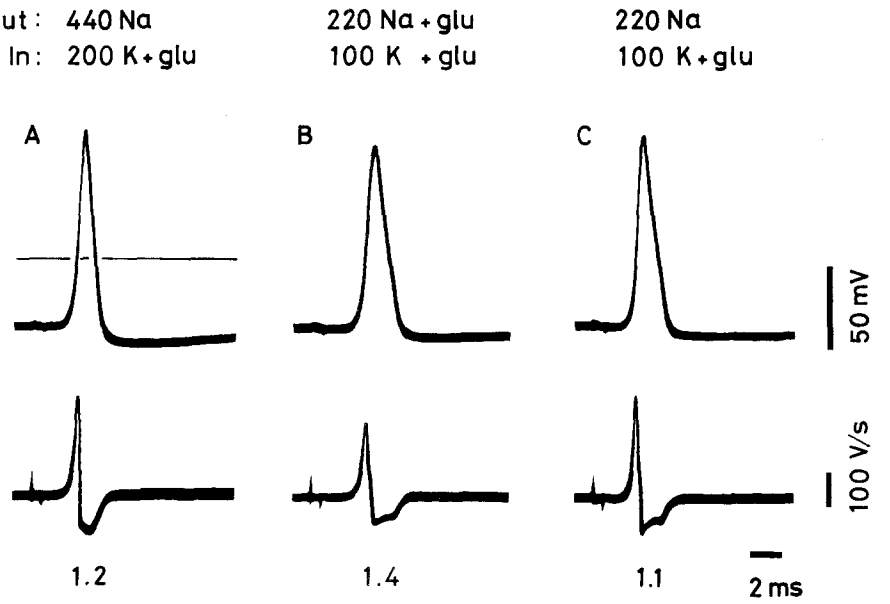

Fig. 10. Effects of increasing the solution resistivity. Ionic concentrations were reduced to one-half with the solution resistivity increased to twofold (records *B* and *C*). The osmolarity was lowered to one-half in case of *C*. Ionic concentrations in external solutions (*Out*) and in internal solutions (*In*) are shown above the records. Glucose concentrations were (in mM) 0 (*A*), 600 (*B*) and 0 (*C*) outside and 810 (*A*), 1010 (*B*), and 370 (*C*) inside. The solution viscosity is shown under each record. The solution temperature was 7.8 °C

B). These solutions contained 220 mM NaCl, 100 mM $CaCl_2$, 5 mM Tris-HCl (pH 8.0) and 600 mM glucose outside and 100 mM K^+ and 1010 mM glucose inside. Ionic concentrations and osmolarities were reduced to one half by reducing the glucose concentration. Glucose concentrations were reduced to 300 mM inside and to 0 mM outside (record *C*). Keeping the ratio of the external sodium and the internal potassium concentrations constant, lowering the ionic concentration did not affect the amplitudes of resting and action potentials largely. The time course of the action potential did not change largely in all solutions. When ionic concentrations were reduced to one half with the solution resistivity increased to

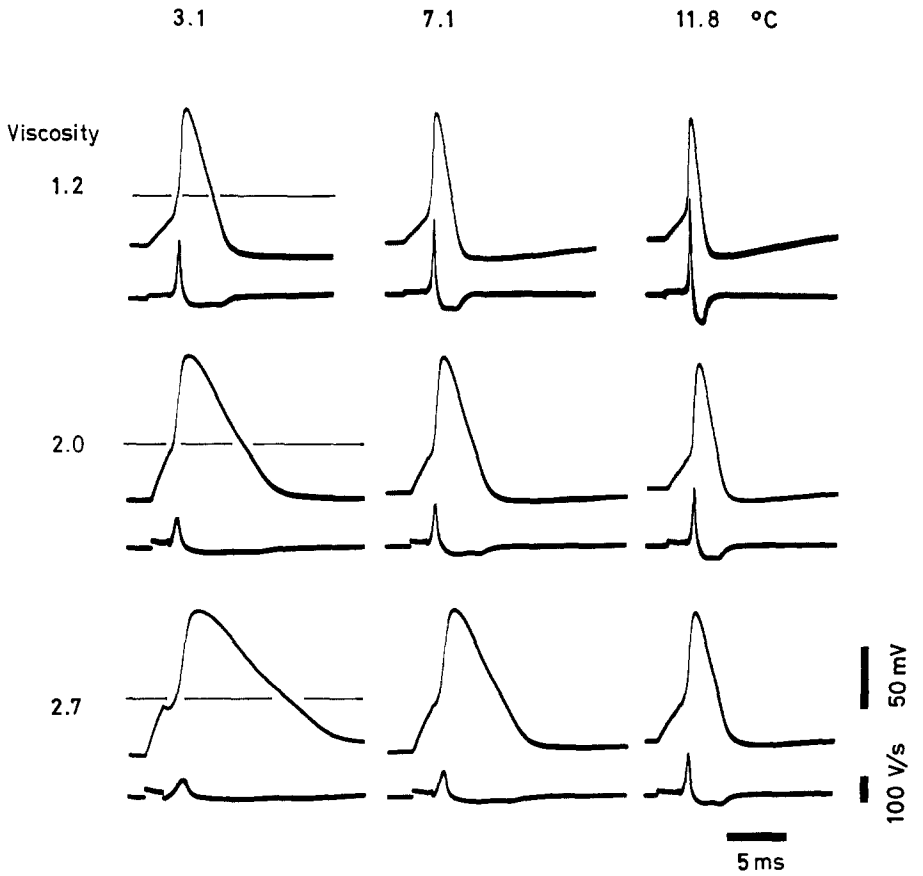

Fig. 11. The effect of the temperature on action potentials and their derivatives at various solution viscosities. The solution temperature is shown above the records. The solution viscosity is shown at the left hand side. External solutions contained (in mM): 450 NaCl, 100 $CaCl_2$, and 10 Tris-HCl. Internal solutions contained (in mM): 180 KF and 20 K^+ as phosphate. Glucose concentrations (in mM) were: 0, 1100, and 1650 outside and 810, 1770, and 2250 inside with the increase of the viscosity

about twofold, the duration of action potential increased by 34% and the maximum rate of rise decreased by 23% (record *B*) but in case of record *C*, the duration increased only by 17% and the maximum rate of rise increased a little. The solution viscosity was 1.4 in case of record *B*, and those in case of records *A* and *C* were 1.2 and 1.1, respectively. Most of the change of the duration and the maximum rate of rise can be explained by the increase in solution viscosity as shown in Fig. 2. The change of solution resistivity by itself did not affect kinetic properties.

Effect of the Temperature on the Viscosity Effects

Action potentials and their derivatives in viscous solutions at various temperatures were compared (Fig. 11). When the temperature was

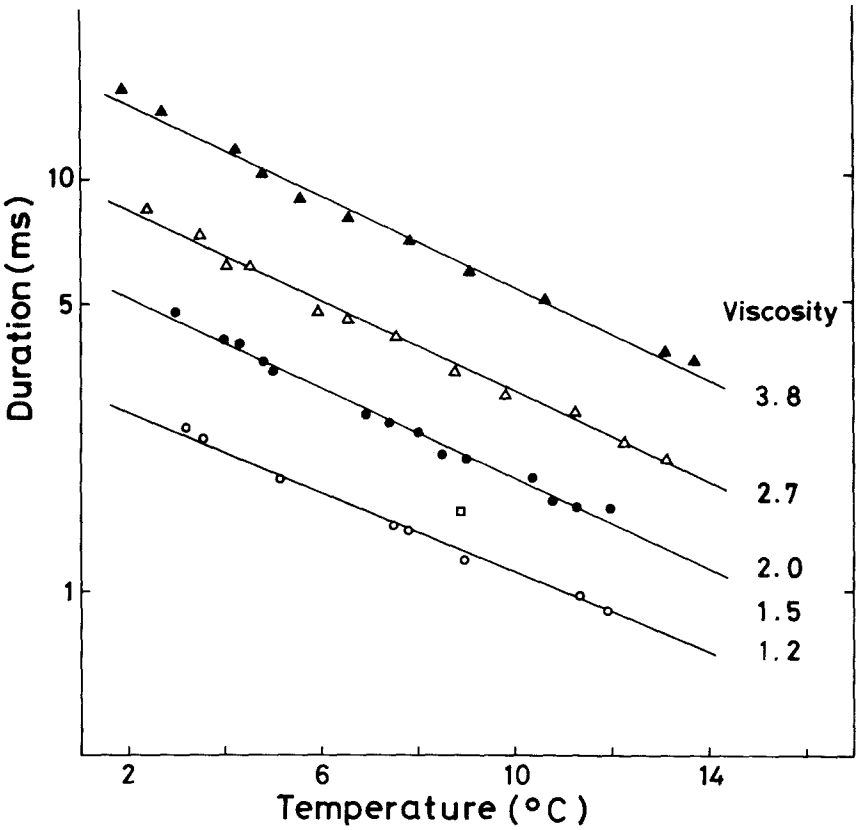

Fig. 12. Relationship between the duration of action potential and the solution temperature. The duration is represented in the ordinate on the logarithmic scale. Records represented in Fig. 11 are plotted

lowered, the duration of action potential increased at the same ratio in solutions of various viscosities and the maximum rate of rise decreased in the same manner. The viscosity, which is the mean of relative viscosities of internal and external solutions determined at 10 °C, are shown. The temperature dependence of the duration and the maximum rate of rise did not change when the solution viscosity increased. All the features of the action potential when the solution viscosity was increased resembled those when the temperature was lowered.

The relationship between the temperature and the duration of action potential on the logarithmic scale is represented in Fig. 12. The temperature dependence was the same in various solutions and Q_{10} values were about 1/3 at all solution viscosity. The temperature dependence tended to become larger and so Q_{10} value tended to become smaller with the

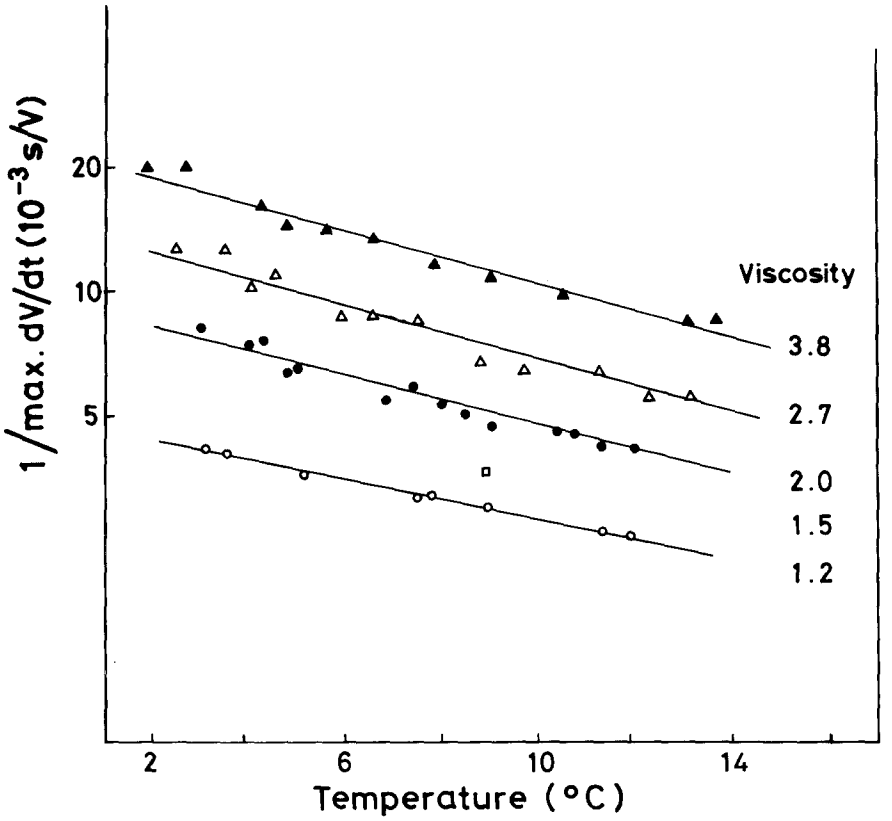

Fig. 13. Relationship between the reciprocal of the maximum rate of rise and the solution temperature. The reciprocal of the maximum rate of rise (1/max.dV/dt) is represented in the ordinate on the logarithmic scale. Records represented in Fig. 11 are plotted

increase of the solution viscosity, because the small increase of the solution viscosity by lowering the temperature gave the secondary effect.

The similar relationship between the temperature and the reciprocal of the maximum rate of rise is represented in Fig. 13. Q_{10} values were about 2.0 at all solution viscosity and tended to become larger as the solution viscosity increased. Effects of the temperature (Hodgkin & Katz, 1949) and the solution viscosity on the duration of action potential were larger than those on the maximum rate of rise. Effects of the temperature and the solution viscosity were very similar. The effect of doubling the solution viscosity corresponded to 8 °C decrease of the temperature. But the mechanisms of the temperature effect and the viscosity effect were independent of each other.

Discussion

Our results showed that the rate constants of the membrane excitation, i.e., the action potential and membrane currents under the voltage clamp, decreased as the solution viscosity increased. At the same time, the membrane resistance at resting and excited states increased. These effects were due to the increase of the solution viscosity but not due to that of the solution osmolarity or that of the solution resistivity. These effects were not specific for the sodium or the potassium channel. This showed that reducing the ionic mobility or ionic flows across the membrane decreased the rates of the membrane excitation. The ionic flows are not only the consequence of the voltage-dependent conductance change in the membrane but also the cause of the time-dependent change of the membrane conductance. Ionic flows play an important role to determine the kinetic properties of the membrane excitation.

Some effects due to the concomitant increase of the series resistance might be included. But we showed that the viscosity effects on the action potential were not due to the increase of the solution resistivity and that of the series resistance by the experiment of lowering the ionic concentrations. The series resistance might affect the results of the voltage-clamp experiment, but the effect on the membrane conductance could be estimated less than 10%. Adelman reported that the replacement of external sodium chloride with sucrose did not affect the time course of membrane currents but somewhat affected the magnitude of membrane currents and current-voltage relations (Adelman & Taylor, 1964). The series resistance by itself might not affect our results on the time course

of membrane currents. If the voltage clamp was not sufficient due to the series resistance, the time to peak inward current at small clamping voltages must decrease as the series resistance increased. Moreover, the time to peak inward current at large clamping voltage, especially, near the reversal potential was not affected by a change in series resistance.

The increase of ionic activities in solutions that contained a high concentration of neutral molecules (Lanier, 1965) may affect the membrane excitation. The increase of ionic activities was small in solutions used, and those in internal solutions and external solutions were almost the same with their ratio unchanged. In this case, kinetic properties were not affected, as in case of changing ionic concentrations with the ratio of external cations and internal cations kept constant as shown in Fig. 10. Therefore, the prolongation of the time course was not due to the increase of ionic activities. The effect of the ionic concentration change is specific for the sodium or the potassium channel (Hodgkin & Huxley, 1952*a*; Narahashi, 1963) and differs from the viscosity effects. Results obtained by comparing effects of glucose and glycerol solutions showed also that these effects were not due to the change of the ionic activities because the content of water in glucose solutions and that in glycerol solutions were almost equal in our experiment and so the ionic activities of these solutions were approximately equal.

Conti and Palmieri (1968) reported that the time course of the excitation of the intact squid giant axon prolonged in D_2O seawater. The increase of the time course was about 40% by 20% increase in solution viscosity and was larger than that in our experiment. Therefore, D_2O effect may be different from the viscosity effect as described by Conti and Palmieri. But comparing with our results, more than half of the increase can be explained by the viscosity of D_2O seawater.

The small change of the surface charge of the membrane in concentrated organic molecule solutions may be considered. But this affects the voltage dependence of the time course of membrane currents under the voltage clamp but does not affect the time course of the action potential. The relationship between the time to peak inward current and clamping voltages was also different from that when the surface charge was changed. The viscosity effect appeared at all clamping voltages, but the surface charge effect appeared only at small clamping voltage (Frankenhaeuser & Hodgkin, 1957; Narahashi, 1963; Hille, 1968). The change of the dielectric constant of solutions by adding glucose or glycerol is very small (Åkerlöf, 1932), so the change of the surface charge need not be considered.

The change of the hydrostatic pressure due to the osmolarity change was less than 1 atm in our experiments and was too small to cause the hydrostatic pressure effect (Henderson & Gilbert, 1975).

Some chemical effects of neutral molecules to the membrane may be included, although glycerol and glucose are inert molecules used by many investigators (Baker *et al.*, 1962; Tasaki & Takenaka, 1964). But these effects were supposed to be saturated when the concentration of neutral molecules was large, more than 1 M. With the increase of the concentration of substances, most chemical effects increased asymptotically to reach the constant level or increase in proportion to the logarithm of the concentration. The viscosity effects increase acceleratively with the increase of the concentration of neutral molecules (Kukita & Yamagishi, 1979),[4] so these effects were different from the chemical effects. Since glycerol was more permeable into the membrane than glucose and the concentration of glycerol was larger than that of glucose in solutions of the same osmolarity, the chemical effects of glycerol solutions must be larger than that of glucose solutions. But the prolongation of the time course was smaller in glycerol solutions than in glucose solutions. Therefore, some contribution of chemical effects can be neglected.

The selectivity of cations at resting and excited states was not affected by the solution viscosity, because the resting potential and the peak amplitude of the action potential did not change in viscous solutions. The temperature dependence of the time course of the membrane excitation did not change either. These results suggest that the elementary processes of the membrane excitation did not change in viscous solutions, while its time course was prolonged. The magnitude of the inward current decreased and the time course of the inward current was prolonged without changing the total charge carried by the inward current. This was different from the temperature effect. The viscosity might affect the cooperative nature of the membrane excitation without any change of the elementary processes. The chemical excitation model (Tasaki, 1968) may explain that the solution viscosity affects the rates of the change in ionic concentrations near the membrane. The stored charged model (Hoyt & Strieb, 1971; Landowne, 1973; Cohen & Landowne, 1974) may explain that the solution viscosity affects the rate of the loss of ions from the membrane. These two models seem to be favorable to explain the viscosity effect but may not explain the independent effects of the

[4] *Ibid.*

temperature and the viscosity. Some of our results suggest that the membrane consists of small aqueous pores dispersed in the membrane. But the mechanism of the prolongation of the time course cannot be explained by a simple pore model.

Although there are many unknown mechanisms, our results showed at least that kinetic properties of the membrane excitation were determined not only by the voltage-dependent nature of the excitable membrane but also by the ionic flows. So a new mechanism to explain the viscosity effect must be considered to clarify the molecular mechanism of the membrane excitation.

The authors thank the members of the Ine Fishery Co-operative for collecting squid and for their kindness in helping us perform the experiment. The authors also thank Dr. K. Furuya for his kind help in performing the experiments.

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