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# Properties of Sodium and Potassium Channels of the Squid Giant Axon Far Below 0 °C

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**Summary.** Squid giant axon could be excited in concentrated glycerol solutions containing normal concentrations of electrolytes, when osmolalities of solutions inside and outside the axon were matched. These glycerol solutions did not freeze at the temperature as low as −19 °C. The nerve excitation in these solutions were observed at this low temperature. The excitation process at this low temperature was slowed down and time constants of the excitation kinetics were several hundredfold larger than those in normal seawater at 10 °C, under which temperature the squid habituated. The temperature coefficients for the electrophysiological membrane parameters under this condition were larger than those in normal seawater above 0 °C. The $Q_{10}$ value for the conduction velocity was 2.0 and that of the duration of the action potential was around 8.5. The time course of the membrane currents was also slowed with the $Q_{10}$ value of around 5 and the magnitude decreased with the $Q_{10}$ value of around 2 as the temperature was lowered. The $Q_{10}$ values for the kinetics of the on process of the Na-channel were around 4.5 and were almost the same as those of the off process of the Na-channel in the wide range of the temperature below 0 °C. The $Q_{10}$ value of the on process of K-channel was around 6.5 and was larger than those for Na-channel. The $Q_{10}$ values increased gradually as the temperature was lowered.

**Key words** squid giant axon · low temperature · Na-channel · K-channel · antifreeze

## Introduction

Until now physiological properties of nerve fibers were examined at temperatures above 0 °C or very slightly below 0 °C, because the conduction of nerve impulses was blocked at and below this temperature (Hodgkin & Katz, 1949; Moore, 1958; Roots & Prosser, 1962; Schwarz, 1979). The time course of the action potential was anomalously prolonged, especially in its falling phase near 0 °C (Hodgkin & Katz, 1949; *our unpublished data*). The axon which was once cooled far below the freezing temperature of the body fluid (around −1 °C) showed a large deterioration of excitability, even when the temperature was raised again above 0 °C. The effects were explained by damages resulting from freezing of axoplasm and physiological solutions (Spyropoulos, 1965; Easton & Swenberg, 1975). On the other hand, the nerve of the hibernating insect is active at the ambient temperature as low as −10 °C and can survive at temperatures as low as −40 °C, since the body fluid of these insects do not freeze, mainly due to the increase in its osmolality (Baust & Miller, 1970). The giant axon isolated from the squid which habituate at temperatures around 10 °C, loses excitability near below 0 °C in normal physiological salt solutions (Spyropoulos, 1965; Easton & Swenberg, 1975). One of the purposes of this report is to show that the excitability of the nerve of the squid, which live at the ambient temperature of around 10 °C, can be maintained below 0 °C without any abnormal prolongation of the action potential near 0 °C when the osmolality of physiological solutions inside and outside the axon is raised.

Another purpose is to examine how the kinetic properties of the nerve excitation change when the fluidity of the nerve membrane and/or the ionic mobility is decreased at temperatures below 0 °C. The transition temperature of the structure of the lipid bilayer which was not reported for the squid giant axon above 0 °C is slightly lower than the ambient temperature at which animals habituate (Cossins & Prosser, 1978; Sandermann, 1978). The nerve membrane of the squid which lives at the ambient temperature of around 10 °C might change the physical state of the lipid bilayer at the low temperature far below 0 °C. The results in this paper show that no such clear discontinuous change in the excitability of the nerve appeared as the temperature was lowered down to −19 °C. $Q_{10}$ values of the temperature dependence of the electrophysiological membrane parameters increased gradually as the temperature was lowered far below 0 °C. The extraordinary increase in the $Q_{10}$ value of the falling phase of the action potential near above 0 °C in normal seawater seemed to appear below −10 °C.

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The difference between the $Q_{10}$ values for the kinetic properties of the sodium and the potassium channels becomes clear below 0 °C, suggesting the difference in molecular structures of these channels. Preliminary results were reported previously (Kukita & Yamagishi, 1981).

## Materials and Methods

Squid (*Doryteuthis bleekeri*) living in the Sea of Japan were obtained at Ine in Kyoto Prefecture, Japan. Hindmost stellar giant axons (450 to 700 μm in diameter) were dissected and used for the intracellular perfusion. The giant axon whose axoplasm was squeezed out with a small roller (Baker, Hodgkin & Shaw, 1962) was mounted horizontally on a Lucite® chamber (20 mm in width). The part of the axon whose axoplasm remained intact so as to make the insertion of the glass column easy and to hinder the leakage of the internal solution through the cut end, was cut on a small block near the left-hand side of the chamber. The inlet glass column (300 μm outside diameter) was inserted into the axon from the cut end and then the internal perfusion was started by raising the hydrostatic pressure of the reservoir of the internal solution (Tasaki, Watanabe & Takenaka, 1962). The outlet glass column (400 μm outside diameter) was inserted from the other end of the axon which was laid on another Lucite® block near the right-hand side of the chamber. The recording and the stimulating electrodes were inserted longitudinally into the axon through the outlet column. The perfusion length was 20 mm.

The temperature of the axon was changed by circulating the cooled external bathing solution, the temperature of which was controlled with the Peltier element. The whole system was kept in a minute cold chamber to increase the efficiency of cooling. The temperature of the axon was measured near the axon with a thermocouple thermometer.

The membrane potential was measured with the glass capillary filled with 1 M KCl and 50% (vol/vol) glycerol connected with Ag–AgCl wire and a floating fine platinum wire was inserted into the capillary to reduce the electrode impedance. The reference electrode was an agar bridge of a solution containing 1 M KCl and 50% (vol/vol) glycerol with Ag–AgCl wire connected. The polarizing current was supplied with a platinized platinum wire (70 μm in diameter) inserted through the perfused section. The external current electrode was 6.5 mm in width, and a pair of guard electrodes were placed at both sides of the electrode. The voltage clamp was performed by the usual methods (Cole & Moore, 1960).

The propagated action potential was measured with the capillary electrode at the end of the perfused section. The long wire current electrode was not inserted and the electrical stimuli were supplied through the inlet column 15 mm apart from the recording electrode.

The concentration of glycerol in the external and the internal solutions was gradually increased, keeping the osmolality of both solutions roughly equivalent in order not to lose the nerve activity (Kukita & Yamagishi, 1979*a*). The glycerol conentrations (in % (vol/vol) of the internal solutions were 10.5, 40.8 and 50.8 and those of the external solutions were 0, 34.2 and 44.2. The external solution contained 440 mM NaCl and 10 mM Na-HEPES (pH 8.0). Since the calcium ion activity was decreased by glycerol, the calcium concentrations (in mM) at the glycerol concentrations (in % (vol/vol) of 0, 34.2 and 44.2 were made 100, 140 and 200, respectively. The internal solutions contained 80 mM KF and 20 mM K-phosphate (pH 7.4). The freezing points of these external solutions were −2.0, −17.5 and −26 °C and those of internal solutions were −4.5, −21 and −30 °C, respectively. The $Q_{10}$ value of the solution conductivity from which we derived the solution microscopic viscosity to explain the slowing down of the nerve excitation in concentrated nonelectrolyte solutions (Kukita & Yamagishi, 1979*b*) increased as the temperature was lowered. The $Q_{10}$'s of the solution conductivity of external solutions containing 40.8 and 50.8% (vol/vol) glycerol were 1.51 and 1.57, respectively, in the temperature range between 0 and 5 °C and those between −15 and −10 °C were 1.72 and 1.86, respectively, while that for the normal seawater without glycerol was 1.27 between 0 and 5 °C. $Q_{10}$'s for internal solutions were somewhat larger and changed in the same manner.

## Results

### *Propagation of Action Potentials Below 0 °C*

Typical records of propagated action potentials at the temperature below 0 °C are shown in Fig. 1. At −19 °C, the resting potential did not change much,

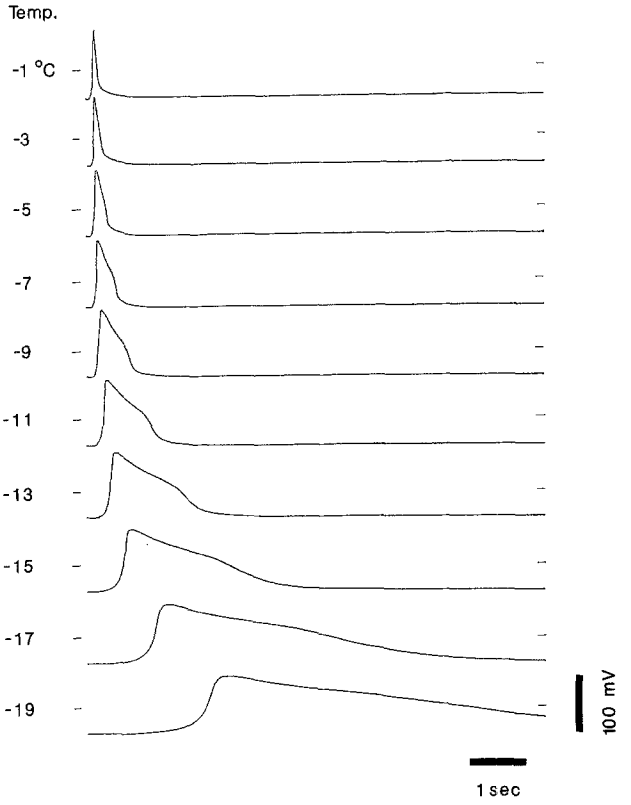


**Fig. 1.** Propagated action potentials below 0 °C. Action potentials were produced by a depolarizing pulse applied though the internal electrode at the end of the perfused section and were recorded internally at the other end. The electrodes were 15 mm apart from each other. At the left end of each trace, the electrical stimulus was applied. The short horizontal bars at both ends of each trace show the external potential level. Temperature is indicated on the left side of each trace. The external solution contained (in mM) 440 NaCl, 200 $CaCl_2$ and 10 Na-HEPES (pH 8.0) and the internal solution contained 80 KF and 20 K-phosphate (pH 7.4). The glycerol concentration (in % (vol/vol)) was 44.2 outside and 50.8 inside. The conduction velocity decreased and the duration of the action potential prolonged as the temperature was lowered

and the action potential could be elicited with the normal strength of the depolarizing electrical current pulse. The conduction velocity decreased as the temperature was lowered. It was 19 cm/sec at −1 °C in these solutions, decreased to 0.9 cm/sec at −19 °C and restored to the original value when the temperature was raised again. This means the conduction velocity at −19 °C was only 0.3% of that in the isotonic physiological solution at 10 °C, the temperature under which the squid live. The duration of the action potential prolonged as the temperature was lowered. The duration at the level of half amplitude was 3.4 sec at −19 °C. The time course of the action potential at −19 °C was a few thousand times as slow as that in the isotonic artificial seawater at 10 °C. The falling phase of the action potential was slowed more prominantly than the rising phase. These changes in the action potential were common to those of nonpropagated membrane action potential. A similar change in the shape of the action potential was reported with axons in normal seawater near and above 0 °C (Hodgkin & Katz, 1949). The $Q_{10}$ value of the change in the duration of the action potential below 0 °C was around 8.5±2.2 (means±SD of 15 experiments). The value was three times as large as that of the normal axons.

In Fig. 2, the change in the conduction velocity relative to that at 0 °C was represented on the logarithmic scale against the temperature. As the temperature was lowered, the conduction velocity decreased exponentially between 15 and −5 °C with the $Q_{10}$ value of 2.0 and then decreased more steeply below −5 °C. The $Q_{10}$ value near −10 °C was around 5. However, there was no clear discontinuous change in the $Q_{10}$ values. The gradual increase in $Q_{10}$ value according to the decrease in the temperature is also observed in experiments above 0 °C and is explained by the Hodgkin and Huxley model for nerve conduction (Easton & Swenberg, 1975). The velocity-temperature curve below 0 °C could be fitted roughly with that for the squid in the North Atlantic (*L. pealei*) (Easton & Swenberg, 1975) by multiplying the $Q_{10}$ value by 1.5 and shifting the temperature by about −16 °C. The $Q_{10}$ value of the conduction velocity in concentrated glycerol solutions was larger than that in normal seawater, mainly because of large $Q_{10}$ values of the solution conductivity inside and outside the axon which were larger by a factor of 1.2 to 1.5 than that of normal seawater and an additional increase in the time constants of kinetics of nerve excitation which were attributed to the increase in the solution microscopic viscosity according to lowering the temperature (Kukita & Yamagishi, 1979*b*).

### *Membrane Currents Above and Below 0 °C*

The membrane currents of the axon in the concentrated glycerol solutions are shown in contrast to those in normal isotonic seawater (Fig. 3). The solutions outside and inside the axon contained 34.2 and 40.8% (vol/vol) glycerol, respectively. The resistivity of these solutions was about fourfold of that of the

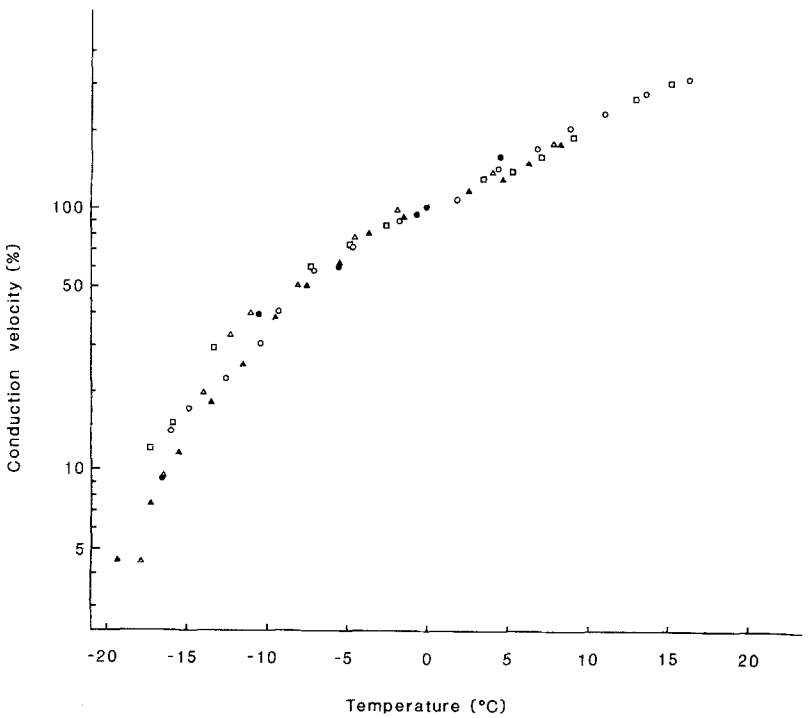


**Fig. 2.** Relationship between conduction velocity and temperature. The conduction velocity relative to that at 0 °C is represented in % on the ordinate. The temperature is represented on the abscissa. Results of the experiments in the same solutions as those of Fig. 1 (△, ▲) and those in the same solutions as those of Fig. 4 (○, ●, □) are plotted. The $Q_{10}$ value above −5 °C was 2.0 and then gradually increased below −5 °C

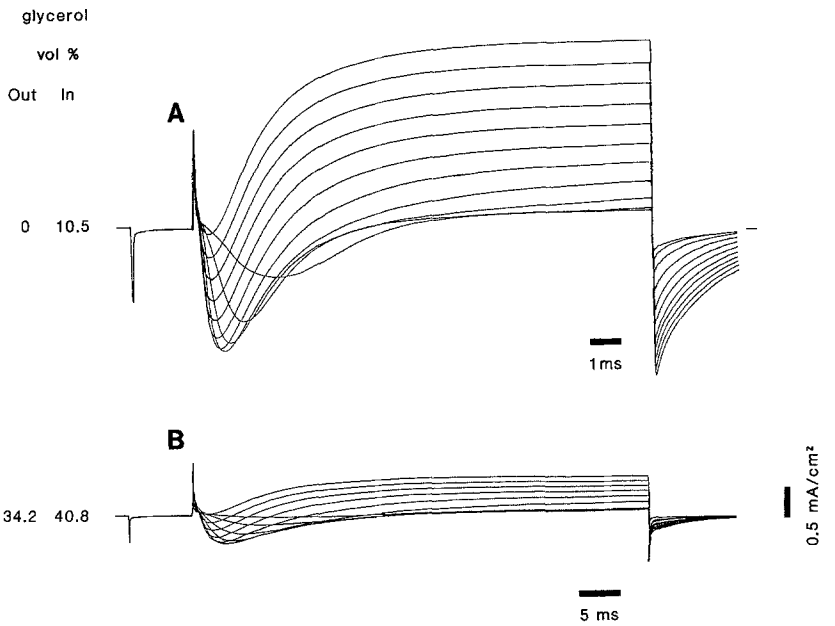


**Fig. 3.** Membrane currents recorded from axons immersed in isotonic and hypertonic glycerol solutions. Traces of membrane currents in hypertonic glycerol solutions (*B*) are represented in comparison with those in isotonic solutions (*A*). The membrane potentials were changed stepwise by 10 mV from −20 to +80 mV. For a short time before application of the depolarizing voltage pulse, the membrane potential was held at −100 mV to reduce the sodium inactivation as shown in the traces. The vertical scale is the same in both *A* and *B*, while the horizontal time scale in *B* is four times as long as that in *A*. The glycerol concentration is shown on the left side of the traces. The external solution contained (in mM) 450 $Na^+$ and 100 $Ca^{2+}$ in case *A* and 450 $Na^+$ and 140 $Ca^{2+}$ in case *B*. Internal solutions contained 100 mM $K^+$. The solution temperature was 3.8 °C

isotonic seawater. The time courses of inward and outward currents in concentrated glycerol solutions (Fig. 3*B*) were prolonged by fivefold of those in normal solutions (Fig. 3*A*), which could be explained by the increase in the solution microscopic viscosity represented with the relative increase in solution resistivity (Kukita & Yamagishi, 1979*b*). The magnitudes of inward and outward currents decreased to 23 and 19%, respectively. The reversal potential decreased by about 10 mV. The leakage current decreased also in the concentrated glycerol solutions.

Records of the membrane currents above and below 0 °C are shown in Fig. 4. The time course of the membrane current was slowed down as the temperature was lowered and that at −15 °C was prolonged to about 30-fold of that at 4.7 °C. The temperature dependence of the time course of the membrane current before the peak inward current was smaller than that after the peak inward current. The larger temperature dependence of the falling phase of the action potential shown in Fig. 1 could be explained by the larger temperature dependence of the membrane currents after the peak inward current. The amplitudes of the inward and outward currents decreased as the temperature was lowered and those at −15 °C were about 15% of those at 4.7 °C. The leakage current decreased as the temperature was lowered. The time courses of the membrane currents were slower by 300-fold than that in normal seawater at 10 °C. The slowing down of the kinetics at low temperature was reversible; when the temperature was raised again, the membrane currents recovered almost completely.

The peak inward current decreased with the $Q_{10}$ value increasing from 2.3 to 3.1 as the temperature was lowered. The slope conductance for the peak inward current decreased in the same manner. The membrane potentials at which the inward currents have their maximum values and the reversal potentials for the inward current changed little. The outward currents at the temperature of 4.7, −0.6, −5.8, −11.0 and −15.0 °C were measured at 60, 150, 300, 600 and 1500 msec after the stepwise depolarization of the membrane potential. These outward currents decreased also as the temperature was lowered with $Q_{10}$ increasing from 2.3 to 4.8. $Q_{10}$ values of the time-to-peak inward current were around 1/5.0. The voltage-*tp* relation curves were almost in parallel with one another, showing that the voltage-dependence of the time constant did not change below 0 °C.

### *Different Temperature Dependence of the Kinetics of Sodium and Potassium Channels*

Experiments described in the previous section were also performed on TTX-poisoned and TEA-perfused

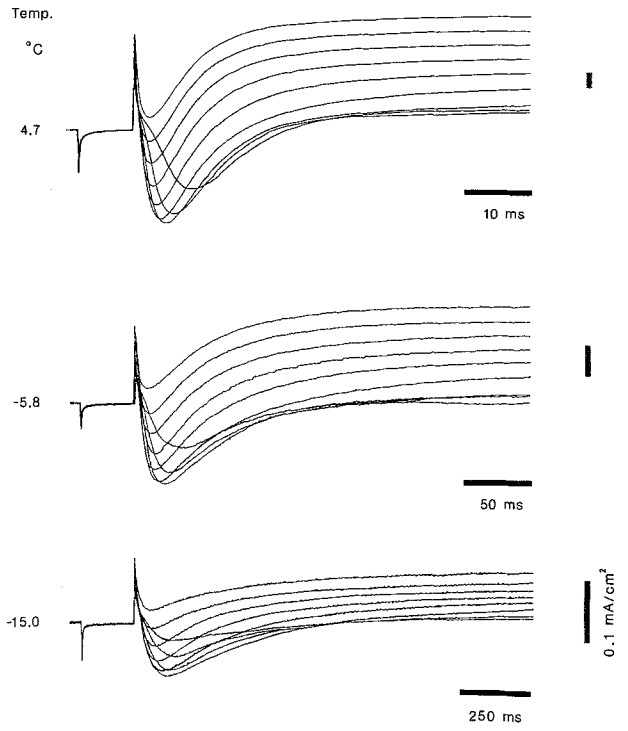


**Fig. 4.** Membrane currents above and below 0 °C. The time course of the membrane currents was prolonged by fivefold with every decrease in temperature by 10 °C. The prolongation of the outward current was larger than that of the inward current. The solutions were the same as those in *B* of Fig. 3. The membrane currents and their slope conductance decreased with the $Q_{10}$ value of around 2 as the temperature was lowered. The time to peak inward current increased as the temperature was lowered with the $Q_{10}$ values of around 5. The membrane potential dependence of *tp* was almost the same at all temperatures

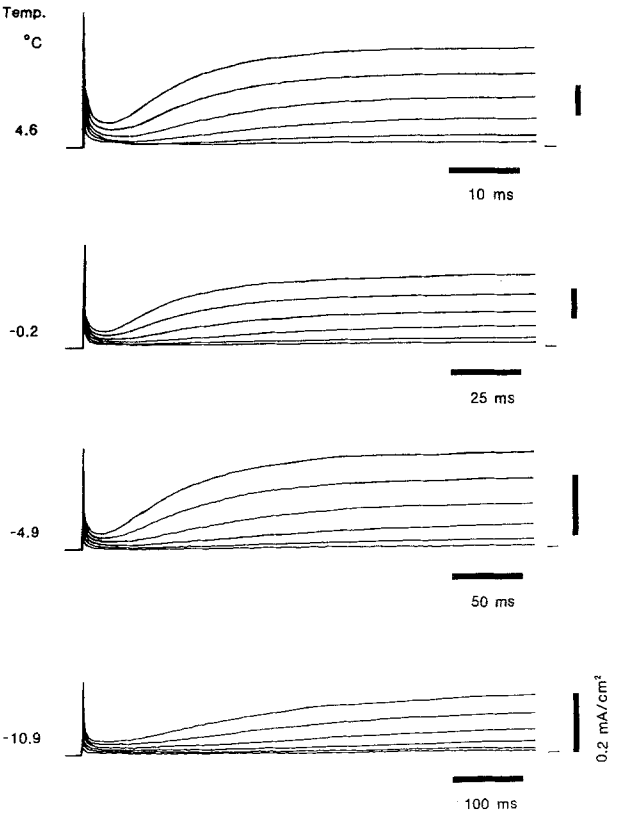


**Fig. 5.** Potassium currents above and below 0 °C. Membrane currents were recorded from an axon immersed is an external solutions containing 100 mM TTX. Except for TTX, the solution compositions were the same as those in Fig. 4. The controlled membrane potential was changed stepwise by 20 mV from −30 to 70 mV. The holding potential was −100 mV. The time course of the potassium current was slowed as the temperature was lowered

giant axons, in order to examine the temperature dependence of the time course of the sodium and the potassium currents below 0 °C separately.

Typical traces of the potassium current measured on the TTX-poisoned axon are represented in Fig. 5. The potassium current was slowed as the temperature was lowered with a $Q_{10}$ of around 6.5. The amplitude of the potassium current at −11 °C was 25% of that at 5.2 °C.

The relationship between the membrane potential and the time to the moment when the potassium current increased to a half of its maximum ($t_K$) is represented in Fig. 6. The $t_K$'s changed in the same manner against the membrane potential at all temperatures.

The current-voltage relation of the potassium current is represented in Fig. 7. The potassium conductance obtained from the linear portion of the current-voltage curve decreased with the $Q_{10}$ value of 1.9 as the temperature was lowered. The current-voltage relation curves shifted slightly in the direction of depolarization.

Typical traces of the sodium current measured on the axon perfused intracellularly with TEA are represented in Fig. 8. The rising and falling phases of the sodium current were slowed in the same manner in the wide range of temperature when the temperature was lowered. The $Q_{10}$ values for them were 1/5.5. They were almost the same as the $Q_{10}$ for the inward current before the peak shown in Fig. 4, but were quite different from that of the potassium channel in Fig. 5 and from that for the inward current after the peak in Fig. 4. The amplitudes of the sodium current and the leakage current decreased as the temperature was lowered. The residual outward current in the trace was mainly the remaining potassium current due to the somewhat lower affinity of TEA to the potassium channel in these concentrated glycerol solutions. The time constant of the on process of the sodium channel ($t_{Na}$) and that of the off process ($t_{1/2}$) at various temperatures are plotted against the membrane potential in Fig. 9. The $t_{Na}$'s at various temperatures lie in parallel with one another as do

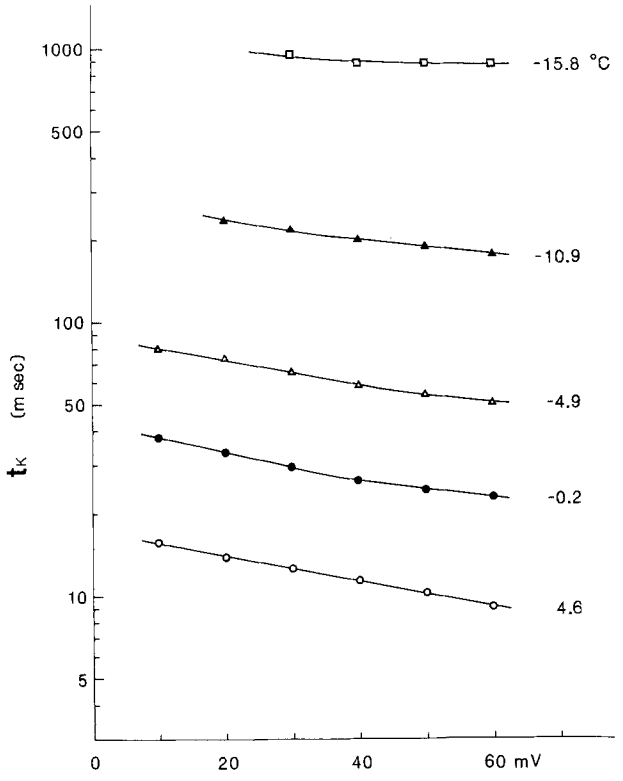


**Fig. 6.** Relations between the time constant of the potassium channel opening and the membrane potential above and below 0 °C. The times from the onset of the depolarizing pulse to the moment when the potassium current reaches half of its maximum ($t_K$) at various temperatures are plotted against the membrane potential. The voltage dependence of $t_K$ was almost the same at all temperatures. The $Q_{10}$ values of $t_K$ was around 6.5

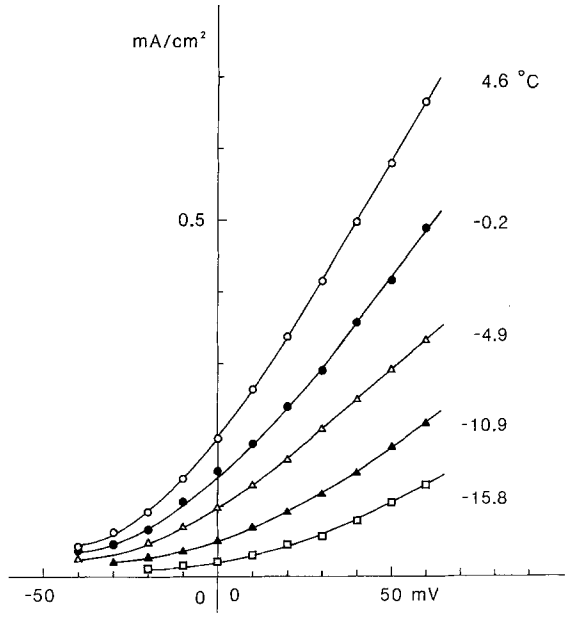


**Fig. 7.** Current-voltage relations for the potassium current above and below 0 °C. The potassium conductance decreased as the temperature was lowered with the $Q_{10}$ value of 2.0. The current-voltage relation curves shifted a little in the direction of depolarization as the temperature was lowered

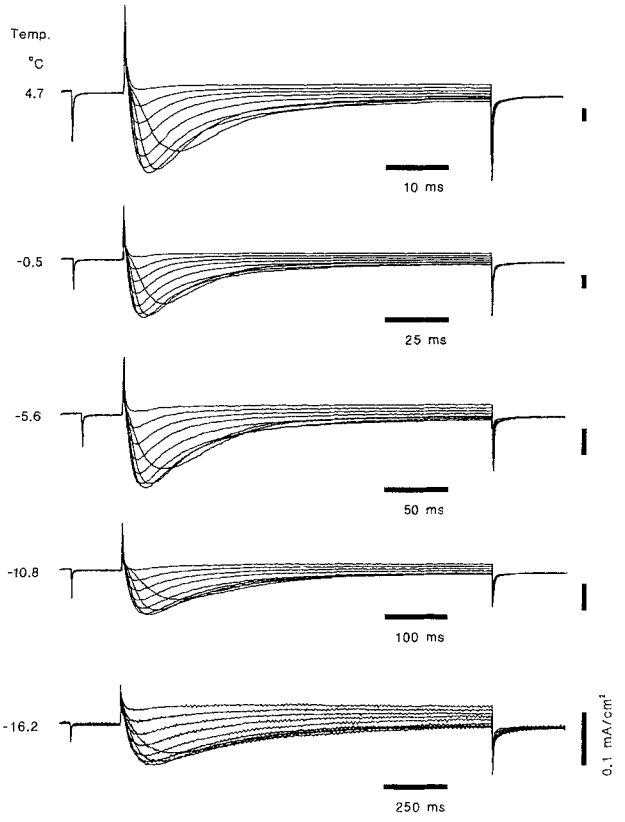


**Fig. 8.** Sodium currents above and below 0 °C. The membrane currents were recorded from an axon intracellularly perfused with the solution containing 10 mM TEA. Except for TEA, the solution compositions were the same as those shown in Fig. 4. The membrane potential was changed stepwise by 10 mV from −10 to 70 mV. The membrane potential was held at −100 mV for the short time before the application of the depolarizing pulses as shown in the traces. The time course of the opening and the closing of the sodium channel was slowed in the same manner according to the decrease in the temperature

the $t_{1/2}$'s. The voltage-dependent kinetics of the on and the off processes changed in the same manner when the temperature was lowered.

The current-voltage relations of the sodium current at various temperatures are represented in Fig. 10. The maximum sodium conductance obtained from the linear portion of the current-voltage relation curve decreased with the $Q_{10}$ value of 2.5 as the temperature was lowered. The reversal potential for the sodium current did not change largely above −10 °C. The reversal potential at −15 °C decreased by 10 mV (the mean of eight axons is 5 mV). The decrease in the reversal potential was partially explained by the error from the remaining outward current which was less sensitive to temperature. The current-voltage relation curves at various temperatures were similar except for their amplitude. This shows that the relationship between the sodium conductance and the membrane potential is the same at all the temperatures examined.

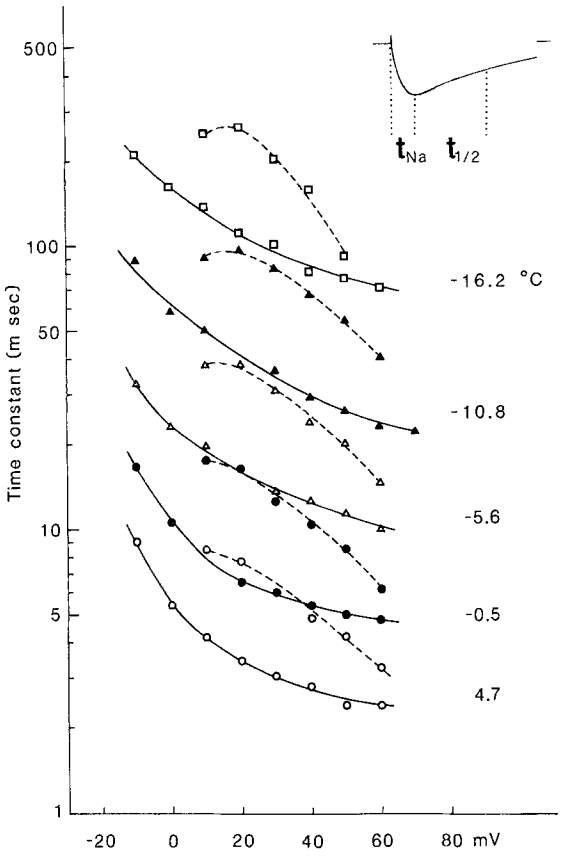


**Fig. 9.** Relationship between the time constants of the opening and the closing of the sodium channel and the membrane potentials above and below 0 °C. The opening time constant ($t_{Na}$) was the time to the peak sodium current from the onset of the membrane potential change. The closing time constant ($t_{1/2}$) was the time which was required for the sodium current to decay from the peak to its half. The definitions for these time constants are shown in the inset. The $t_{Na}$'s at each temperature are shown by solid line (—) and the $t_{1/2}$'s by broken line (---). The voltage dependence of the $t_{Na}$ was almost the same at each temperature and that of the $t_{1/2}$ was also the same at each temperature

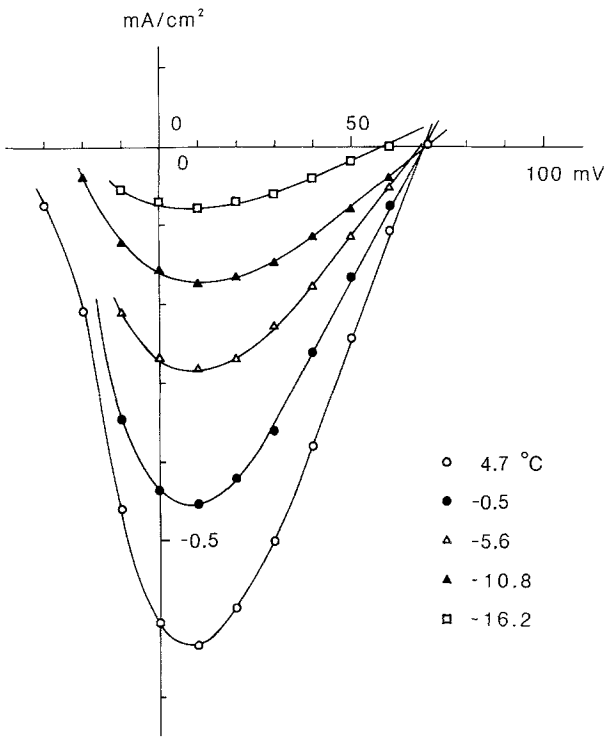


**Fig. 10.** Current-voltage relations for the sodium current above and below 0 °C. The peak inward currents recorded from the TEA-perfused axon shown in Fig. 8 are plotted against the membrane potentials. The sodium currents decreased as the temperature was lowered, while the reversal potential changed little. The maximum sodium conductance obtained from the slope of the linear portion of the current-voltage curve decreased with the $Q_{10}$ value of around 2.5 as the temperature was lowered

The relationship between the membrane parameters for the sodium and the potassium channels and the reciprocal of the temperature (in K) is represented in Fig. 11. The slope of change is almost linear around 0 °C and there is no extraordinarily large change in the membrane parameters frequently observed in the axon in normal seawater near 0 °C. The time constants of the opening and the closing of the sodium channel increased almost in the same manner when the temperature was lowered but that of the opening of the potassium channel increased more steeply. The $Q_{10}$ values of the temperature dependence of the sodium channel were quite different from that of the potassium channel (Table 1). The difference of $Q_{10}$ values became clear as the temperature was lowered. The changes in the time constants were almost linear against the reciprocal of the absolute temperature above −5.5 °C but became larger under the lower temperature. The resistance of the potassium channel increased linearly against the reciprocal of the absolute temperature, while the change in the resistance of the sodium channel became larger as the temperature was lowered. The $Q_{10}$ value for the sodium conductance was somewhat larger than that for the potassium conductance (Table 1). Some of the differences between channel conductances might be due to the increase in the leakage current at low temperature, although the leakage current decreased also as the temperature was lowered. Especially at the lowest temperature below −15 °C the long time course of the membrane current frequently caused an increase in leakage current due to the large amount of the ionic flow during the nerve excitation. Anyway, the membrane parameters for the sodium channel changed against the temperature in a manner different from that for the potassium channel. The $Q_{10}$ values of kinetics in normal seawater were around 3.0 and much smaller than $Q_{10}$'s listed. Although the large $Q_{10}$ in concentrated glycerol solutions and far below 0 °C were qualitatively explained by the increase in

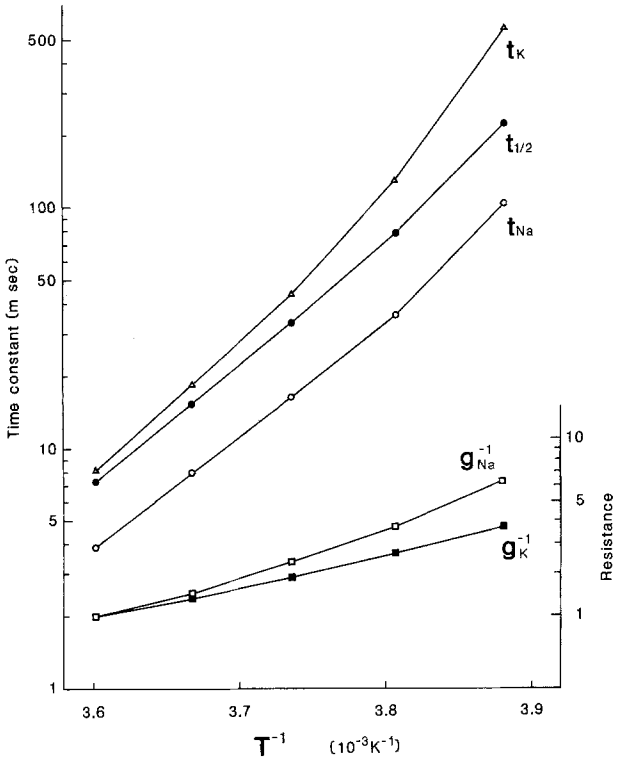


**Fig. 11.** Arrhenius plot of the membrane parameters for the sodium and the potassium channels. The time constants ($t_{Na}$ and $t_{1/2}$ at the membrane potential of 30 mV, and $t_K$ at the membrane potential of 50 mV) in msec (left-hand scale) and the relative change (right-hand scale) in the reciprocal of the maximum channel conductance ($1/g_{Na}$, $1/g_K$) are plotted against the reciprocal of the absolute temperature. The temperature of the results plotted were (in °C) 4.5, −0.5, −5.5, −10.5 and −15.5. The means of the results of eight axons are plotted for the sodium channel and those of nine axons for the potassium channel. The standard deviations of the results are about 20% for the $t_{Na}$'s and they increase to 35% for the $t_{1/2}$'s and to 44% for $t_K$'s as the temperature is lowered. The temperature dependences of the membrane parameters for the sodium channel were different from those for the potassium channel. There is no clear break in the slope of Arrhenius plot but the slope increases as the temperature is lowered

**Table 1.** $Q_{10}$ values of membrane parameters[a]

| Temp. (°C) | −0.5 | −5.5 | −10.5 |
|---|---|---|---|
| Na-channel: | | | |
| Rate of On | | | |
| at 10 mV | 3.9±0.5 | 4.4±0.6 | 6.3±0.8 |
| at 30 mV | 4.2±0.6 | 4.6±0.6 | 6.1±0.7 |
| Rate of Off | | | |
| at 10 mV | 4.5±1.2 | 4.5±0.3 | 6.0±1.6 |
| at 30 mV | 4.3±1.0 | 4.9±0.7 | 6.6±0.7 |
| $g_{Na}$ | 2.3±0.2 | 2.5±0.3 | 2.8±0.6 |
| K-channel: | | | |
| Rate of On | | | |
| at 30 mV | 5.2±0.7 | 6.6±1.4 | 9.8±1.5 |
| at 50 mV | 5.4±0.9 | 6.5±1.3 | 10.7±2.1 |
| $g_K$ | 1.9±0.2 | 2.0±0.3 | 2.1±0.4 |

[a] The $Q_{10}$ values of the rates of on process ($1/t_{Na}$) and off process ($1/t_{1/2}$) of the sodium channel, of the rate of on process of the potassium channel ($1/t_K$), of the sodium conductance ($g_{Na}$) and of the potassium conductance ($g_K$) are listed. The $Q_{10}$ value at each temperature is the ratio of the value 5 degrees above to that 5 degrees below that temperature. The means ±SD of eight axons for the sodium channel and those of nine axons for the potassium channel are listed. The difference of the $Q_{10}$ values for the sodium and potassium channels becomes clear below −5 °C.

the solution microscopic viscosity near ionic channels, only around one-third of the difference could be explained by this. Therefore, the large $Q_{10}$'s might be attributed to the molecular stability of ionic channels under these conditions.

## Discussion

The nerve fiber of the squid was excitable far below 0 °C, when the osmolality of solutions inside and outside the nerve was raised and the freezing point of these solutions was lowered. Although the squid live at a temperature of around 10 °C, their nerve was active far below 0 °C when the osmolality of the physiological solutions was raised in much the same way as that reported for the physiological change in the hibernating insects during winter (Baust & Miller, 1970; Zachariassen, 1980). These results show that the blockage of the nerve conduction after freezing the physiological solution (Hodgkin & Katz, 1949; Roots & Prosser, 1962; Spyropoulos, 1965; Easton & Swenberg, 1975) is not the structure change in the membrane by lowering the temperature but the secondary effect caused by the freezing of the physiological solutions or the axoplasm.

The moderate fluidity of the membrane or the lipid bilayer is considered to be important for the membrane protein to work efficiently because the temperature at which the membrane loses its fluidity is frequently related to that at which the biological activity changes sharply and is reflected by the living temperature of animals. Some investigators reported that the change in the ionic conductance of the muscle membrane against the temperature showed the discontinuity (Anderson, Cull-Candy & Miledi, 1977; Fischbach & Lass, 1978; Schwarz, 1979) and that the changes in the sodium conductance and the kinetics of the off process of the sodium channel in the rabbit myelinated nerve (Chiu, Moore & Ritchie, 1979) and the kinetics of the on and off process of the sodium channel in the frog myelinated nerve (Schwarz, 1979) became larger as the temperature was lowered below a critical temperature of around 6 °C. Although these changes were explained by the phase transition in lipid bilayer, there is no trait of the break near 0 °C in the Arrhenius plot of the mem-

brane parameters of the squid nerve and a large amount of glycerol seemed to lower the transition temperature far below 0 °C as shown in Figs. 2 and 11. The squid nerve membranes have no clear transition temperature in the temperature range between 20 and −19 °C with a tendency of the gradual increase in $Q_{10}$'s of the membrane parameters at lower temperature. Somewhat larger change below −10 °C might suggest that some critical point exists around there but it is not a clear one. The possible reasons which can be considered are the following. The transition temperature of the lipid bilayer does not lie in this temperature range, since the decrease in the transition temperature by adding an antifreeze is considered to be small (Steim, Tourtelotte, Reinert, McElhaney & Rader, 1969). The change in the physical state of lipid bilayer against the temperature is a gradual one, and/or the activity of the ionic channels on the squid axolemma was not affected sharply by the physical state of the lipid bilayer in the neighbor of ionic channels. As represented in the results by other investigators (Chiu et al., 1979; Schwarz, 1979), the transition of the kinetic parameters of nerve excitation was too small to distinguish between the break and the continuous change around the critical temperature.

The $Q_{10}$ values of membrane parameters were larger than the well-known $Q_{10}$ value of 3.0 in normal seawater, because the $Q_{10}$ values in concentrated glycerol solutions were the combination of the $Q_{10}$ value of 3.0 in the normal membrane and the prolongation by the increase in the solution microscopic viscosity due to lowering the temperature (Kukita & Yamagishi, 1979*b*). A part of the gradual increase in $Q_{10}$ values at lower temperature can be explained by the increase in $Q_{10}$ value of the microscopic viscosity but it might be attributed to the stabilization of the ionic channel molecules under these conditions. At least in the squid nerve the break in the temperature dependence did not become clear when the temperature range of the experiment was expanded far below 0 °C as described by Schwarz (1979) and so it is safely said that the temperature dependence of membrane parameters of the excitation of squid giant axon is the gradual one.

Considering the result of the heat production at the upstroke phase of the action potential (Howarth, Keynes & Ritchie, 1968) and the abolition of the action potential by heat (Spyropoulos, 1961), some investigators proposed the model that the nerve excitation is the exothermic response (Tasaki & Spyropoulos, 1957) and the action potential was produced by cooling the nerve (Inoue, Kobatake & Tasaki, 1973). The experimental result reported here shows that the nerve stays at the resting state at the temperature as low as −19 °C without the decrease in the threshold potential for the excitation and the spontaneous firing does not occur in solutions containing a relatively high concentration of calcium. So, I could not demonstrate the spontaneous firing by cooling the axons, probably because of the difference in solution compositions.

Some difference between the $Q_{10}$ value of the time constant of the on process and that of the off process of the sodium channel was reported with respect to the excitation of the myelinated nerve (Frankenhauser & Moore, 1963; Schwarz, 1979). However, the clear difference between the $Q_{10}$ value for the sodium channel and that for the potassium channel has not yet been reported. Our result shows for the first time that the $Q_{10}$ values for the opening and closing kinetics of the sodium channel on the squid axolemma are very close to each other and they are significantly different from the opening kinetics of the potassium channel at the temperature far below 0 °C. This result strongly suggests that the opening and the closing mechanisms of the sodium channel possess the common structure which is independent of that for the potassium channel.

For practical use, the very slow kinetics of the nerve excitation below 0 °C serves as a valuable tool for some biophysical measurements of the excitable membrane, because the detection of the structural change in the ionic channels during the nerve excitation could be performed with apparatus of a lower time resolution. Although the prolonged action potentials may be produced in squid axons intracellularly perfused with TEA (Armstong & Binstock, 1965; Tasaki & Hagiwara, 1957) with proteases (Takenaka & Yamagishi, 1969; Armstrong, Bezanilla & Rojas, 1973), or with dilute salt solutions (Narahashi, 1963), all these procedures do not slow the opening of the sodium channel. Cooling the axon and adding a large amount of nonelectrolytes into solutions (Kukita & Yamagishi, 1979*b*) are the only methods known until now to slow the opening kinetics of the sodium channel large enough for practical use. A combination of these expands the temperature range for the experiment below subzero temperature to increase this advantage to an enormous extent, because the duration of the single action potential can now be prolonged by a factor of thousands in comparison with that elicited in normal seawater at the usual room temperature.

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