BRIEF COMMUNICATION

EXCITATION OF SQUID GIANT AXONS BELOW 0°C

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ABSTRACT The excitation of the squid giant axon that had been perfused intra- and extracellularly with solutions containing a high concentration of glycerol could be observed below 0°C. The action potential could be elicited at normal strengths of electrical stimuli. The time-course of the action potential was slowed, whereas the resting potential and the amplitude of the action potential changed only slightly. The membrane current under the voltage clamp at −6.3°C was about 100-fold slower than that in normal sea water at 8.7°C because of the large viscosity of glycerol solutions and the low temperature. The Q_{10} values of the magnitude and the time-course of the membrane current were 2.3 and 1/4.0, respectively.

Because nerve excitation is such a fast phenomenon, measurements of electrical or physical properties are sometimes difficult. The main purpose of this report is to show that normal nerve excitation can be observed on a prolonged time scale by lowering the temperature of the axon below 0°C. Until now it has been impossible to perform this experiment on either an intact nerve or an intracellularly perfused giant axon because the cytoplasm or the perfusing solutions froze (Spyropoulos, 1965; Easton and Swenberg, 1975). By using nonfreezing solutions inside and outside the axon we have been able to observe nerve excitation below 0°C. As reported previously (Kukita and Yamagishi, 1979*a*), nerve excitation can be maintained in solutions containing a high concentration of glycerol. Such solutions do not freeze until the temperature is lowered to −15°C. Both the high concentration of glycerol and the low temperature prolonged the time-course of nerve excitation to about 100-fold that in normal isotonic artificial sea water at room temperature.

The giant axon of squid (*Dorytheutis bleekeri*) was intracellularly perfused according to the method of Tasaki and his collaborators (Tasaki et al., 1962) with some modification. The concentration of glycerol in both the internal and the external solutions was increased gradually to a final concentration of 41 vol% inside and 34 vol% outside. The external solution contained 370 mM NaCl, 80 mM $CaCl_2$, and 10 mM Na-HEPES (pH 8.0); the internal solution contained 64 mM KF and 16 mM K^+ (as phosphate buffer at the pH of 7.4). The temperature of the axon was lowered by circulating cooled external solutions around it. These solutions were cooled either with a peltier cooling system or by blowing cold nitrogen gas vaporized from liquid nitrogen. The temperature was measured near the axon with the thermocouple thermometer. The glass capillary internal electrode and the external electrode were filled with a solution containing 1 M KCl and 50 vol% glycerol. The resistance of the internal electrode was decreased to about 200 KΩ at room temperature by inserting the thin

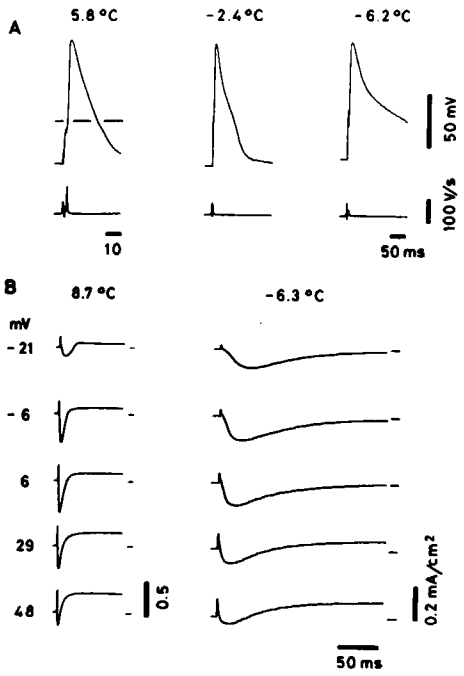

FIGURE 1 The action potentials and the membrane currents under the voltage clamp above and below 0°C. *A*, the action potentials and their derivatives at 5.8°, −2.4°, and −6.2°C. The time scales of the records above and below 0°C shown by the horizontal bar were 10 and 50 ms, respectively. The duration of the action potential prolonged to sixfold when the temperature was lowered from 5.8° to −6.2°C. *B*, traces of typical records of membrane current at 8.7° and −6.3°C. The membrane potential controlled was shown on the left. The holding potential was −90 mV. The records above and below 0°C were represented on the same time scale. The vertical bars on the right of the records above and below 0°C indicate 0.5 and 0.2 mA/cm^2, respectively. The membrane current slowed by 10-fold and its amplitude decreased to 30% when the temperature was lowered from 8.7° to −6.3°C. The internal solution contained 60 mM KF, 16 mM K^+ (as phosphate buffer at pH 7.4) and 41 vol% glycerol. The external solution contained 370 mM NaCl, 80 mM $CaCl_2$, 10 mM Na-HEPES (pH 8.0) and 34 vol% glycerol.

platinum wire. The voltage clamp was performed according to the usual method using a long platinized platinum wire for the current supply.

Fig. 1 shows typical records of action potentials and membrane currents under the voltage clamps above and below 0°C. The action potentials and their derivatives at 5.8°C, −2.4°C, and −6.2°C are shown in Fig. 1*A*. Below 0°C, action potentials could be elicited by depolarizing current pulses of normal amplitude. These action potentials had a prolonged time-course and normal amplitudes (Fig. 1*A*). Both the rising and falling phases of the action potentials were slowed, with the duration prolonged by six-fold and the maximum rate of rise decreased to 23%. Fig. 1*B*(left) shows the membrane currents in solutions of high concentration of glycerol at 8.7°C. The time-course of these currents was slowed by about 10-fold with respect to those in normal isotonic solutions containing the same concentrations of salts. This prolongation was mainly due to the increase in the viscosity of the solution (Kukita and Yamagishi, 1979*b*). Both the inward and the outward currents were slowed and decreased to a similar extent, so that the shape of the membrane currents was similar to that in normal sea water. Application of TTX and TEA under this condition showed that both the sodium and

the potassium channels were affected at the usual concentrations. As the temperature was lowered, the time-course was prolonged further. As shown in the records on the right, at −6.2°C the time-course was prolonged by about 10-fold and a decrease in amplitude of membrane currents to 30% was observed. The Q_{10} values of the magnitude and of the duration of the membrane current were 2.3 and 0.25, respectively. These were normal values, although they were somewhat larger than those in normal sea water near 0°C (Hodgkin and Katz, 1949; Moore, 1958; Spyropoulos, 1965). Although the membrane currents had similar shapes at both temperatures, their time-course was prolonged by 10-fold and their magnitude decreased to one-fourth their value at −6.3°C.

An action potential could also be elicited at ~ −10°C. When the temperature was lowered to < −20°C by blowing cold nitrogen gas, the membrane was depolarized and no regenerative potential change could be induced by a depolarizing or a hyperpolarizing current pulse. The action potential appeared with a little deterioration when the temperature was raised again.

Fig. 2 shows the current-voltage relations of nerves at 8.7° and −6.3°C. The inward current at the voltage where it had its maximum value decreased to 29% of its original value and the outward current at 50 mV decreased to 32% of its original value when the temperature was lowered by 15°C. The sodium and the potassium potentials changed little. The membrane potential at which the inward current was largest shifted by 10 mV along the voltage axis in the direction of depolarization at the lower temperature. The slope conductance for the inward current decreased to 30% and that for the late outward current decreased to 35%.

Fig. 3 shows the time to the peak inward current (t_p) and the time from the onset of the membrane potential change to the moment when the inward current decayed to one-half of its maximum value ($t_{1/2}$) at 8.7° and −6.3°C as the function of the membrane potential. The

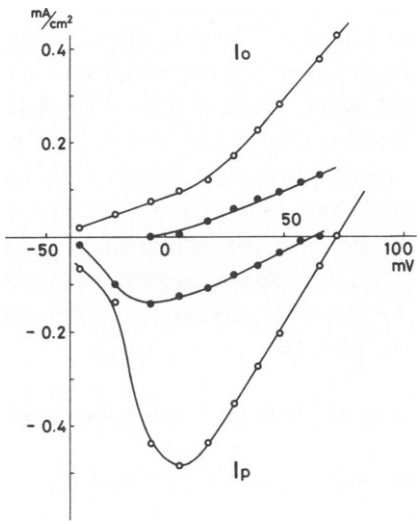

FIGURE 2 The current-voltage relations of nerve excitation above and below 0°C. Open circles show the peak inward current (I_p) and the outward current 20 ms after the onset of the potential change (I_o) at 8.7°C. Filled circles show the peak inward currents (I_p) and the outward current 200 ms after the onset of the potential change (I_o) at −6.3°C. Both the inward and outward currents decreased to 30% when the temperature was lowered by 15°C.

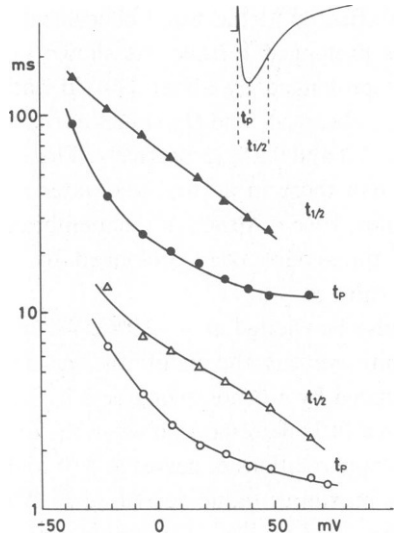

FIGURE 3 The time-course of the inward current as the function of the membrane potential. On the ordinate are represented the time to the peak inward current (t_p) and the time required for the inward current to decay to reach the half of its maximum ($t_{1/2}$) whose definition are shown in the inset. The membrane potential is represented on the abscissa. The temperature was 8.7°C (○ △) and −6.3°C (● ▲). The time constants t_p and $t_{1/2}$ increased by 10-fold with lowering the temperature by 15°C.

values t_p and $t_{1/2}$ increased about 10-fold at all membrane potentials when the temperature was lowered by 15°C. The voltage dependence of t_p and $t_{1/2}$ were almost the same at both temperatures. Thus we have shown that the characteristics of the sodium and potassium channels are the same below 0°C or at normal temperature.

At this low temperature we can observe normal nerve activity on a time scale about 100 times slower than those usually recorded. Although prolonged action potentials can be elicited by using TEA (Tasaki and Hagiwara, 1957), protease (Takenaka and Yamagishi, 1969), or solutions of low ionic concentrations (Narahashi, 1963), these procedures prolong only their falling phase, leaving their rising phase unchanged. Thus only under the experimental conditions described here can changes in the physical or electrical properties related to the nerve excitation, especially the gating of the sodium channel, be slowed down enough to be easily detected. Furthermore, this procedure suggested the possibility of long-term storage of nerve fibers without loss of the fibers' normal activity by freezing them with an appropriate amount of glycerol added to the solutions.

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