J. exp. Biol. (1975), 62, 265–275
With 9 figures
Printed in Great Britain

THE EFFECT OF TEMPERATURE ON THE OUTWARD CURRENTS IN THE SOMA OF MOLLUSCAN NEURONES IN VOLTAGE-CLAMP CONDITIONS

BY I. S. MAGURA, A. E. VALEYEV AND I. Z. ZAMEKHOVSKY
Department of General Physiology of Nervous System, Bogomoletz Institute of Physiology, Ukrainian Academy of Sciences, Kiev-24, 252601, USSR

(*Received 27 June* 1974)

SUMMARY

The delayed outward current in snail neurones was separated into two components with different temperature sensitivity: (i) a persistent component and (ii) a transient (inactivating) component. The effect of cooling on the value of the transient current is strongly dependent upon the value of the conditioning potential. It was supposed that cooling causes a decrease in the negative surface potential in the vicinity of the potassium pathways and removes their inactivaton. Simultaneously cooling depresses the potassium conductance. The effect on surface potential is more distinct with conditioning potentials at which a significant fraction of the transient outward current is inactivated. The effect of cooling on the transient component of the fast outward current was similar to that on the transient component of the delayed outward current.

INTRODUCTION

The main purpose of this investigation is to describe the effects of temperature on the outwardly directed currents transient in molluscan neurones during long-lasting depolarization in voltage-clamp conditions. The outward currents are presumably carried by potassium ions. These currents are suppressed by tetraethylammonium (Hagiwara & Saito, 1959; Neher & Lux, 1972) which specifically blocks potassium channels (see Hille, 1970, for review). Besides this, it was recently shown that spike activity or voltage-clamp pulses caused changes in extracellular potassium concentration in the vicinity of molluscan neurones (Neher & Lux, 1973).

When rather large long-lasting voltage-clamp steps are applied to the somatic membrane a relatively fast inactivation of delayed outward currents is observed. After the delayed outward current reaches its peak, it decays towards a final value (Alving, 1969; Leicht, Meves & Wellhoner, 1971; Connor & Stevens, 1971*a*; Gola & Romey, 1971; Magura, Krishtal & Valeyev, 1971; Neher & Lux, 1971). Similar observation was made on supramedullary neurones of puffer fish (Nakajima, 1966; Nakajima & Kusano, 1966) and on stretch receptor neurones (Nakajima & Onodera, 1969).

The time course and magnitude of potassium currents in molluscan neurones are strongly dependent upon the conditioning level of the membrane potential (Connor & Stevens, 1971*a*; Gola & Romey, 1971; Magura, Krishtal & Valeyev, 1971). When a

conditioning hyperpolarization is used, the subsequent depolarization causes the appearance of a fast transient outward current in some neurones (Hagiwara, Kusano & Saito, 1961; Connor & Stevens, 1971*b*; Neher, 1971*a*). They are activated below −40 mV. Activation of delayed outward current occurs when the membrane potential is more positive than −40 mV. It is assumed that the appearance of the fast outward current is a result of the removal of inactivation of the fast potassium channels. These channels are, on several criteria, operationally distinct from those of the delayed outward current (Connor & Stevens, 1971*b*; Neher, 1971*a*).

The tentative conclusion of this paper is that the temperature changes cause, not only a direct effect on the mechanisms controlling potassium currents in the soma of molluscan neurones, but also probably change in surface membrane potential in the vicinity of potassium pathways.

METHODS

The experiments were performed on giant nerve cells in the visceral complex of *Helix pomatia* ganglia. The preparation has been described in an earlier report (Magura, 1967).

Ringer solution used in experiments had the following composition (mM): NaCl, 80; KCl, 4; $CaCl_2$, 10. The pH was adjusted to 7·5 with Tris-HCl. Temperature regulation was carried out by passing Ringer solution through reservoirs where temperatures were maintained by thermoelectric devices. The temperature of the bath was measured by a thermistor placed close to the preparation.

Two micro-electrodes filled with 3 M-KCl were inserted simultaneously into the same nerve cell. One micro-electrode (resistance 4–5 MΩ) was used to feed current the other (resistance 5–7 MΩ) to measure the membrane potential. The apparatus used for voltage clamping was similar to that described by Chamberlain & Kerkut (1969). Voltage-clamp devices suitable for molluscan neurones have also been described by Gedulding & Gruener (1970) and Gola & Romey (1971).

In our experiments the observed rise time of the membrane voltage pulse was usually less than 500 μsec and the decay time of the capacitive charging current between 1 and 2 msec (the membrane capacitance was 10–20 nF).

RESULTS

Effect of cooling on delayed outward current inactivation

The effects of cooling upon the early inward and delayed outward currents is demonstrated in Fig. 1. Decrease in value and in rate of rise of delayed outward current was observed when the temperature was lowered from 20 to 10 °C. A better estimation of the effect of cooling upon the rate of rise of delayed outward current could be obtained from the record at the inward current equilibrium potential. Somewhat similar changes in early inward current occurred.

A long-lasting, rather large depolarization of the somatic membrane produces a delayed outward current which declines from its maximum to a final value. Fig. 2(*a*) shows that inactivation of delayed outward current is less distinct when the temperature is lowered (see also Fig. 3). The peak value of delayed outward current, rather than its final value, is depressed more effectively by cooling.

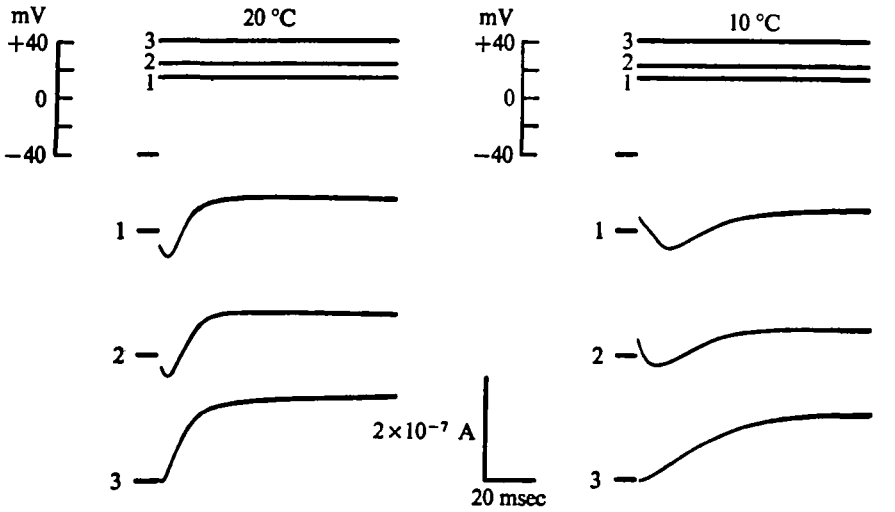

Fig. 1. The effect of temperature on the membrane currents. Membrane potential, above, and currents, below. Holding potential, 40 mV.

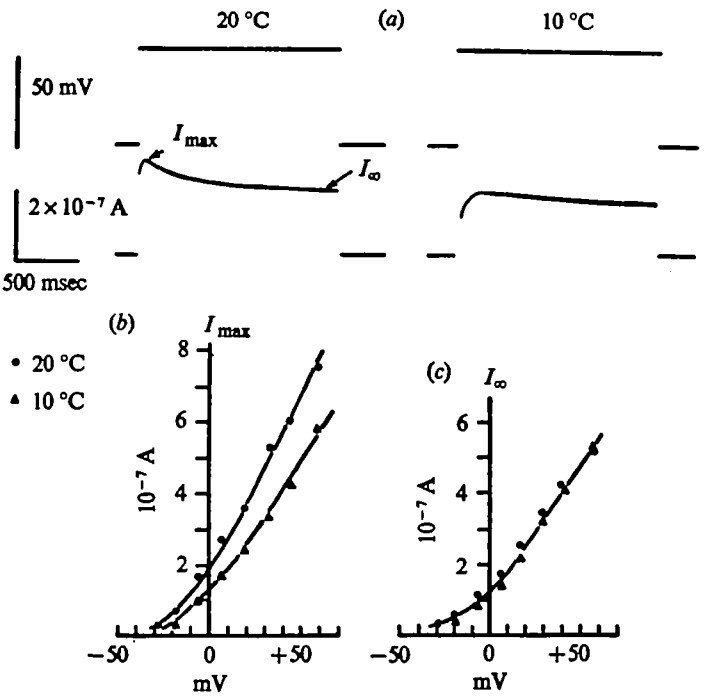

Fig. 2. (*a*) Effect of cooling on the delayed outward current. Membrane potential, above, and current, below. Resting potential, 40 mV, holding potential, 50 mV. (*b*) Current–voltage relations for the peak of the delayed outward current at 20 °C (filled circles), and at 10 °C (triangles). (*c*) Current–voltage relations for the final value of the delayed outward current at 20 °C (filled circles) and at 10 °C (triangles).

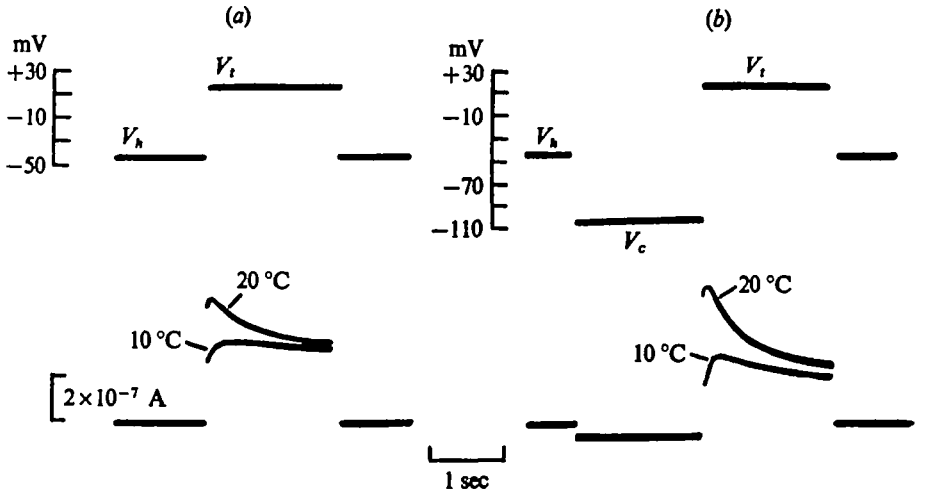

Fig. 3. Comparison of the effect of cooling on the delayed outward current, without conditioning hyperpolarization (*a*) and with conditioning hyperpolarization (*b*). Above, membrane potentials, below, membrane currents. V_h, holding potential; V_c, conditioning potential; V_t, test potential.

In Fig. 2(*b*, *c*) the peak value (I_{max}) of delayed outward currents and their final value (I_∞) taken from an experiment on the same cell at 20 and 10 °C was plotted as a function of the membrane potential. Difference in the effect of cooling on I_{max} and on I_∞ is clearly seen (see also Fig. 5). The curve for I_{max} at 20 °C is somewhat steeper than that at 10 °C.

The effect of cooling upon a delayed outward current may be considerably altered by a preceding shift of the membrane potential (Figs. 3–5). Fig. 3 shows that the effect of cooling, to depress the peak value of the delayed outward current, was increased when a conditioning hyperpolarization was used. (It is also seen that a conditioning hyperpolarization increases the fraction of the outward current system which is available.)

Fig. 4 also demonstrates an increase in the effect of cooling on the peak value of delayed outward current and on the peak of membrane conductance when a conditioning hyperpolarization is used. In this cell we did not observe the appearance of the fast outward current after a conditioning hyperpolarization. As can be seen in Fig. 4 the conditioning hyperpolarization significantly increases the slope of the current voltage relation of the peak value of delayed outward current at 20 °C.

The peak value of conductance $G_{k(max)}$ was calculated by the equation

$$G_{k(max)} = \frac{I_{max}}{E_m - E_k};$$

E_m, membrane potential; E_k, potassium equilibrium potential. E_k was taken as −70 mV (Magura, Krishtal & Valeyev, 1971).

The Q_{10} for $G_{k(max)}$ was approximately 1·5 without a conditioning hyperpolarization. Conditioning hyperpolarization increased the Q_{10} for $G_{k(max)}$ to 2.

The effect of cooling upon the delayed outward current is quite different from that demonstrated in Figs. 2 and 3 when a conditioning depolarization is used. As can be

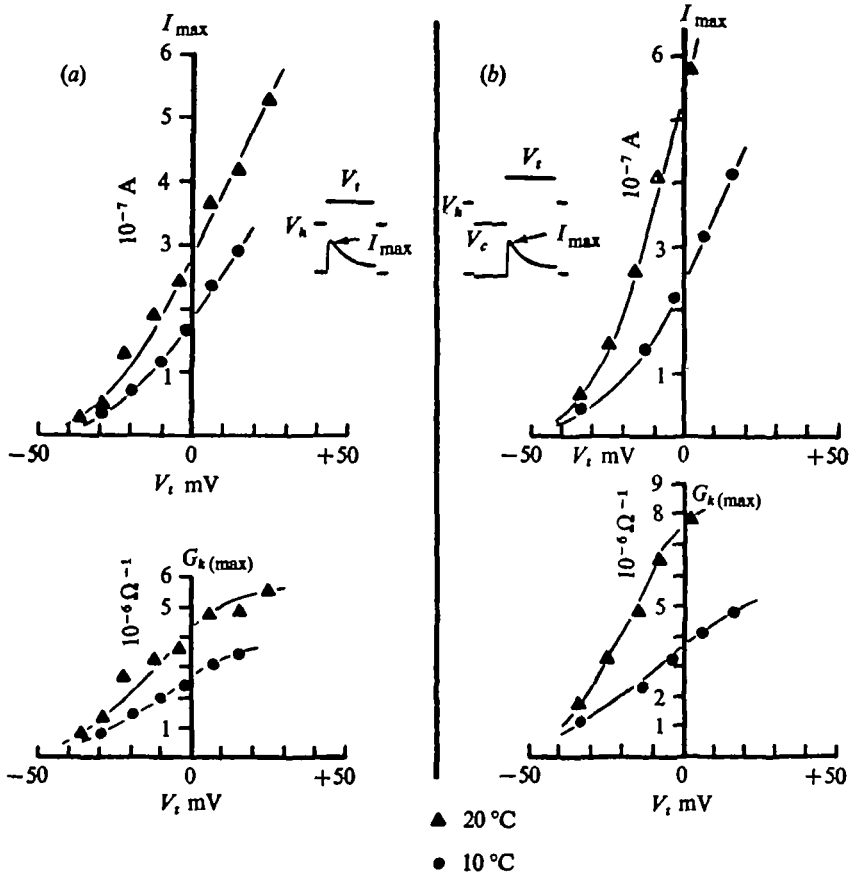

Fig. 4. The effect of cooling on a current–voltage relation for the peak value of delayed outward current (I_{max}) and on the peak value of conductance ($G_{k(max)}$): (*a*) without conditioning hyperpolarization; (*b*) with conditioning hyperpolarization lasting 1·5 sec. Holding potential (V_h) = −45 mV, conditioning potential (V_c) = −105 mV, test potential (V_t). The inset diagrams illustrate a method of measuring I_{max}. The conductance was calculated from the equation given in the text.

seen from Fig. 5(*a*), with a conditioning depolarization of −32 mV, cooling increased the peak value of the delayed outward current. (Note that its final value decreased.) In such conditions the inactivation of the delayed outward current was more distinct at 10 °C than at 20 °C. Cooling caused a decrease in the peak value of outward current in this neurone when a conditioning hyperpolarization was applied (−65 mV). Fig. 5(*b*) shows that the depressing effect of cooling on the peak value of the delayed outward current was most distinct with complete removal of the steady-state inactivation (at a conditioning potential more negative than −100 mV). When the conditioning potential was more positive than −50 mV the cooling increased the peak value of the outward current. Fig. 5(*b*) also demonstrates that the effect of cooling upon the final value of the delayed outward current was negligible.

The results shown in Fig. 5 can be explained if one supposes that cooling not only depresses the peak value of the delayed outward current but decreases its steady-state value of inactivation (see Effect of cooling on the fast transient outward current).

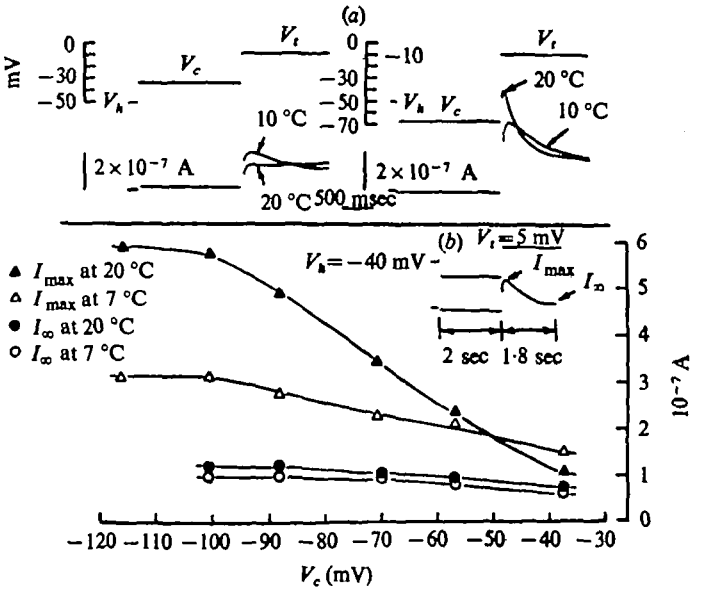

Fig. 5. (*a*) Dependence of the effect of cooling upon the conditioning level of membrane potential. Membrane potentials, above, and delayed outward currents, below. (*b*) Dependence of the peak (I_{max}) and final (I_∞) values of the delayed outward current upon the conditioning membrane potential at 20 and 7 °C. The inset illustrates the method of measuring I_{max} and I_∞.

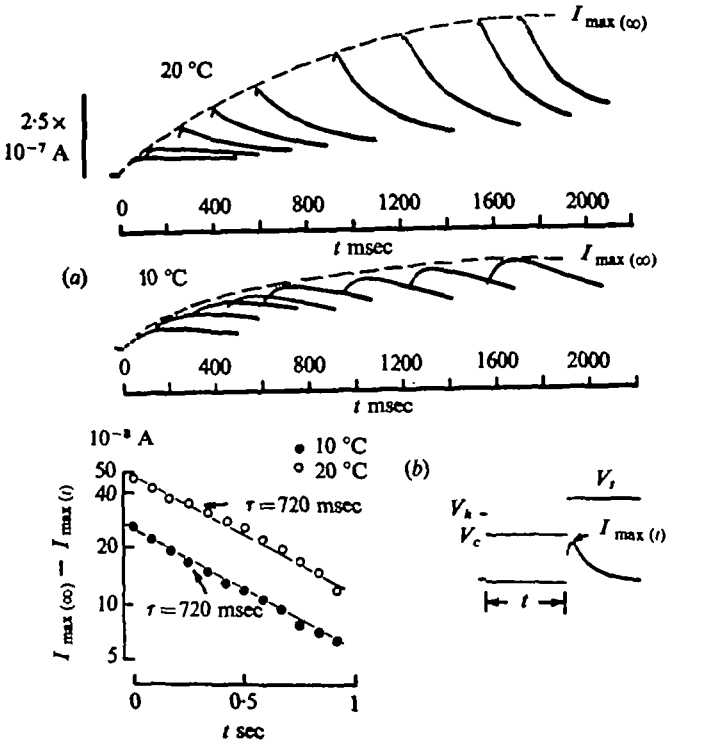

Fig. 6. (*a*) Effect of temperature on the time course of removal of delayed outward current inactivation. The inset illustrates the method for determination of this time course. V_h = −45 mV; V_c = −100 mV; V_t = −15 mV. (*b*) Analysis of a time course plotted semilogarithmically.

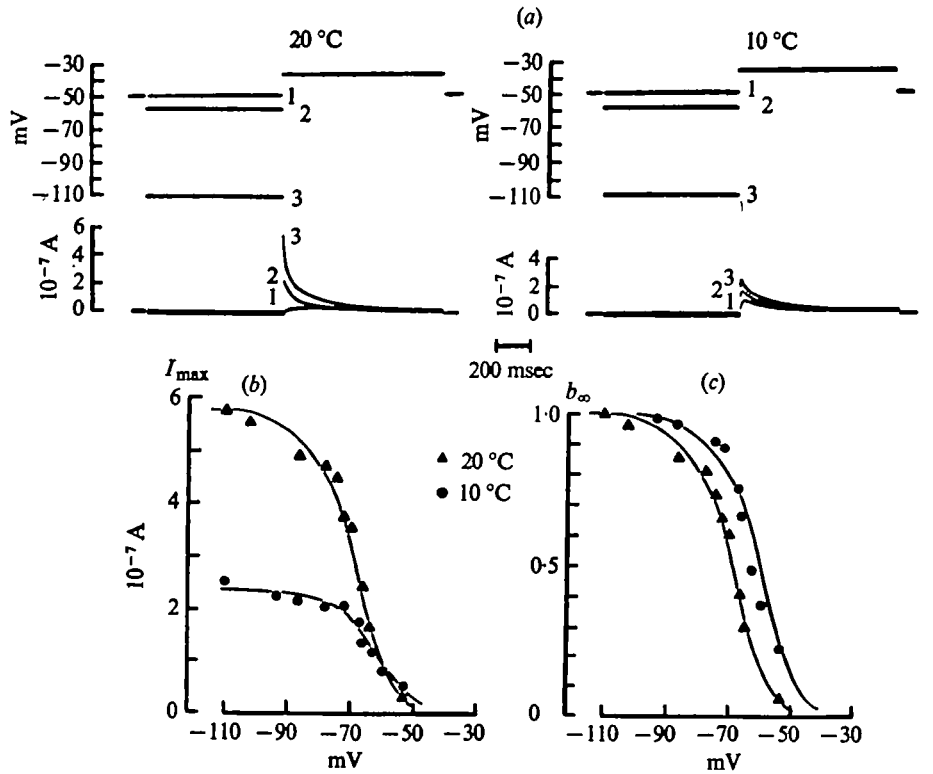

Fig. 7. (*a*) Comparison of the fast transient current traces from two step experiments at 20 and 10 °C. Membrane potential, above, and currents, below. (*b*) Effect of cooling on the steady-state relation between the peak of the fast outward current and membrane potential during the first step. (*c*) Steady-state relation for inactivation of fast outward current at 20 and 10 °C (from results presented in *b*).

The time course of the delayed outward current inactivation is exponential with a time constant (τ) of 300–800 msec (at 20 °C). The Q_{10} of τ^{-1} was about 2·5. These observations seem difficult to reconcile with the results presented in Fig. 6. This Figure demonstrates that the effect of cooling on the time course of removal of the delayed outward current inactivation (during conditioning hyperpolarization) was negligible. Somewhat similar results were obtained when fast outward current was investigated (Fig. 9).

Effects of cooling on the fast transient outward current

In some molluscan neurones a conditioning hyperpolarization causes the appearance of a fast transient outward current during the test depolarization (Hagiwara, Kusano & Saito, 1961; Connor & Stevens, 1971*b*; Neher, 1971*a*). Fig. 7(*a*, *b*) demonstrates the dependence of the peak value of the fast outward current upon the conditioning level of the membrane potential at 10 and 20 °C. The potential was changed from −50 to −110 mV. Test depolarization was constant (−35 mV). At such depolarization the activation of the delayed outward current system was negligible. During a test depolarization the inactivation of outward current was nearly complete. (Note the appearance of a transient outward current at 10 °C when the conditioning potential was −50 mV.)

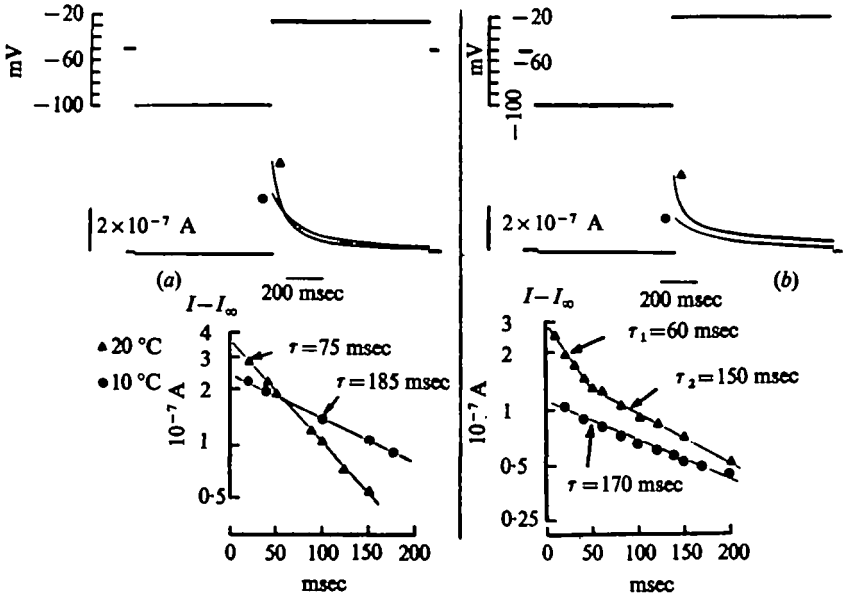

Fig. 8. Comparison of the fast transient current traces obtained from two neurones (*a*, *b*) at 20 and 10 °C. Two step potential, above, current traces, below. In the lower part of the Figure the decaying phase of transient fast outward current is plotted semilogarithmically. Note that recording conditions from the two neurones were identical.

It is clearly seen from Fig. 7(*b*) that the effect of cooling depends on the conditioning value of the membrane potential. The depressing effect of cooling was most distinct when the conditioning potential was −100 mV, when nearly complete removal of fast outward current inactivation occurred. When the conditioning potential was more positive than −65 mV, cooling slightly increased the peak value of the fast outward current. Somewhat similar effect of cooling on the delayed outward current was described above (Fig. 5).

These results were normalized and inactivation curves were obtained (Fig. 7*c*). They show that cooling causes the inactivation curve to shift in the direction of a more positive membrane potential. A similar effect has been observed when the external calcium concentration is increased (Neher, 1971*b*).

The inactivation curves in Fig. 7(*c*) may be calculated by an equation similar to that used by Hodgkin & Huxley (1952):

$$b_\infty = \frac{1}{1+\exp\left(\frac{V-V_h}{k}\right)},$$

where V = membrane potential, V_h = potential at which $b_\infty = 0{\cdot}5$, $k = 6$ mV. At 20 °C $V_h = -70$ mV; at 10 °C $V_h = -60$ mV.

One may assume that cooling causes a decrease in the negative surface potential in the vicinity of the potassium pathways and removes their inactivation. Simultaneously cooling depresses the potassium conductance. This second effect of cooling was dominant with a conditioning pulse more negative than −65 mV.

The upper part of Fig. 8(*a*, *b*) shows the records of membrane potential and fast

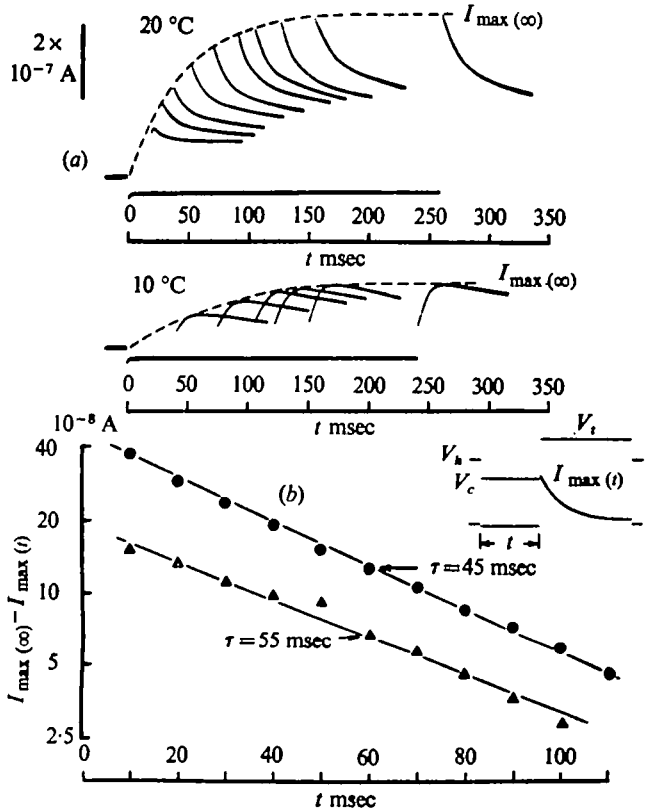

Fig. 9. (*a*) Effect of temperature on the time course of removal of fast outward current inactivation. The inset illustrates the method for determination of this time course. $V_h = -50$ mV; $V_c = -100$ mV; $V_t = -5$ mV. (*b*) Analysis of a time course on a semilogarithmic plot. 20 °C, filled circles; 10 °C, triangles.

outward current in two neurones at 20 °C and 10 °C. In Fig. 8(*a*) semilogarithmic plots of the decaying phase of these traces show that the time course of inactivation is exponential. The time constants (τ) at 20 and 10 °C were respectively 75 and 185 msec. The Q_{10} of the reciprocal time constant (τ^{-1}) was about 2·5. In Fig. 8(*b*) at 20 °C the time course of outward current inactivation was complex and was determined by two time constants. The time constant of inactivation at the beginning of the decay was 60 msec (τ_1). After 50 msec the time constant had increased to 150 msec (τ_2). At 10 °C the time course of inactivation was determined by only one time constant.

One may suggest that the time course of inactivation in Fig. 8(*b*) is related to the activation of two different fractions of potassium channels, where the time constants of inactivation are different (the fast potassium channels and delayed outward current channels). Such a suggestion is difficult to bring into accord with the observation that the time constant of the slow phase of outward current inactivation is often shorter than the time constant of delayed outward current inactivation (Fig. 8*b*). It is difficult to exclude the possibility of a slow phase of fast outward current inactivation in some neurones. Fozzard & Hiraoka (1973) recently described a somewhat similar time course of inactivation of chloride currents in cardiac Purkinje fibres.

Experiments with two pulses have indicated that fast outward current inactivation

is removed exponentially with a voltage-dependent time constant (Neher, 1971*a*). Fig. 9 demonstrates that a lowering of temperature from 20 to 10 °C only slightly prolongs the time constant for removal of fast outward current inactivation (at a conditioning potential of −100 mV). This is clearly seen in a semilogarithmic plot (Fig. 9*b*). The Q_{10} of the reciprocal time constant of inactivation removal was only 1·3. In this neurone the Q_{10} of developing fast outward current inactivation was about 2·5 (at a test potential of −30 mV). It seems reasonable to suppose that interpretation of these results requires a detailed investigation of the effect of cooling on the Hodgkin–Huxley rate coefficients (α and β) for outward current inactivation.

DISCUSSION

Upon the results of the above experiments it seems reasonable to separate the delayed outward current in molluscan neurones into two components: (i) a persistent component which is maintained during long-lasting depolarization; (ii) a transient component which inactivates relatively quickly during depolarization. Temperature changes have a more significant effect on the value of the transient component than on the value of the persistent one.

The effect of temperature on the peak value of the transient component of the delayed outward current is clearly dependent upon the membrane potential which precedes the test depolarization. Similar observation was made on the fast transient outward current. A negative shift of the preceding membrane potential (conditioning hyperpolarization) increases the effect of cooling to depress the peak of transient outward currents. It reaches a maximum with conditioning polarizations at which a complete removal of potassium inactivation is observed (about −100 mV). We suppose that cooling not only depresses the peak value of the transient potassium conductance but also decreases the negative surface potential of the membrane in the vicinity of potassium pathways. This potential is created by the negative fixed charges of the membrane (see Hille (1968) for review).

Decreasing the negative surface potential by cooling causes the inactivation curve for fast outward current to shift in the direction of more positive membrane potential. It also increases the fraction of transient potassium channels which are available. Such an effect of cooling is clearly dominant at conditioning potentials at which a significant fraction of the potassium channels are inactivated.

It seems reasonable to suppose that changes in surface potential by cooling may cause certain changes in the kinetic of potassium inactivation.

In these investigations we did not estimate the outward current density and conductance in Ω^{-1} cm^{-2}. Such estimation is difficult because of the complexity of the surface of the giant neurone soma. The invasion of the giant cells by strands and trabeculae of glial cells increases enormously the membrane surface in certain places, especially in the axon hillock region (Bullock, 1961). A calculation of the surface of the soma using its capacity (assuming a value of I μF/cm^2) shows that the surface of the soma is about 10 times as large as that of sphere having the same diameter (Magura, 1967; Magura, Grobova & Zamekhovsky, 1972). This value is in accord with the data obtained by light and electron microscopic examinations of sections of molluscan giant neurones (Mirolli & Talbot, 1972). If this is correct the density of potassium

current through the somatic membrane of molluscan neurones is, according to our calculation, much lower than in the giant squid axon. The limiting conductance for delayed outward current is approximately $8 \cdot 10^{-4}$ Ω^{-1}/cm^2 (20 °C). This value is roughly 1/40 of that in the giant squid axon.

We thank Professor P. G. Kostyuk for his kind support and valuable advice. We should like to thank Miss Elena Dolgaya for technical assistance.

REFERENCES

ALVING, B. O. (1969). Differences between pacemaker and nonpacemaker neurons of *Aplysia* on voltage-clamping. *J. gen. Physiol.* **54**, 512–31.

BULLOCK, T. H. (1961). On the anatomy of the giant neurons of the visceral ganglion of *Aplysia*. In *Nervous Inhibition* (ed. E. Florey), pp. 233–8. Oxford: Pergamon Press.

CHAMBERLAIN, S. G. & KERKUT, G. A. (1969). Voltage clamp analysis of the sodium and calcium inward currents in snail neurones. *Comp. Biochem. Physiol.* **28**, 781–801.

CONNOR, J. A. & STEVENS, C. F. (1971*a*). Inward and delayed outward membrane currents in isolated neural somata under voltage-clamp. *J. Physiol., Lond.* **213**, 1–19.

CONNOR, J. A. & STEVENS, C. F. (1971*b*). Voltage clamp studies of a transient outward membrane current in gastropod neural somata. *J. Physiol., Lond.* **213**, 21–30.

FOZZARD, H. A. & HIRAOKA, M. (1973). The positive dynamic current and its inactivation properties in cardiac Purkinje fibres. *J. Physiol., Lond.* **234**, 569–86.

GEDULDIG, D. & GRUENER, R. (1970). Voltage clamp of the *Aplysia* giant neurone: early sodium and calcium currents. *J. Physiol., Lond.* **211**, 217–44.

GOLA, M. M. & ROMEY, G. (1971). Responses anomales a des courants sousliminaires de certaines membranes somatiques (neurones geants d'*Helix pomatia*). *Pflügers Arch. ges. Physiol.* **327**, 105–31.

HAGIWARA, S., KUSANO, K. & SAITO, N. (1961). Membrane changes of *Onchidium* nerve cell in potassium-rich media. *J. Physiol., Lond.* **155**, 470–89.

HAGIWARA, S. & SAITO, N. (1959). Voltage current relations in nerve cell membranes of *Onchidium verruculatum*. *J. Physiol., Lond.* **148**, 161–70.

HILLE, B. (1968). Charges and potentials at the nerve surface. Divalent ions and pH. *J. gen. Physiol.* **51**, 221–36.

HILLE, B. (1970). Ionic channels in nerve membranes. In *Progress in Biophysics*, vol. 21 (ed. J. A. Butler and D. Noble), pp. 3–32. Oxford: Pergamon.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol., Lond.* **117**, 500–44.

LEICHT, R., MEVES, H. & WELLHONER, H. (1971). Slow changes of membrane permeability in giant neurones of *Helix pomatia*. *Pflügers Arch. ges. Physiol.* **323**, 63–79.

MAGURA, I. S. (1967). On quantitative evaluation of ionic currents through the membrane of giant neurons of *Planorbis corneus* during action potential generation. *Biofizika* **12**, 456–61. (In Russian.)

MAGURA, I. S., GROBOVA, E. V. & ZAMEKHOVSKY, I. Z. (1972). Electrical characteristics of giant mollusc neurons. *Neurophysiology* **4**, 651–8. (In Russian.)

MAGURA, I. S., KRISHTAL, O. A. & VALEYEV, A. G. (1971). Behaviour of delayed current under long duration voltage clamp in snail neurones. *Comp. Biochem. Physiol.* **40**A, 415–22.

MIROLLI, M. & TALBOTT, S. R. (1972). The geometrical factors determining the electrotonic properties of a molluscan neurone. *J. Physiol., Lond.* **227**, 19–34.

NAKAJIMA, S. (1966). Analysis of K inactivation and TEA action in the supramedullary cells of puffer. *J. gen. Physiol.* **49**, 629–40.

NAKAJIMA, S. & KUSANO, K. (1966). Behaviour of relayed current under voltage clamp in the supra-medullary neurons of puffer. *J. gen. Physiol.* **49**, 613–28.

NAKAJIMA, S. & ONODERA, K. (1969). Membrane properties of the stretch receptor neurones of crayfish with particular reference to mechanisms of sensory adaptations. *J. Physiol., Lond.* **200**, 161–87.

NEHER, E. (1971*a*). Two fast transient current components during voltage clamp on snail neurons. *J. gen. Physiol.* **58**, 36–53.

NEHER, E. (1971*b*). A new current component appropriate for the study of electrically-induced permeability changes in nerve membrane. *First European Biophysics Congress*, Baden, pp. 301–4.

NEHER, E. & LUX, H. D. (1971). Properties of somatic membrane patches of snail neurones under voltage clamp. *Pflügers Arch. ges. Physiol.* **322**, 35–8.

NEHER, E. & LUX, H. D. (1972). Differential action of TEA^+ on two K^+ current components of a molluscan neurone. *Pflügers Arch. ges. Physiol.* **336**, 87–100.

NEHER, E. & LUX, H. D. (1973). Rapid changes of potassium concentration at the outer surface of exposed single neurons during membrane current flow. *J. gen. Physiol.* **61**, 385–99.