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TEMPERATURE ACCLIMATION: INFLUENCE ON TRANSIENT OUTWARD AND INWARD CURRENTS IN AN IDENTIFIED NEURONE OF *HELIX POMATIA*

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SUMMARY

1. To gain insight into the mechanisms underlying compensatory changes in excitability induced by thermal acclimation we examined the effects of short-term and long-term temperature transition on a transient outward potassium current (A-current) and the transient inward current in an identified neurone (Br) of the land snail *Helix pomatia*.

2. The A-current in neurones from snails acclimated to 20 °C was suppressed by cooling to 3 °C, with a Q_{10} of 4.5 between 10 °C and 20 °C. This is consistent with suppression of the ability of these neurones to fire repetitively at low temperatures.

3. Neurones acclimated to low temperature (5 °C) partially recovered their ability to fire repetitively, but exhibited no A-current, at this temperature.

4. Neither the equilibrium potential for the A-current (E_A), the steady-state inactivation parameters of the A-current (B_O), nor the ratio of the time constants of A-current activation and inactivation (τ_A/τ_B) were affected by cooling, leading to the conclusion that the reduction of the A-current by cooling was due to a decrease in the A-current activation parameters ($G_A A_\infty$).

5. Cooling reduced the maximum peak inward current, with a Q_{10} of 2, in neurones from warm-acclimated animals. After acclimation to 5 °C, maximum peak inward current partially recovered.

6. Cooling neurones from warm-acclimated animals slowed the time course of the recovery of inward current from inactivation. Acclimation to the cold caused a partial, compensatory shortening of the inactivation removal process.

INTRODUCTION

The physiological and ecological importance of adaptation to different temperatures is clear and some effects of thermal acclimation on the function of the nervous system have been described (Lagerspetz, 1974; Prosser & Nelson, 1981).

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However, the mechanisms by which temperature and thermal acclimation modify the biophysical properties of excitable membranes are not well understood.

We have previously described (Zečević & Levitan, 1978, 1980) the effects of temperature and thermal acclimation on some physiological properties of an identified nerve cell of the land snail, *Helix aspersa*. We found that spontaneous bioelectrical activity and the ability of the cell to sustain repetitive discharge upon stimulation were blocked in neurones from warm-acclimated (20 °C) snails when they were abruptly cooled to about 5 °C. After acclimation to 5 °C for 2 weeks a 'perfect compensation' (Precht, 1958) in the frequency of spontaneous action potentials was observed, and the excitability of the neurone substantially recovered. Clear compensatory effects of cold acclimation on spontaneous bioelectrical activity of this same identified neurone have also been reported by Langley (1979).

To further examine the mechanisms underlying these changes in excitability we have studied the effects of temperature and thermal acclimation on the voltage-dependent ionic conductances of the membrane. A transient, outward, temperature-sensitive potassium current (A-current) has been associated with a neurone's ability to fire repetitively (Connor & Stevens, 1971*c*; Partridge & Connor, 1978), and the transient inward current influences a neurone's response to depolarization (Hodgkin & Huxley, 1952*b*). We examined these currents in an identified neurone from two groups of land snails, one acclimated to 5 °C and the other to 25 °C.

We found that complete recovery of spontaneous repetitive firing after thermal acclimation was not accompanied by recovery of the transient potassium conductance. The amplitude of the peak inward current, which was reduced by abrupt cooling, and the effective duration of the inactivation removal process, which was prolonged by abrupt cooling, both partially recovered after cold acclimation.

MATERIALS AND METHODS

Helix pomatia were collected in the Botanical Garden in Belgrade and acclimated to 5 or 25 °C for at least 2 weeks, as previously described (Zečević & Levitan, 1980). All experiments were conducted on the neurones from dormant animals. After removal from an animal the complex of ganglia was transferred to a small chamber (0·5 ml) with a temperature-controlled physiological solution. The dissection procedure and temperature control have been described previously (Zečević & Levitan, 1980). The composition of normal physiological solution was (in mmol l^{-1}) KCl, 4; NaCl, 80; CaCl, 10; $MgCl_2$, 5; and Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl), 5; buffered to pH 7·8. An identifiable giant neurone (Br) in the right parietal ganglion was used in all experiments. This cell is analogous to cell A in *Helix aspersa* (Kerkut & Meech, 1967).

Conventional glass microelectrodes were used to record membrane potential and to pass current. Both recording and current passing electrodes were filled with 3 mol l^{-1} KCl and had resistances in the range of 2–5 MΩ. The solution in the chamber was connected *via* a Ag/AgCl electrode to a virtual ground circuit, effectively grounding the bath. Membrane potential was recorded differentially between the intracellular microelectrode and an extracellular Ag/AgCl electrode which was maintained at room

temperature and connected to the chamber by an agar bridge containing normal snail solution.

For recording membrane potential and currents under voltage-clamp conditions a second current-passing microelectrode was inserted into the nerve cell and connected to a voltage-clamp device (Model 8500, Dagan, Minneapolis, Minnesota). To reduce capacitive coupling between the current and voltage electrodes a grounded aluminium shield was manipulated between the two microelectrodes, and an aluminium shield 'driven' by the unity gain output of the preamplifier was placed around the voltage-recording electrode. The solution in the bath was lowered to within 0·5 mm of the cell surface. Voltages and currents were displayed on an oscilloscope (Tektronix 5113, Beaverton, Oregon) and photographed.

To minimize the contribution of the currents from the axon which could not be space-clamped with electrodes in the cell body, the neurone was either ligated with a silk thread near the soma-axon junction, or undercut as described by Connor (1977).

The tail of the capacitive current and the leakage current (corresponding to a resistance of 2–10 MΩ) significantly interfered with inward ionic current at command potentials more positive than 0 mV. The currents were corrected by subtracting the current produced by a hyperpolarizing pulse scaled to the appropriate voltage. This procedure assumes linear leakage over the range of membrane potentials of interest and will lead to a slight underestimate of the peak inward current if the leakage current rectifies to some extent. Since A-currents are relatively slow compared to capacitive currents, only the leakage current was subtracted in the calculation of A-currents.

The resistance in series with the membrane was determined from the initial jump in the voltage transient in response to a brief current step (Connor & Stevens, 1971*a*). Since the series resistance was less than 10 KΩ, the error in the recorded membrane potential due to this resistance was neglected.

RESULTS

At 20 °C, Br neurones from warm-acclimated animals typically responded to a transmembrane depolarization with a repetitive discharge (Fig. 1A) whose frequency was dependent upon stimulus strength (Fig. 1D). When cooled to 5 °C, the same neurones responded to depolarization with a single action potential, and the multiple discharges could not be provoked by further depolarization (Fig. 1B). Thus abrupt cooling completely eliminated the ability of the warm-acclimated neurones to sustain repetitive discharge.

Thermal acclimation of animals to 5 °C induced partial recovery of the neurones' ability to fire repetitively at low temperature. Depolarization of these cells at low temperatures induced repetitive firing (Fig. 1C), and the frequency of firing was dependent on the stimulus strength (Fig. 1D). These results were confirmed in more than 20 neurones from warm- and cold-acclimated animals.

Since the adaptive phenomena described above must be based on changes in ionic membrane conductances, we examined the thermal characteristics of the transient inward and outward currents. Both the inward current (Hodgkin & Huxley, 1952*b*) and transient outward current (A-current, Connor & Stevens, 1971*c*) have been shown to influence the neuronal response to depolarization.

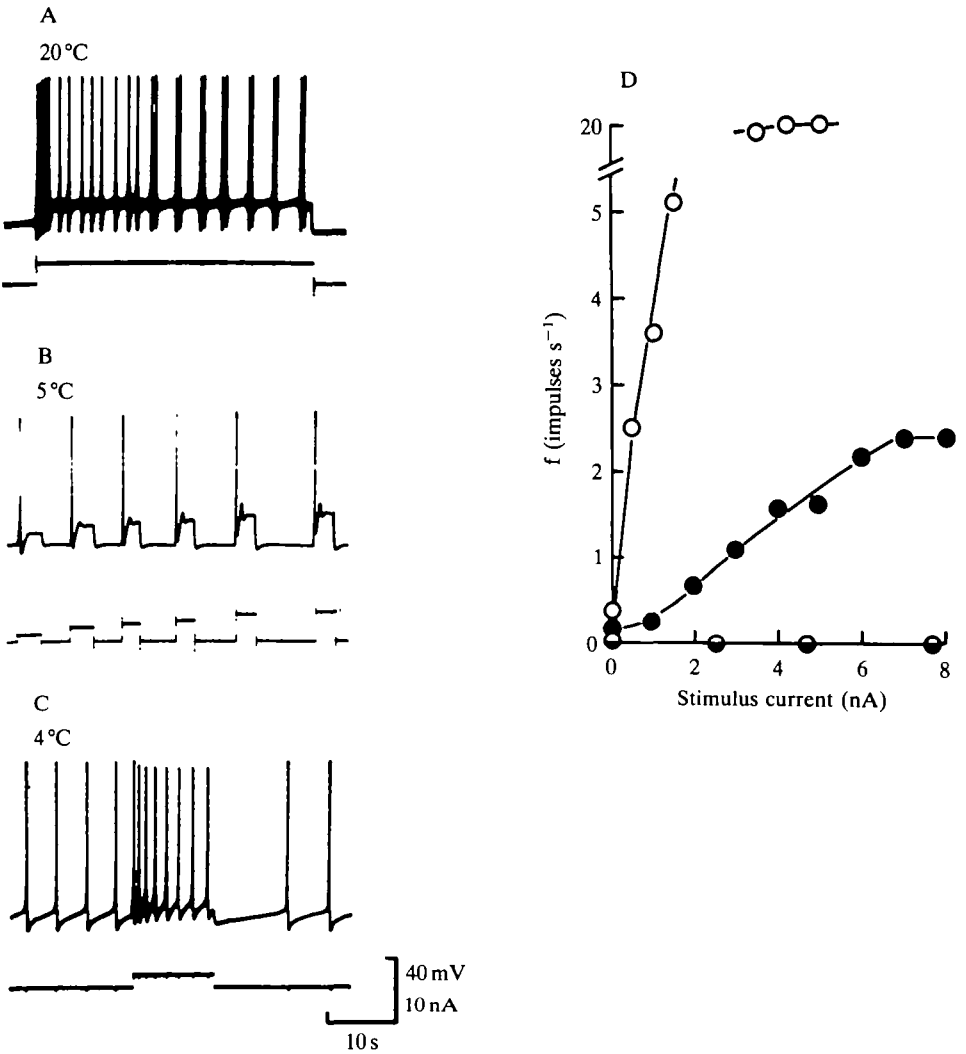


Fig. 1. Response of Br neurone to depolarization under different thermal condtions. (A) Warm-acclimated cell depolarized at 20 °C from the membrane potential of −48 mV in between two spontaneously occurring bursts of action potentials. (B) Warm-acclimated cell depolarized at 5 °C from the membrane potential of −50 mV. (C) Cold-acclimated cell depolarized at 4 °C from the membrane potential of −51 mV. In A, B and C the upper trace is membrane potential, and the lower trace is transmembrane stimulus current. (D) Initial frequency of firing (f) (calculated from the first interspike interval) as a function of stimulus current. Neurone from warm-acclimated animal at 20 °C (open circles) and at 5 °C (half-filled circles). Neurone from cold-acclimated animal tested at 4 °C (filled circles).

A-current

When a Br neurone from a warm-acclimated animal was depolarized from a holding potential of between −90 and −100 mV, towards the resting membrane potential (about −45 mV), a transient outward current was observed at 20 °C (Fig. 2). During

depolarization the current rose to a peak which increased with more positive command potentials and then decayed exponentially. This current is apparently analogous to the A-current described by Connor & Stevens (1971*b*) in other molluscan neurones.

Effects of cooling

After abrupt cooling of this same neurone to 5 °C, the A-current could not be initiated (Fig. 2). The temperature dependence of the A-current was characterized using the temperature coefficient, Q_{10}:

$$Q_{10} = \left(\frac{I_2}{I_1}\right)^{10/(T_2-T_1)},$$

where I_1 is the value of the peak outward current at temperature T_1, and I_2 is the value of the peak outward current at temperature T_2, with $T_2 > T_1$. A-current magnitude, determined over the temperature range from 10–20 °C, decreased with temperature with a Q_{10} of $4{\cdot}5 \pm 0{\cdot}2$ (mean ± S.E.M.) in six neurones from warm-acclimated animals. Below 10 °C, the currents were smaller than to be expected from the above value of Q_{10}, indicating that temperature dependence was larger at lower temperatures. However, the A-currents intitiated below 10 °C were small and close in magnitude to the possible errors introduced by leakage subtraction (Fig. 3). For this reason, determination of Q_{10} for temperatures below 10 °C was not attempted.

The blockade of the repetitive firing response to depolarization is thus accompanied by the elimination of the A-current when neurones are abruptly cooled. This is in accordance with the previously established correlation between repetitive discharge and transient potassium conductance in other molluscan neurones (Connor & Stevens, 1971*c*).

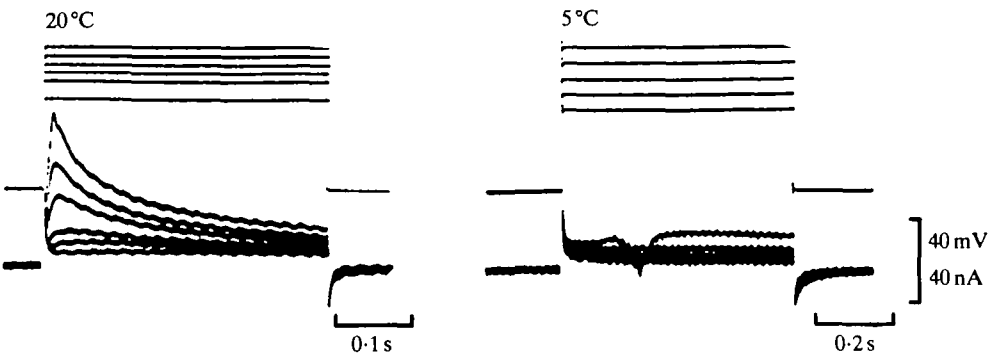


Fig. 2. Membrane currents associated with depolarizing voltage steps from a constant holding potential of −100 mV to various membrane potentials at 20 and 5 °C in a Br neurone from a warm-acclimated animal. Transient outward current is absent at low temperature. In this and all subsequent figures, upper traces show voltage steps; lower traces show membrane currents; upward deflections represent positive-going voltage changes and outward currents.

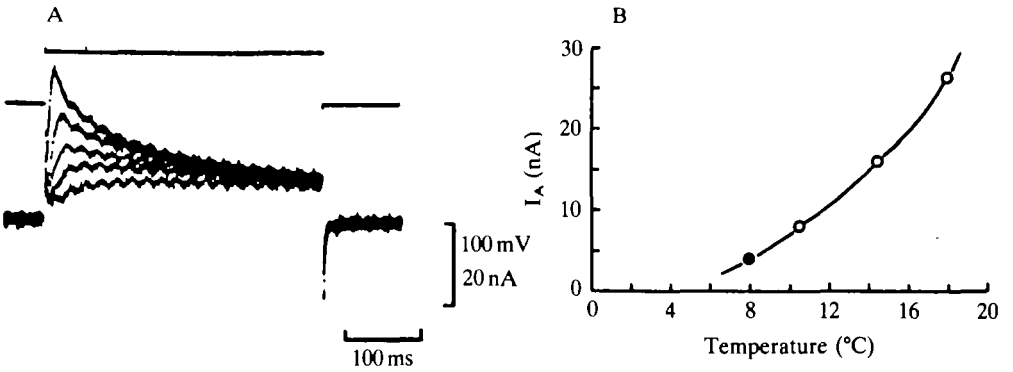


Fig. 3. (A) Transient outward currents (lower traces) initiated by the same depolarizing voltage step from the holding potential of −95 mV at different temperatures. (B) Peak A-current magnitude as a function of temperature as determined in an experiment on the warm-acclimated neurone shown in Fig. 3A. Filled circle, representing I_A magnitude at 8 °C, is not reliable for reasons explained in the text.

The A-current has been formally described (Connor & Stevens, 1971*a,b,c*) by the following equation:

$$I_A = K[1 - \exp(-t/\tau_A)]^4 \exp(-t/\tau_B), \tag{1}$$

where τ_A and τ_B are the time constants of activation and inactivation and K is a scale factor described by:

$$K = G_A A_\infty B_O (V_m - E_A), \tag{2}$$

where $G_A A_\infty$ represents an activation term, B_O is steady-state inactivation parameter, V_m is the membrane potential, and E_A is the equilibrium potential for the A-current. Equation 1 may be rearranged to give the form:

$$\ln\left[1 - \left(\frac{I_A}{K\exp(-t/\tau_B)}\right)^{1/4}\right] = \frac{t}{\tau_A}, \tag{3}$$

which can be used for experimental determination of the time constants (see below).

To investigate the mechanisms by which temperature influences the peak amplitude of the A-current, we analysed the effects of fast cooling on some of the parameters that determine the height of the A-current: the reversal potential of A-current, E_A; the ratio of the time constants of A-current activation and inactivation, τ_A/τ_B; and the steady-state inactivation parameter, B_O (Partridge & Connor, 1978). In these series of experiments, neurones from warm-acclimated animals were cooled to 10–12 °C, a temperature range in which A-currents of reasonable amplitude could still be evoked (Fig. 3).

The reversal potential for the A-current, E_A, was determined from the potential at

which the direction of the tail currents reversed (cf. Adams & Gage, 1979*b*). The value of this parameter did not change with abrupt temperature transition from 20 °C to 11 °C (Fig. 4).

The steady-state inactivation parameter, B_O, was determined from the ratio of the peak amplitude of I_A evoked by depolarization from a given holding potential to the I_A generated by depolarizing from a very hyperpolarized level at which inactivation is minimal or absent. The holding potential was established by setting the membrane potential to different values for at least 3 s before superimposing a depolarizing pulse. The variation of steady-state inactivation parameter with membrane potential was unaffected by fast cooling from 20 °C to 12 °C (Fig. 5).

To evaluate the effect of temperature on the ratio τ_A/τ_B (see equation 1), time constants for activation and inactivation were determined at different temperatures.

The time constant for inactivation of I_A (τ_B) was determined directly from the semilogarithmic plot of the exponentially decaying phase of the current (Fig. 6A). The inactivation kinetics were strongly dependent on temperature, and in four different neurones τ_B was found to be $93{\cdot}2 \pm 1{\cdot}5$ ms at $20 \pm 0{\cdot}5$ °C, and $241 \pm 9{\cdot}3$ ms at $12 \pm 0{\cdot}2$ °C (± S.E.M.). The increase in τ_B with cooling corresponds to a thermal coefficient, Q_{10}^{-1}, of 3·3.

τ_A was estimated by plotting

$$\ln\left[1 - \left(\frac{I_A}{K\exp(-t/\tau_B)}\right)^{1/4}\right] \tag{4}$$

as a function of time (see equation 3). The scale factor, K, was determined from equation 1, which reduces to

$$I_A = K\exp(-t/\tau_B)$$

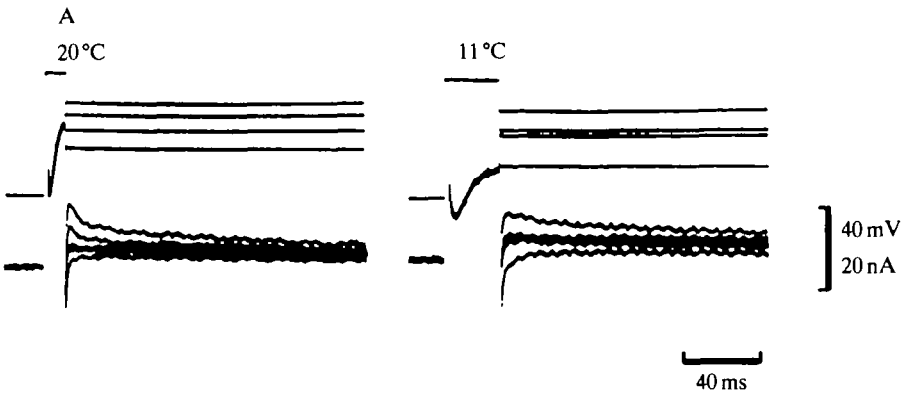
for $t >> \tau_A$. Since τ_B is known from the decaying phase of the A-current, K can be estimated and its value is obviously dependent on the potential to which the membrane is stepped.

That the data on Fig. 6B fall on a straight line supports the use of the power 4 in equation 1 (Connor & Stevens, 1971*b*) and the slope of the line ($-1/\tau_A$) leads to an estimate of τ_A as a function of temperature.

The time constant for activation, τ_A, estimated for four different neurones, was $2{\cdot}9 \pm 0{\cdot}4$ ms at $20 \pm 0{\cdot}5$ °C, and $7{\cdot}7 \pm 0{\cdot}8$ ms at $12 \pm 0{\cdot}2$ °C (± S.E.M.). The difference in τ_A at two different temperatures corresponds to $Q_{10}^{-1} = 3{\cdot}4$.

These results show that τ_A and τ_B varied similarly with temperature and that the ratio τ_A/τ_B, which was 0·029 at 20 °C and 0·030 at 12 °C, was essentially independent of temperature.

Since neither the reversal potential of the A-current, E_A, the steady-state parameter, B_O, nor the ratio of the time constants for A-current activation and inactivation, τ_A/τ_B, changed with abrupt cooling, it was concluded that the reduction in A-current in the Br neurone was due to the temperature sensitivity of the activation parameter, $G_A A_\infty$.



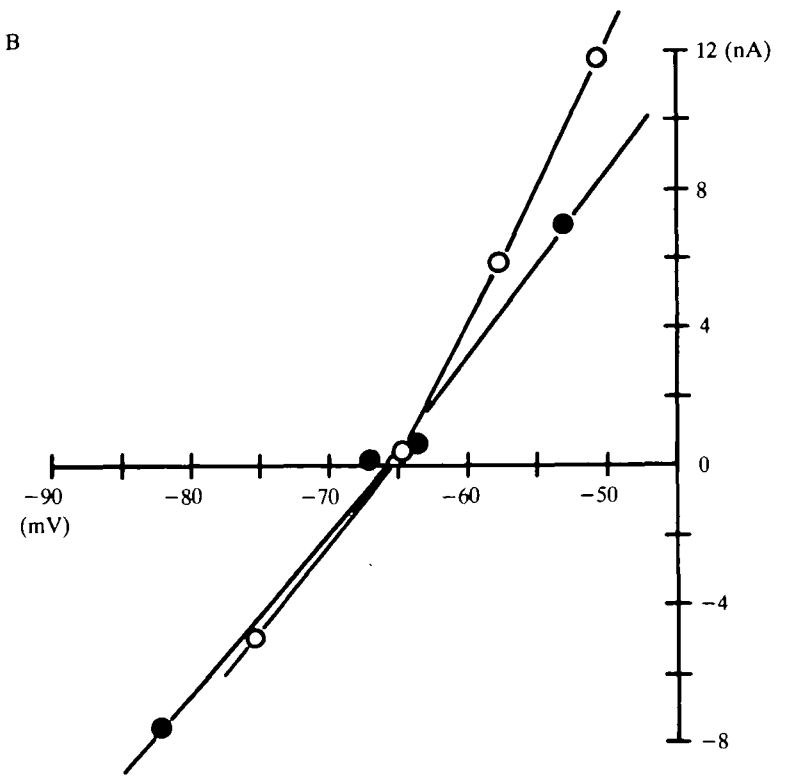


Fig. 4. (A) Effect of membrane potential on I_A tail currents elicited by repolarizing the membrane to different levels at the peak of transient outward current at 20 and 11 °C. Holding potential, −100 mV. (B) Tail current amplitude as a function of membrane potential. Reversal potential determined from the graph (−66 mV) did not change with temperature.

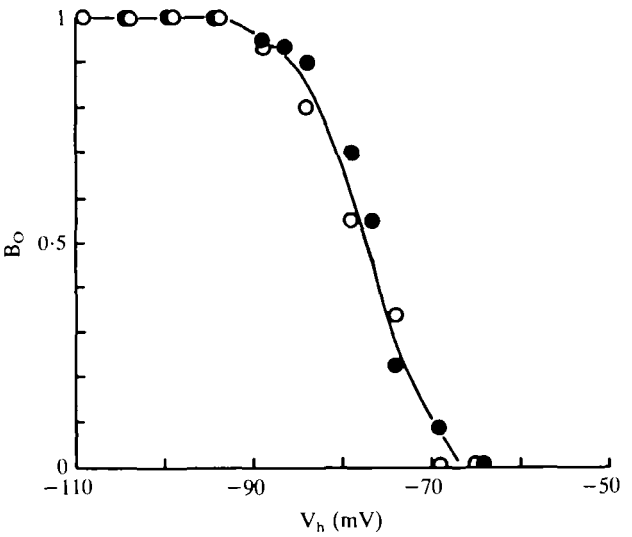


Fig. 5. Steady-state inactivation parameter (B_O) of I_A as a function of holding potential (V_h) in a Br neurone from a warm-acclimated animal, at 12 °C (open circles) and 20 °C (filled circles).

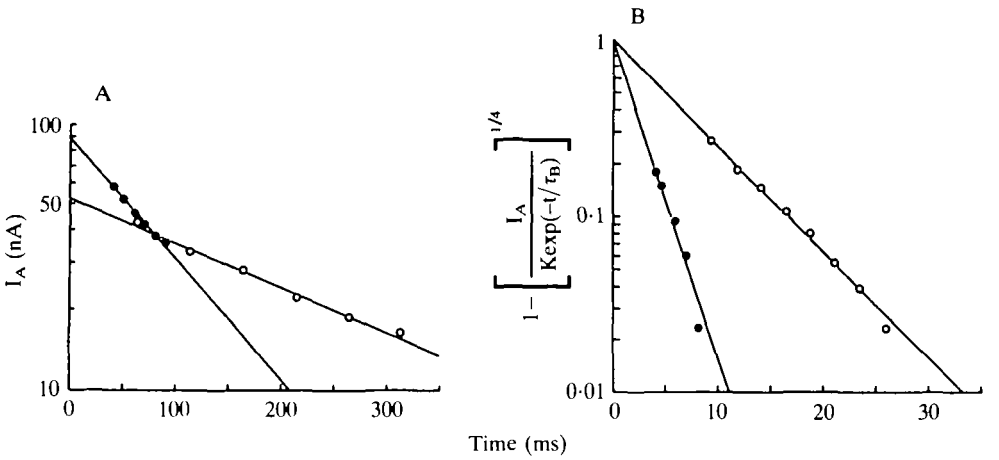


Fig. 6. The time constant of inactivation of I_A (τ_B) determined in a Br neurone from a warm-acclimated snail was 94·6 ms at 19·5 °C (filled circles) and 258 ms at 12 °C (open circles). (B) The time constant of activation of I_A (τ_B), in the same neurone as in A, was 2·6 ms at 19·5 °C (filled circles) and 7·1 ms at 12 °C (open circles).

Effects of acclimation to cold

Neurones from cold-acclimated animals were spontaneously active at 5 °C (Zečević & Levitan, 1980) and responded to depolarization with repetitive discharge. The recovery of repetitive firing with acclimation was however not accompanied by recovery of the transient potassium conductance characteristics, since no A-currents could be recorded at this low temperature (Fig. 7).

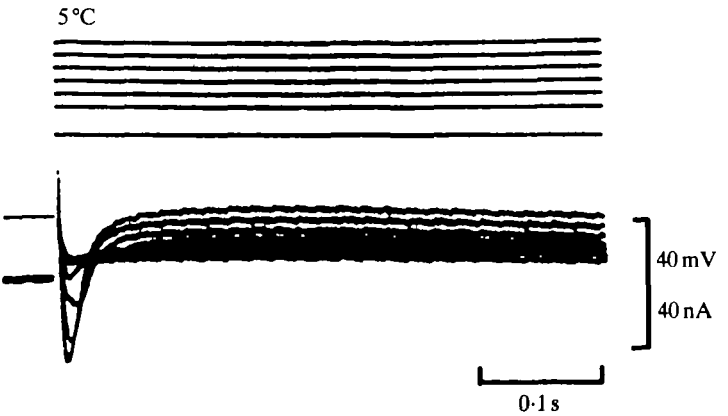


Fig. 7. Membrane currents at 5 °C associated with depolarizing voltage steps from a fixed holding potential of −90 mV to various membrane potential levels in a Br neurone from a cold-acclimated snail. Transient outward currents are absent.

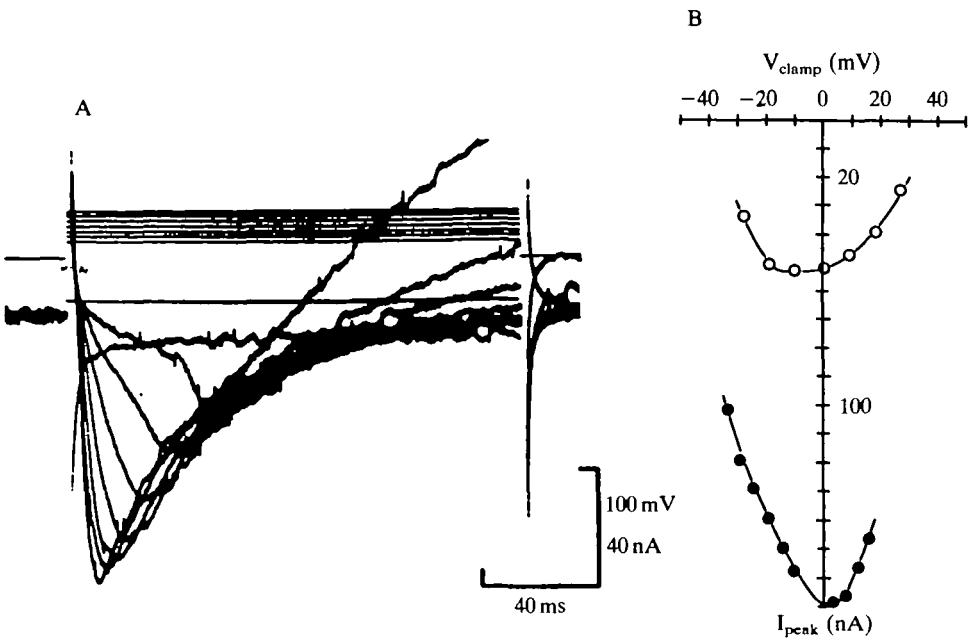


Fig. 8. Membrane currents associated with depolarizing voltage steps from a holding potential of −50 mV to various clamping potentials. The membrane current evoked by one hyperpolarizing voltage step, scaled to the appropriate voltage, was used to determine the capacitive and leakage currents. (B) Peak inward current (I_{peak}) recorded in the Br neurone from a warm-acclimated animal at 22·5 °C (filled circles) and 3 °C (open circles) plotted as a function of potential to which membrane was clamped (V_{clamp}). Holding potential, −50 mV.

Inward current

To determine whether transient inward currents play an important role in the recovery of repetitive firing with acclimation, variations in the characteristics of this current were examined as a function of temperature and thermal acclimation. No attempt was made to separate the inward current into its Na and Ca components.

The effects of temperature and thermal acclimation on inward currents were determined by monitoring changes in (1) the amplitude of maximum peak inward current and (2) the time course of the recovery of inward current from inactivation; two parameters that could be measured with reasonable accuracy without pharmacological separation of inward and outward currents.

The characteristics of the inward current in the Br neurone (Fig. 8) were similar to those reported previously for the squid giant axon (Hodgkin, Huxley & Katz, 1952) and for *Helix* neurones (Standen, 1975; Meech, 1979).

Effects of cooling

The effect of abrupt cooling on the inward current amplitude was characterized by calculating the Q_{10} for the maximum peak inward current, determined from the I-V relationship for the peak inward current (Fig. 8B). Abrupt cooling of warm-acclimated neurones from 23 °C to 3 °C reduced maximum peak inward current with a Q_{10} that varied from 1·9 to 2·1 in five neurones. The dependence of Q_{10} for inward current on temperature has not been examined.

Effects of acclimation to cold

To explore the effects of temperature acclimation on maximum peak inward current, it was necessary to compare results obtained from warm- and cold-adapted neurones. Since these neurones differed in diameter and thus in their effective membrane area, the inward current was expressed per unit surface area of membrane. The effective surface area of each neurone was estimated from measurements of effective membrane capacitance, C_m, where it was assumed that the specific membrane capacitance of Br neurones was not significantly different from that found in other systems, approximately $1\,\mu F\,cm^{-1}$ (Cole, 1949). The effective capacitance of the neurone was calculated from the effective membrane resistance, R_m, and the time constant, τ, of the exponential change in membrane potential to a hyperpolarizing current pulse, where $C_m = \tau/R_m$.

The surface area of Br neurones, estimated from C_m, ranged from 0·007 to 0·023 cm^2.

The diameter of each nerve cell was also determined visually, through the dissection microscope, and used to estimate the surface area of the equivalent smooth sphere. The ratio between the surface area of the neurone estimated from C_m and the area of the smooth sphere of equivalent diameter reflects the degree of membrane infolding (Marmor, 1971; Eaton, Russell & Brown, 1975). The degree of membrane infolding in neurones from cold-acclimated animals was significantly greater than that from warm-acclimated animals (Table 1), suggesting a higher surface to volume ratio in neurones acclimated to cold. The difference in infolding does not, however,

Table 1. *Cell radius, membrane surface area and the degree of membrane infolding determined at 3 °C in Br neurones from warm-acclimated and cold-acclimated animals*

	Warm-acclimated, $N = 11$	Cold-acclimated, $N = 15$
Cell radius, r (μm)	112 ± 4	118 ± 4
Membrane surface area, Ar ($cm^2 \times 10^{-3}$) estimated from r	1·6 ± 0·3	1·8 ± 0·1
Membrane surface area, A_{C_m} ($cm^2 \times 10^{-3}$) estimated from C_m	14 ± 0·8	20 ± 1
Infolding factor, F, Ar/A_{C_m}	8·8 ± 0·6*	11·1 ± 0·1*

N, number of cells examined; C_m, membrane capacitance; results are mean ± S.E.M.
*Significantly different, $P < 0·02$ (t-test).

influence the calculation of inward current density since membrane capacitance was used to estimate membrane surface area. Neither the effective membrane capacitance nor the cell radius changed within 3 h of an abrupt temperature transition.

Peak inward current amplitude is also a function of holding potential because this potential determines the value of steady-state inactivation (h_o). The steady-state inactivation parameter for inward current was determined as a function of membrane potential by recording inward currents associated with depolarizing voltage steps

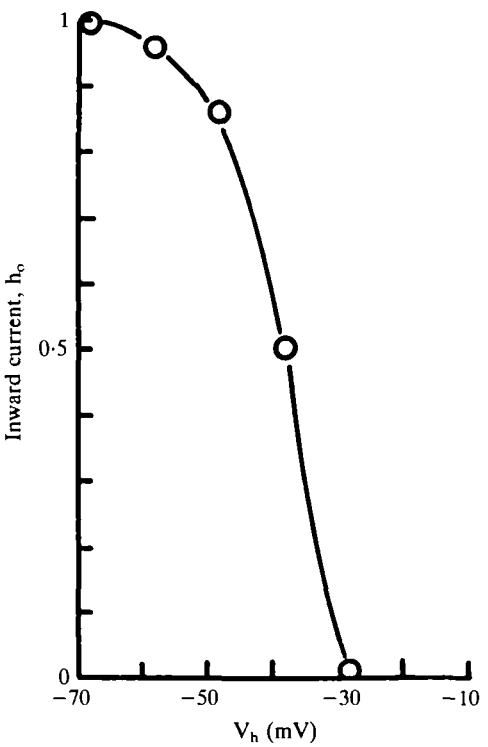


Fig. 9. The steady-state inactivation parameter for inward current (h_o) as a function of holding potential (V_h).

from various holding potentials to a fixed clamping potential (cf. Adams & Gage, 1979*a*). Steady-state inactivation as a function of membrane potential was determined at 3 °C and 24 °C for three neurones from warm-acclimated animals and two neurones from cold-acclimated snails. It was found that the relationship between h_o and membrane potential was essentially unaffected by either abrupt temperature transitions or by thermal acclimation. To minimize differences in the peak inward current due to small variations in the holding potential (which was always made equal to resting membrane potential), all of the results were corrected to the condition h = 1 on the basis of a typical steady-state inactivation curve (Fig. 9).

The maximum peak inward current density at 3 °C, corrected to the condition h = 1, was 5·1 ± 0·4 (S.E.M., $N = 11$) for neurones from warm-acclimated snails, significantly different ($P < 0{\cdot}05$, *t*-test) from the value of 7·3 ± 1·0 (S.E.M., $N = 5$) for cold-acclimated snails (Fig. 10). Thus cold acclimation induces a partial recovery of the peak inward current from the effect of abrupt cooling. The effect of such a change in inward current upon repetitive firing is difficult to predict in the absence of enough data for the complete simulation of neuronal excitability, but we recently reported that voltage threshold for evoking action potentials in the Br neurone undergoes complete recovery during temperature acclimation (Zečević & Pašić, 1983).

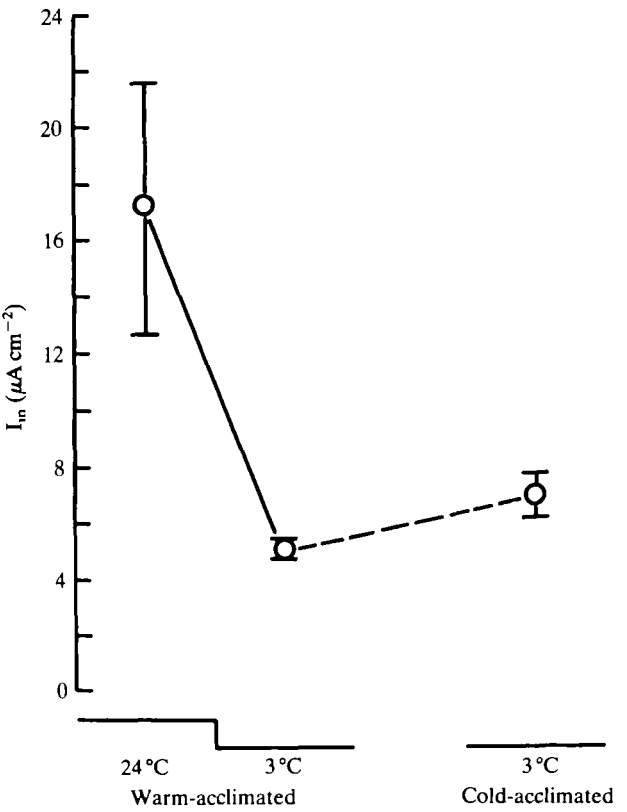


Fig. 10. The effect of temperature (solid line) and cold acclimation (dashed line) on maximum peak inward current density (I_{in}). Means ± S.E.M.

Recovery of inward current from inactivation

To determine whether the difference in the ability of neurones from warm- and cold-acclimated animals to fire repetitively at low temperature (Fig. 1) might be partially due to long-term thermal effects on the time course of inactivation, the rate of recovery of inward current from inactivation was examined under different thermal conditions.

Inward currents generated in response to pairs of constant-amplitude depolarizing pulses were recorded at different temperatures as a function of the interval between pulses (Hodgkin & Huxley, 1952*a*; Adams & Gage, 1979*b*) (Fig. 11). The time course of recovery of inward current fom inactivation was indicated by the ratio of the amplitude of the second to the first peak inward current, I_2/I_1, as a function of the interval between them.

Effects of cooling

Abrupt cooling of neurones from warm-acclimated animals caused an increase in the time needed for inward current to recover from inactivation (Fig. 12). Since the mean time to recover 50 % of peak inward current was 70 ms at 24 °C and 359 ms at 3 °C in neurones from warm-acclimated snails, the $Q_{\bar{10}}^{1}$ of the process was 2.

Effects of acclimation to cold

The recovery of inward current from inactivation at 3 °C was generally made quicker by cold acclimation (Fig. 13). Time needed to recover 20–50 %, and 80 %, of the peak inward current was significantly affected ($P < 0{\cdot}05$).

The shortening of the time to recover from inactivation which occurs during acclimation to the cold is of an adaptive nature since the effect is opposite to that induced by abrupt cooling (Fig. 14).

DISCUSSION

We have examined some properties of an identified snail neurone to determine

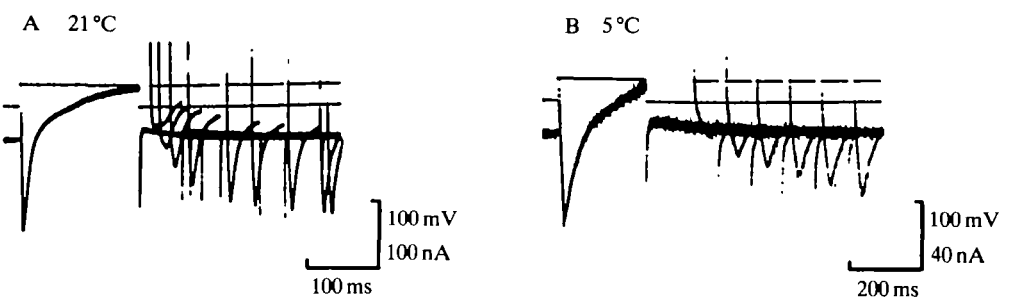


Fig. 11. The recovery of inward current from inactivation determined by double pulse technique in a Br neurone from a warm-acclimated animal. (A) Inward currents at 21 °C in response to nine pairs of depolarizing pulses. Both pulses are of the same amplitude but the second pulse occurred at various times after the first. (B) Inward currents at 5 °C in response to six superimposed pairs of depolarizing pulses of the same amplitude at varying intervals. In both A and B the holding potential was −45 mV and the inward current was evoked by clamping to −10 mV.

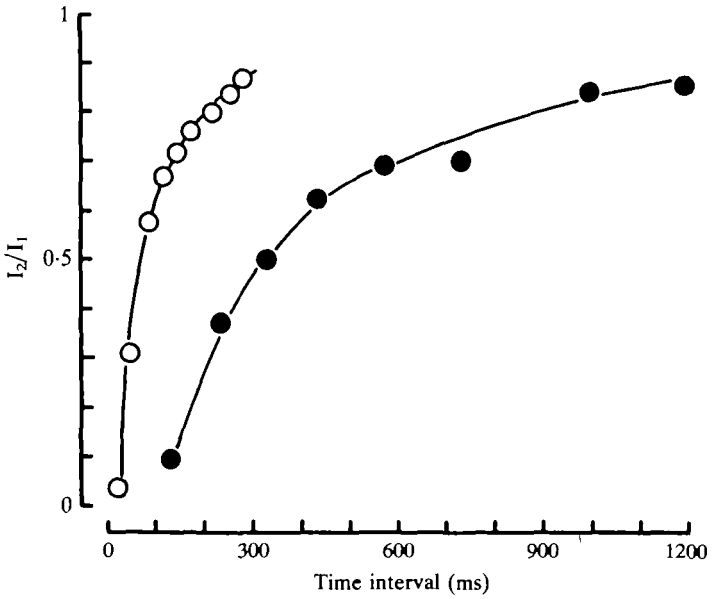


Fig. 12. Inactivation removal curve for inward current at 24 °C (open circles) and 3 °C (filled circles) in a Br neurone from a warm-acclimated snail. I_2/I_1, the ratio of the amplitude of inward currents in response to the second and first depolarizing command pulses, is plotted as a function of the time interval between the end of the first pulse and the beginning of a second pulse of equal amplitude.

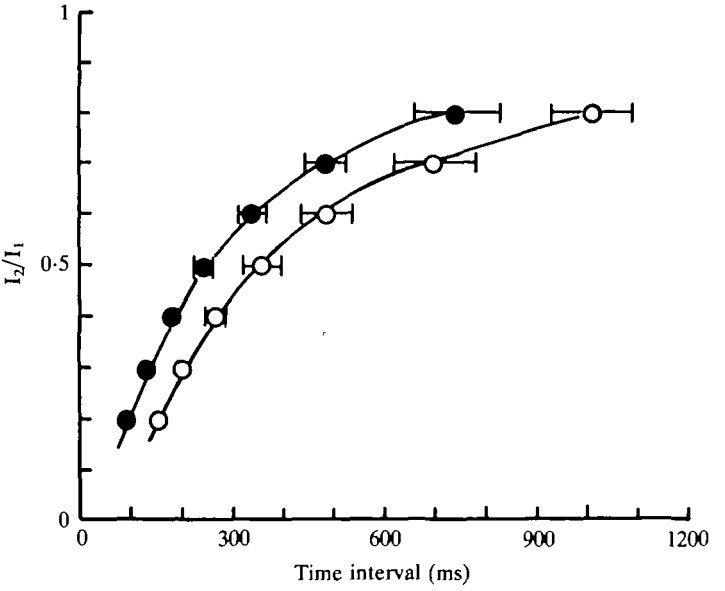


Fig. 13. Inactivation removal curve for inward current at 3 °C in Br neurones from warm-acclimated animals (open circles, $N = 12$) and cold-acclimated animals (filled circles, $N = 7$). Means ± S.E.M.

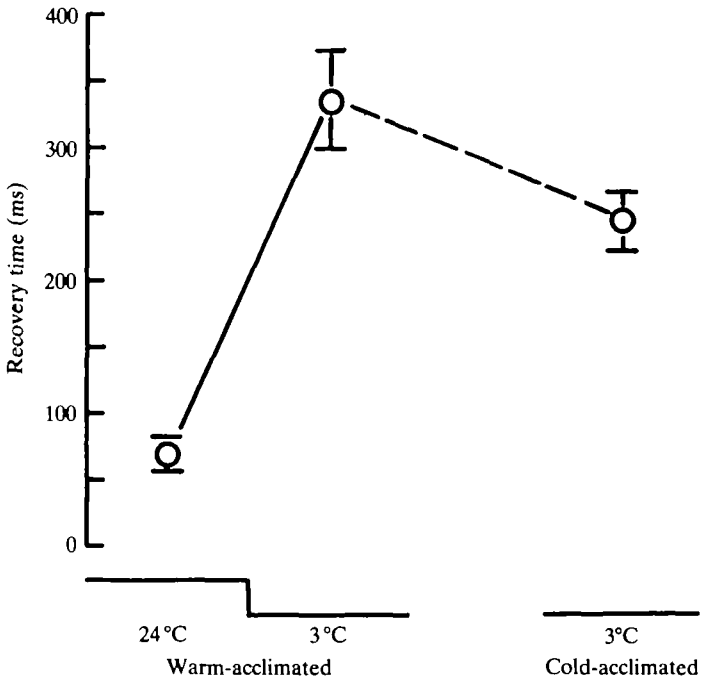


Fig. 14. The effect of temperature (solid line) and cold acclimation (dashed line) on the time to recover to 50 % of control inward current after inactivation. Means ± S.E.M.

whether changes induced by thermal acclimation could be observed at the level of voltage-dependent conductances. This work was planned as an initial step in the analysis of conductance changes under different thermal conditions in snail 'bursting' neurones. Our results are restricted to the effects of acclimation to low temperature. No experiments were conducted on cold-acclimated neurones that had been abruptly warmed. It is clear from previous work, however, that acclimation to warm temperatures also has a profound effect on the characteristics of endogenous, pacemaker activity in this same nerve cell and hence on membrane voltage-dependent conductances (See Langley, 1979; Zečević & Levatin, 1980).

A-current

Both the amplitude and kinetic characteristics of the A-current in the bursting neurone of *Helix pomatia* are highly temperature dependent. The reduction of A-current amplitude with temperature is probably due to a decrease in the effective conductance of ionic channels since other parameters determining A-current amplitude remained essentially unaltered after a rapid temperature decrease. These observations are comparable in many respects to those reported previously in neurones from the marine molluscs *Archidoris* and *Anisodoris* (Partridge & Connor, 1978).

A principal difference between the two molluscan systems appears to be that at 3–5 °C the A-current and repetitive firing are present in neurones from marine molluscs but absent in neurones from the warm-acclimated land snail. The absence

of an A-current at the low temperature may be due to a weaker G system in the *Helix pomatia* neurones than in those from marine molluscs. In addition, although Q_{10} values for A-current amplitude are essentially similar in *Helix* Br neurone and *Anisodoris* nerve cells in the range from 10 to 20 °C, our results on the *Helix* neurones indicate a stronger dependence of A-current magnitude at lower temperatures. A variable Q_{10} has been obtained in other excitable systems (Kimura & Meves, 1979; Hagiwara & Yoshii, 1980).

Since repetitive firing can be provoked at low temperatures (even below 0 °C: D. Zečević, H. Levatin & M. Pašić, unpublished observation) in neurones from cold-acclimated animals in which A-currents are absent, it is likely that other voltage-dependent conductances must play a significant role in the compensatory process.

Inward current

The maximum peak inward current of Br neurones from warm-acclimated animals has a Q_{10} of 2, a value close to that reported in other systems. In the giant axon of the squid (Hodgkin & Huxley, 1952*b*; Wang, Narahashi & Scuka, 1972) and *Myxicola* (Schauf, 1973), for example, and in myelinated nerve fibres (Frankenhauser & Moore, 1963) the peak Na-current has Q_{10} close to 1·5. In *Anisodoris* (Partridge & Connor, 1978) and *Aplysia* (Adams & Gage, 1979*a*), neurones in which the inward current has components due to Na and Ca flux, Q_{10} values of 1·7 and 2 have been reported. The inward current in the Br neurone of *Helix pomatia* also has Na and Ca components (Zečević & Levitan, 1980), but the temperature sensitivities of the Na and Ca conductances have not been explored separately.

The reduction of inward current magnitude with temperature might be due not only to a decrease in the effective conductance of ionic channels but also to the temperature sensitivity of the voltage-dependence of activation and inactivation processes (Wang *et al.* 1972; Kimura & Meves, 1979) and/or the ratio of their time constants (Frankenhauser & Moore, 1963; Chandler & Meves, 1970; Kimura & Meves, 1979).

The adaptive effect of the long-term temperature transition on inward current amplitude has not been previously explored. Which of the above-mentioned parameters determining inward current magnitude are modified in a compensatory way by cold acclimation remains to be explored.

Recovery of inward current from inactivation

Fast cooling of neurones from warm-acclimated snails resulted in an increase in the time needed for the removal of inactivation of inward current. Such an effect might have a role in the observed changes in the excitability that are most pronounced after the generation of the first action potential.

The temperature dependence of the recovery from inactivation had a $Q_{10}^{-1} = 2$, a value which is in agreement with results obtained previously from giant axons of squid (Hodgkin & Huxley, 1952*b*) and *Myxicola* (Frankenhauser & Moore, 1963) where inactivation was dependent on temperature with $Q_{10}^{-1} = 2$–3.

Although the mechanism of inward current inactivation is not well understood, some data suggest that thermal effects on the part of this process that depends on membrane potential may be related to membrane viscosity. In squid giant axon,

depolarization has been found to immobilize polar molecules in the membrane that are functionally connected to ionic channels (Meves & Vogel, 1977). The recovery of inward current from inactivation is related in time to the recovery of charge movement from the immobilized state (Armstrong & Bezanilla, 1977). Temperature might influence this process through its effects on membrane viscosity. An effect of membrane fluidity on asymmetrical charge movement has been postulated (Hammel & Zimmerman, 1970; Kimura & Meves, 1979; Schwartz, 1979).

The effect of thermal acclimation on the recovery of inward current from inactivation has not been previously explored. The mechanism by which cold acclimation promotes a compensatory shortening of the time course of inactivation removal might also be partially dependent on the known compensatory effect of thermal acclimation on lipid composition and fluidity of the membrane (Sinensky, 1971; Cossins, 1977; Cossins & Prosser, 1978; Willis, 1979).

Although changes induced by temperature and thermal acclimation in the characteristics of voltage-dependent conductances could be thought of, to some extent, in terms of direct effects of temperature on physico-chemical features of the membrane such as fluidity, there is evidence to suggest that thermal effects might be more complex. Pacemaker molluscan neurones have complex excitability characteristics and it appears that five independent current components are responsible for endogenous 'bursting'. Some of those different conductance systems depend on the rate at which intracellular ionized calcium accumulates during excitation (Gorman & Hermann, 1982) as well as on the rate at which calcium returns to its resting level. In addition, the removal of ionized free Ca depends on the metabolic state of the neurone (Meech, 1979). For example, it has been shown that low temperatures slow the uptake of Ca ions in molluscan neurones and therefore increase the amount of free intracellular Ca^{2+} (Tillotson & Gorman, 1981). An increase in free Ca^{2+} at low temperatures results in an increase in Ca^{2+}-activated K^+ current – a condition that tends to hyperpolarize the membrane (Gorman & Hermann, 1979; Gorman, Hermann & Thomas, 1982). Therefore, it would be interesting to investigate to what extent similar conditions are present in *Helix* Br neurone and whether thermal acclimation has an effect on intracellular Ca^{2+}-buffering.

Also, inactivation of Ca^{2+}-current in molluscan neurones appears to depend on Ca^{2+} entry into the cell rather than on membrane potential (Tillotson, 1979). It is thus possible that recovery of inward current from inactivation following temperature acclimation is in part due to changes in the mechanisms responsible for short-term Ca^{2+} removal in the cytoplasm.

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