Short communication

Temperature-Dependent Properties of Delayed Outward Current Channels in Somatic Membrane of Snail Neurones

I. S. MAGURA, N. B. PREVARSKAYA and J. ZACHAR

1 Department of General Physiology of the Nervous System, Bogomoletz Institute of Physiology, Academy of Sciences of the Ukr. SSR, Kiev-24, 252601, USSR
2 Department of General Physiology, Centre of Physiological Sciences, Slovak Academy of Sciences, Vlárska 5, 833 06 Bratislava, Czechoslovakia

Our study has been aimed at characterizing the effects of temperature on delayed outward current (I_K) in voltage clamp experiments with internally perfused nerve cell bodies from snails Helix pomatia. This study has been prompted by the fact that temperature is a useful probe of the channel kinetic states and its physico-chemical environment (Beam and Donaldson 1983).

The technique of cell isolation and intracellular perfusion did not differ from that already described (Kostyuk et al. 1981). The intracellular solution contained (in mmol.l⁻¹): KF 125; HEPES 10; pH 7.3. The external sodium-free solution had the following composition (in mmol.l⁻¹): Tris-Cl 100; CaCl₂ 7; MgCl₂ 5; KCl 4. The pH was adjusted to 7.4. The temperature was maintained constant by passing external solution through a reservoir where temperature was maintained by a thermoelectric device.

In our experiments, the Ca current has completely decayed after 15 min of intracellular perfusion: I_K (studied without overlapping inward currents) has persisted for several hours.

The effects of cooling on I_K are illustrated in Fig. 1A: the peak amplitude decreases as the temperature is lowered, the steady state currents remaining relatively unaffected (see Magura et al. 1975). The decaying phase of these traces showed an approximately exponential time course of inactivation. The time constants of inactivation (τa) at 20 and 10 °C were, respectively, 300 and 600 ms. Such effect of cooling on the inactivating component of the delayed outward current may be attributed to a decrease in membrane fluidity (see a review by Chapman 1975). A similar effect of cooling on the sodium current was observed in squid giant axon (Matteson and Armstrong 1982).

The temperature dependence of I_K activation was determined. For this purpose, I_K was described according to the Hodgkin-Huxley model. The time course of the K channel activation was quantified by fitting the measured currents to n⁴ kinetics. At holding potentials more negative than −45 mV, steady-state
Fig. 1A: Delayed outward currents recorded during steps to $-5$ mV at 20 and 10 °C (obtained in the order 20 and 10 °C). The holding potential was $-55$ mV. B: Effect of temperature on time constant $\tau$. C: Arrhenius plot of $\tau$ at $-12; 0$ and 9 mV. Ordinate: time constant $\tau$, on a logarithmic scale. Abscissa: reciprocal of the absolute temperature $T$ (lines were drawn by eye).

Activation of delayed outward current channels is expected to be zero. Thus, for currents activated by depolarizing steps from this holding potential, $n_0 = 0$. Since the time constant of inactivation $\tau_h \gg \tau$, and $t$ does not exceed the time when $I_K$ reaches a maximum:

$$I_K(t) = I_{K_{\infty}} \left[ 1 - \exp \left( \frac{t}{\tau_n} \right) \right]$$

where $I_{K_{\infty}}$ is the peak $I_K$, and $\tau_n$ is the voltage dependent time constant of the hypothetical parameter $n$ (see Beam and Donaldson 1983).

Eq. (1) was fitted to individual currents by adjusting the values of $\tau_n$ and $I_{K_{\infty}}$.

Fig. 1B shows values of $\tau_n$ for potentials between $-13$ and $+20$ mV at four different temperatures. The effect of temperature on $\tau_n$ was much greater in the cold than in the warm: $\tau_n$ had a $Q_{10}$ of nearly 6 at temperatures below 8°, but a $Q_{10}$ of nearly 3 over the range of 20—8 °C. The values for the activation energy were 159.1 kJ/mol at temperatures below 8 °C and 83.7 kJ/mol at temperatures above 8 °C. Similar results were reported for $\tau_n$ in rat skeletal muscle (Beam and
Donaldson 1983). The $\tau_n$ values for $-12$; $0$; and $+9$ mV are plotted in an Arrhenius plot in Fig. 1C. For all the three potentials the $\tau_n$ values in the Arrhenius plot are represented by two straight lines each, of different slopes. Similar results were reported for gating parameters of sodium channels in squid giant axons (Kimura and Meves 1979) and in node of Ranvier (Chiu et al. 1979). Abrupt changes in the Arrhenius plot have been interpreted to mean that the membrane lipids of these cells undergo a phase transition (Chiu et al. 1979).

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References


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