Short communication

Interaction of Sodium Ions with Potassium Channels of Mollusc Neuronal Somatic Membrane

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The effects of internal Na^+ ions on noninactivating delayed outward and transient outward currents were studied in internally perfused nerve cell bodies from snails *Helix pomatia* using voltage clamp. These outward currents are carried predominantly by K^+ ions. The interaction of an Na^+ ion with a K channel makes the former a useful probe of some K channel properties (Bezanilla and Armstrong 1972; French and Wells 1977; Hille and Schwarz 1978; Lattore and Miller 1983).

The technique of cell isolation and intracellular perfusion did not differ from that described earlier (Kostyuk et al. 1981). The intracellular solution contained (in mmol/l): KF 75; Tris-Cl 50; Tris F 10; pH 7.3. Sodium ions were introduced into the internal medium by equivalent replacement of Tris ions. The external sodium-free solution had following composition (in mmol/l): Tris-Cl 100; $CaCl_2$ 7; $MgCl_2$ 5; KCl 4; pH 7.4. The experiments were done at room temperature (20—22 °C).

The Ca current has completely decayed after 15 min of perfusion; K currents persisted for 1.5—2 hours. We have studied outward currents without overlapping inward currents. The holding potential was set to -100 mV in order to abolish inactivation of the transient outward current. With 75 mmol/l K^+ plus 50 mmol/l Na^+ in the internal solution a blocking of the transient outward current and noninactivating outward current occurred (Fig. 1*A*). Fig. 1*B* shows the effect of internal Na^+ (50 mmol/l) on the peak of the outward currents. The negative slope region in the I-E plot is produced by voltage dependent blocking of the transient fraction of the potassium current by Na^+. The voltage dependence may imply that the binding site is within the membrane field. The block occurred at a more positive voltage when the external K^+ concentration increased.

For any single Na concentration, a fit to the I-E relation could be obtained using the following equation:

$$I_{K+Na}(E) = I_K(E) \,/\, 1 + \frac{Na^+}{K_B} \exp \frac{z'FE}{RT} \qquad (1)$$

where I_{K+Na} is the current recorded in the presence of intracellular Na^+ ions; I_K is

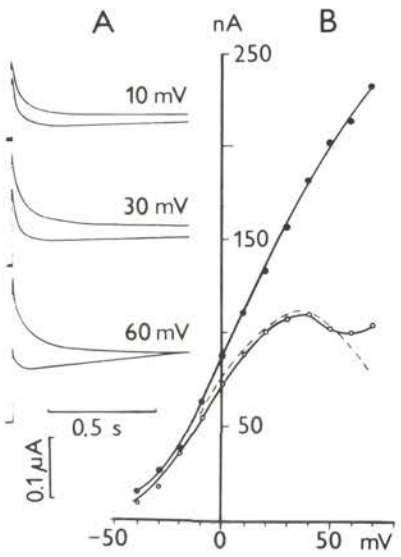

Fig. 1. Blocking effect of internal Na on outward currents. (**A**) currents recorded on stepping from −100 mV to potentials indicated in the figure. Upper traces: control ($[Na]_i = 0$); lower traces: membrane currents at $[Na]_i = 50$ mmol/l. (**B**) current-voltage relationship at peak of the outward current; full symbols: $[Na]_i = 0$; empty symbols: $[Na]_i = 50$ mmol/l. The dashed line represents theoretical current according to Eq. (1); $z' = 0.9$.

the current in the absence of intracellular Na^+ ions; E is the membrane potential; K_B is the concentration of Na^+ required to block 50 % of the channels at $E = 0$; z' is the effective valence of the blocking reaction given by the valence of the blocking ion multiplied by the fraction of the total potential drop through which it moves; F, R, and T are the usual thermodynamic quantities.

The dashed line in Fig. 1*B* has been derived from equation (1), $z' = 0.9$; $K_B = 355$ mmol/l. The normally linear instantaneous I-E relationship for the transient outward current was transformed by internal Na^+ (4—50 mmol/l) into an N-shaped (Fig. 2*A*). At high transmembrane voltages (≥ 60 mV) a second region of increasing current was observed. It was suggested that, at high voltage, Na^+ not only enters the K channels but it can pass through them with relative ease (French and Wells 1977).

Noninactivating delayed outward current was recorded during depolarization of the membrane from a holding potential of −35 mV. Fig. 2*B* illustrates the blocking effect of internal Na^+ (50 mmol/l) on the instantaneous I-E relationship of noninactivating delayed outward current channels. The blocking action of internal Na^+ ions on the noninactivating delayed outward current showed no significant voltage dependence.

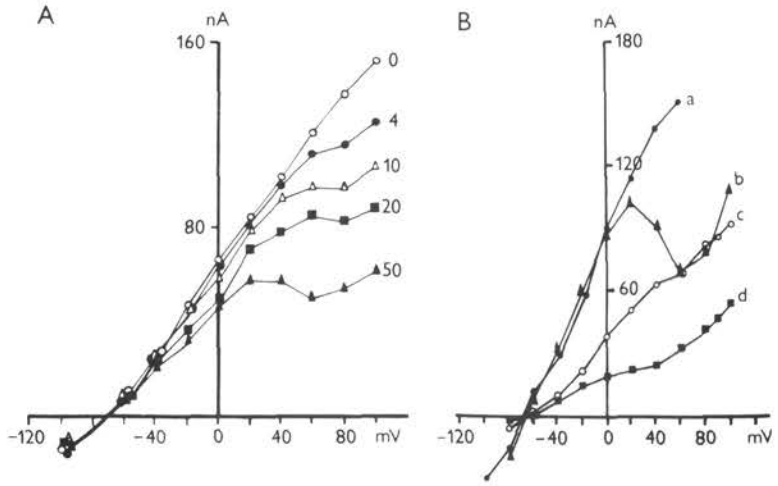

Fig. 2A: Comparison of instantaneous I-E relationships of the transient outward current at various intracellular concentrations of Na^+ ions (0; 4; 10; 20; 50 mmol/l). Figures indicate the respective intracellular sodium concentration (in mmol/l). **B**: Comparison of instantaneous I-E relationships of the same neuron for transient (curves *a* and *b*) and noninactivating (curves *c* and *d*) outward currents prior to the introduction of 50 mmol/l Na^+ ions intraneuronally (curves *a* and *c*), and after it (curves *b* and *d*).

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