Short communication

Interaction of Potassium Ions and Tetrodotoxin (TTX) with Inactivated Sodium Channels in Isolated Rat Myocardial Cells

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Sodium currents, I_{Na}, in enzymatically isolated myocytes of adult rats have been studied using a version of the whole-cell patch technique described earlier (Zilberter et al. 1982). Ca^+ and K^+ channels were blocked by addition of 1 mmol/l Mn^{2+} and 1 mmol/l 4-aminopyridine to the control Tyrode's solution in the suction pipette.

Removal of K^+ or addition of 1.5×10^{-6} mol/l TTX to the control solution in the suction pipette did not affect appreciably the kinetics of the fast ('ordinary') reactivation of Na^+ channels after a 50- ms conditioning depolarizing pulse. By contrast the slow phase of Na^+ channels reactivation present after a 2-s depolarizing step was very sensitive to both K^+ and TTX (Fig. 1). The time constant of this

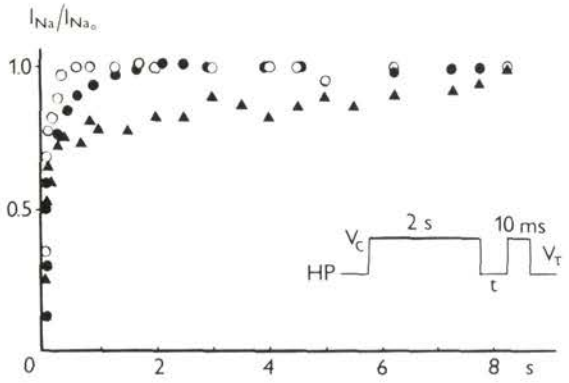

Fig. 1. The time course of recovery of Na^+ channels from inactivation after a 2-s depolarizing step. The magnitude of both conditioning and test pulses were 40 mV from the holding potential $V = -60$ mV (V is a displacement of the membrane potential from its resting value, $E_r \sim -65$ mV). The cell was bathed in the normal (control) Tyrode's solution, containing (in mmol/l): 130 NaCl; 5.4 KCl; 1.2 $MgSO_4$; 0.9 $CaCl_2$; II Glucose; 20 MOPS; pH 7.4; 1 mmol/l $MnCl_2$ was added to block Ca^{++} channels. All solutions in the suction pipette contained 1 mmol/l $MnCl_2$ and 1 mmol/l 4-aminopyridine. Temperature 20 °C. ● control solution (5.4 mmol/l K); ○ K^+ -free solution; ▲ K^+ -free solution containing 1.5×10^{-6} mol/l TTX. Cell 15.03.83.

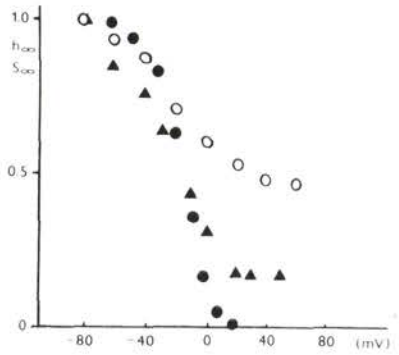

Fig. 2. Steady-state voltage-dependence of the fractions of Na^+ channels free from fast (h_∞) and 'slow' (s_∞) inactivation. ● and ○ correspond to h_∞ and s_∞ (respectively) in control solution containing 1.5×10^{-6} mol/l TTX. ▲ s_∞ was determined as described in Khodorov et al (1976). Temperature 20 °C. Cell 19.03.83.

slow reactivation was: ~130 ms in nominally K^+-free solution; 500 ms in the control (5.4 mmol/l K^+) solution; and ~5s in K^+-free solution containing TTX (at holding potential $E_h \leq -120$ mV and temperature 20 °C). The fraction of Na^+ channels free from K^+- or TTX -induced slow inactivation (s_∞) at the end of a 2 -s conditioning step decreased with increasing depolarization and approached a non-zero steady-state level at potentials close to those where the fraction of Na^+ channels free from the fast inactivation (h_∞) approached zero (Fig. 2). Decelaration of the reactivation of the Na^+ channels by K^+ or TTX resulted in a cumulative ('use-dependent') inhibition of I_{Na} during repetitive pulsing of the membrane. The rate and depth of this use-dependent block increased with pulse duration over the range 10 to 100 ms (5 Hz). In TTX-treated membrane the use-dependent inhibition of I_{Na} developed on the background of the 'tonic block' ($K_D \approx 1.8 \times 10^{-6}$ mol/l TTX). These effects of TTX resembled those observed by Cohen et al. (1981) in rabbit Purkinje fibers.

The time- and voltage-dependence of the effects of K^+ and TTX, described above, suggest these chemicals are capable to interact with inactivated Na^+ channels in the membrane of myocardial cells. The 'slow inactivation' resulting from such interaction is qualitatively similar to that previously found by studying the effects of external K^+ (Peganov et al. 1973) and local anesthetics (Khodorov et al. 1973) on Na^+ channels in myelinated nerves. Since TTX cannot reach the inner channel receptor (Hille 1977) the ability of this toxin to induce 'slow Na inactivation'can be considered as additional evidence in favour of the notion that the Na^+ channel responsible for this process is located near to the outer side of the membrane (Khodorov et al.1976; 1979)

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