Short communication

Evidence for Voltage-Dependent Inactivation of Slow Currents through Calcium Channels in Frog Muscle Membrane

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Two mechanisms of inactivation of slow inward current through calcium channels in frog skeletal muscle have been described: voltage dependent inactivation similar to that of classical sodium channels (Cota et al. 1982, 1984; Stanfield 1977); and calcium ions depletion inside the T-tubules as a result of inward current flowing through the T-membrane. In the latter case the inactivation is current dependent (Almers et al. 1981).

The present communication describes an additional potential-dependent inactivation mechanism of calcium channels in skeletal muscle. The currents were studied in isolated twitch muscle fibre segments from *Rana temporaria* (*m. ileofibularis*) by means of the vaseline-gap voltage clamp method (Zachar et al. 1982) at 18—20 °C. The current through calcium channels was elicited by more permeable Ba^{2+} ions. The external solutions containing different Ba^{2+} concentrations used were (in mmol/l): 1. $BaCl_2$ 10; (TEA)Br 99; KCl 2.5; TRIS-HCl 10; glucose 5.6; and 2. $BaCl_2$ 76; KCl 2.5; glucose 5.6; TRIS-HCl 10. The internal solution contains: (TEA)Br 60; CsCl 50; EDTA 0.1; TRIS-HCl 10; pH of all solutions was 7.5.

Barium inward currents, which are known to pass through calcium channels in T-membrane, were recorded in 14 experiments. Values of 100—340 $\mu A/cm^2$ (mean $\pm$ S.E.M. 193 $\pm$ 39 $\mu A/cm^2$) in 10 mmol/l Ba^{2+} solution and 280—720 $\mu A/cm^2$ (460 $\pm$ 42 $\mu A/cm^2$) in 76 mmol/l Ba^{2+} solution were measured.

Fig. 1 shows families of voltage clamp Ba^{2+} currents recorded in 10 and 76 mmol/l Ba^{2+} and corresponding current-voltage relations. The records show some distortion suggesting that the voltage clamp was not entirely tight. The localization of calcium channels on the T-tubular membrane apparently is the cause of this complication (Cota et al. 1983). I_{Ba} current-voltage curve in 10 mmol/l Ba^{2+} was close to that obtained by Cota and Stefani (1984), and it was shifted by +15 mV in 76 mmol/l Ba^{2+} in agreement with the effect of the surface charge on the voltage dependent calcium channel activation. The correct shape

of current-voltage curves suggests that steady-state properties of I_{Ba} can be adequately described.

Fig. 2*A* illustrates the effect of a conditioning depolarizing pulse of Ba^{2+} current during the test pulse in 10 and 76 mmol/l Ba^{2+}. A current decrease is evident over a range of conditioning depolarization between −70 mV and −30 mV. During the test pulse, I_{Ba} is reduced to about 80 % of the control value, without any detectable inward current during conditioning pre-pulse. Fig. 2*B* shows the dependence of h_∞ on membrane potential in 10 and 76 mmol/l Ba^{2+}. At high barium concentration, the curve for steady-state inactivation shifted by 14 mV to more positive potentials, but its slope remained unchanged. This voltage shift is in accord with the shift of current-voltage curve in 76 mmol/l Ba^{2+} (Fig. 1*B*).

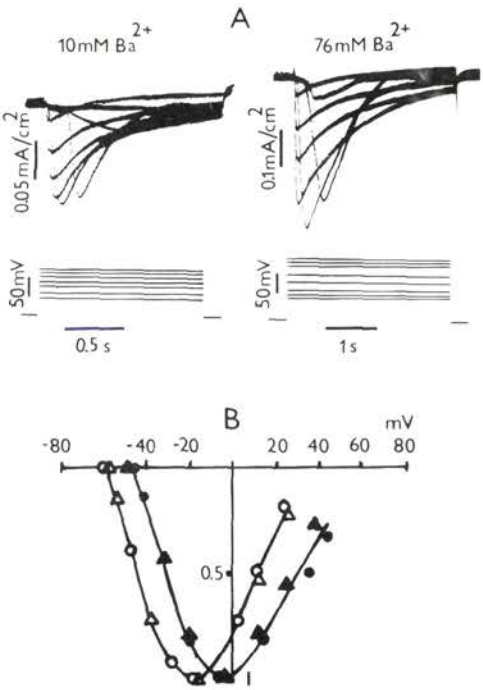

Fig. 1. Barium currents in a twitch muscle fibre segment in 10 and 76 mmol/l Ba^{2+}. *A*: superimposed voltage clamp records for voltage steps as shown below. Note the difference in gain and time scale. *B*: normalized current-voltage relations, obtained from two different fibres (circles — records shown in *A*). Open symbols: 10 mmol/l Ba^{2+}, filled symbols: 76 mmol/l Ba^{2+}. The curves were drawn by hand.

The experimental points for each curve can be fitted to the relation:

$$h_\infty = (1 + \exp((E_m - V_h)/k_h))^{-1}$$

where E_m is the transmembrane potential, $k_h = 6$ mV, $V_h = 51$ mV for 10 and 37 mV for 76 mmol/l Ba^{2+}.

During exit of Ba^{2+} from T-tubules, the role of depletion in current inactivation in 76 mmol/l Ba^{2+} would be less pronounced than in 10 mmol/l, because of

the saturation effect of calcium channels at high concentrations of permeant ions, and the slope of h_∞ on membrane potential-dependence would be less than at low concentrations. The similarity of slopes at low and high Ba^{2+} concentrations suggests that the potential-dependent mechanism is the most likely explanation of the current inactivation through calcium channels.

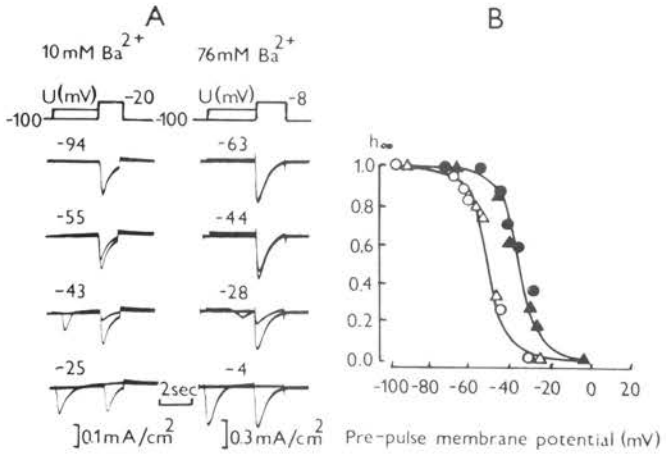

Fig. 2. Steady-state inactivation of Ba^{2+} current in voltage clamp experiments on the same fibres as shown in Fig. 1. Voltage (*uppermost*) and currents (*below*) during step depolarization in 10 and 76 mmol/l $BaCl_2$ (*A*) and steady-state inactivation of the currents (*B*). On each record the first traces show the current in response to conditioning pulse only, and the second traces correspond to responses to prepulses as indicated (in mV). Open and filled symbols: 10 and 76 mmol l Ba^{2+} respectively. The curves were drawn by hand.

In would be reasonable to suppose that the current-dependence of inactivation of divalent ions current (Almers et al. 1981), which suggests a depletion of divalent ions in T-tubules, could appear as a result of a high (isotonic) EGTA concentration in the internal solution. If the concentration of EGTA is high, it probably permeates the membrane and divalent cations outside, as suggested by Kostyuk et al. (1977) for *Helix* neurones.

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