SOME ELECTROPHYSIOLOGICAL PROPERTIES OF SHARK MYOCYTES

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Single ventricular cells were isolated from shark heart (*Squalus acanthias*) using previously described procedures (Maylie & Morad, Am. J. Physiol. 269:H1695-H1703, 1995). Dissociated myocytes were placed in a chamber on the stage of an inverted microscope and superfused with elasmobranch solution containing (mM) 270 NaCl, 4 KCl, 3 $MgCl_2$, 0.5 KH_2PO_4, 0.5 Na_2SO_4, 350 Urea, 10 HEPES, 0.5 glucose, 2 $CaCl_2$, pH 7.2. Whole-cell currents were measured using 3-5 MΩ pipette attached to the input of a patch clamp amplifier (Dagan 8900). The pipette solution contained (in mM) 200 KCl, 60 NaCl, 300 Urea, 10 HEPES, 5 Mg-ATP, 0.2 EGTA, pH 7.2 with KOH.

When the membrane voltage was held at –90 mV, voltage-clamp steps from –120 to +105 mV activated a number of superposed currents illustrated in Fig. 1A. Voltage clamp steps from –120 to –90 mV activated inward current, which had a property similar to those associated with inwardly rectifying K^+ current (I_{K1}). Clamp pulses from +45 to +105 activated a slowly activating outward current which increased with increasing depolarization (Fig. 1A).

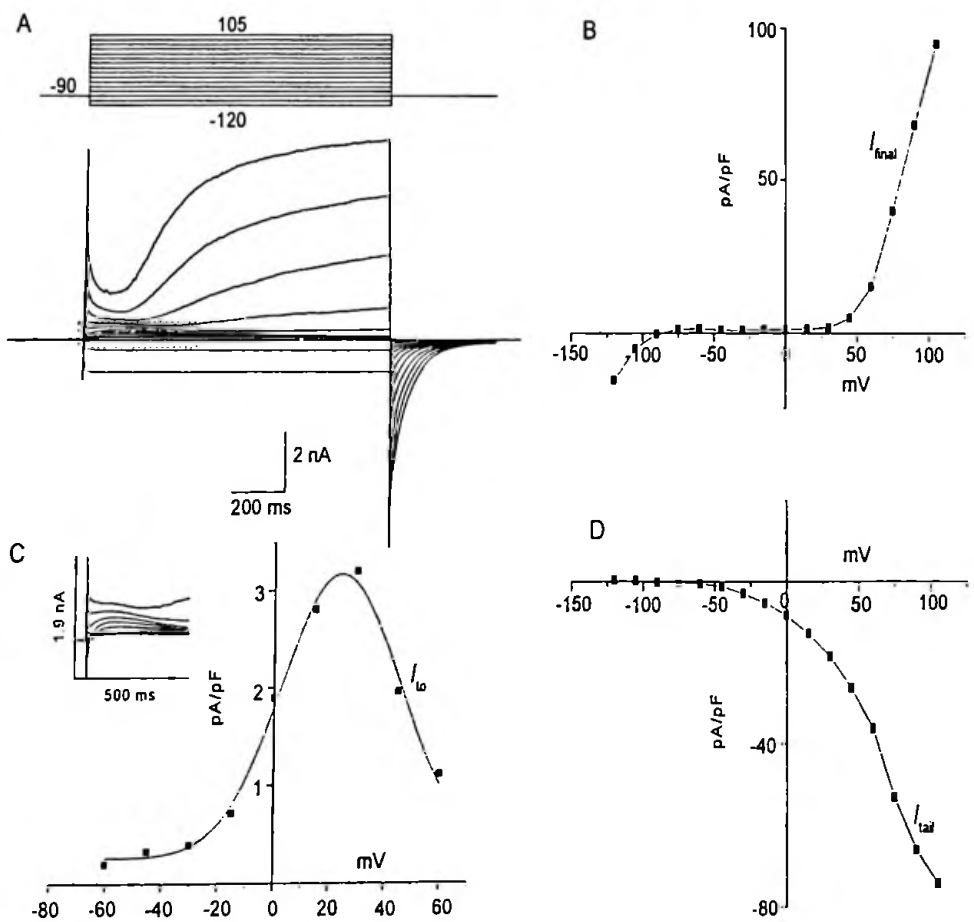

Figure 1. Activation of some currents in shark ventricular myocyte. A, pulse protocol and whole cell currents. B and D, current density-voltage relations of the final currents and tail currents, respectively. C. Current density-voltage relation of the peak currents illustrated in the inset. The inset was made by magnifying the currents within the box in A.

Clamp pulses in the range of –30 to +60 mV activated a transient outward current which followed the activation of fast transient sodium and calcium current (inset of Fig. 1C). This transient current reached its peak value at about 150 ms following the onset of depolarization. The magnitude of this current was measured as the peak outward current relative to the maintained component of outward current. The transient current had a bell-shape voltage-dependence with a maximum at +25 mV. The bell-shape current-voltage relation reflects the voltage-dependence of I_{Ca}, suggesting that the transient outward current is activated by rise of $[Ca^{2+}]_i$.

An inward tail current was measured following clamp steps to positive potentials. As the membrane voltage gradually increased, the magnitude of the tail current also increased and the tail current inactivated with a slower kinetics (Fig. 1A). The larger membrane depolarizations most likely induce a rise in $[Ca^{2+}]_i$ secondary to activation of the Ca^{2+} channel and the Ca^{2+} influx mode of the Na^+–Ca^{2+} exchanger. The slow inward tail current may therefore represent the efflux of Ca^{2+} on the exchanger. Consistence with this assumption, the tail current was suppressed by a novel blocker of Na^+-Ca^{2+} exchanger, KB-R7943 (Fig. 2A, B and D). Since, KB-R7943 inhibits also I_{Ca} (data not shown), the suppression of the transient outward current (panel B and E of Fig. 2) suggests that this outward current may represent a Ca^{2+} activated Cl^-current. More critical experiments are, however, required to identify the ionic nature of this transient outward current. (Supported by NIH #16152)

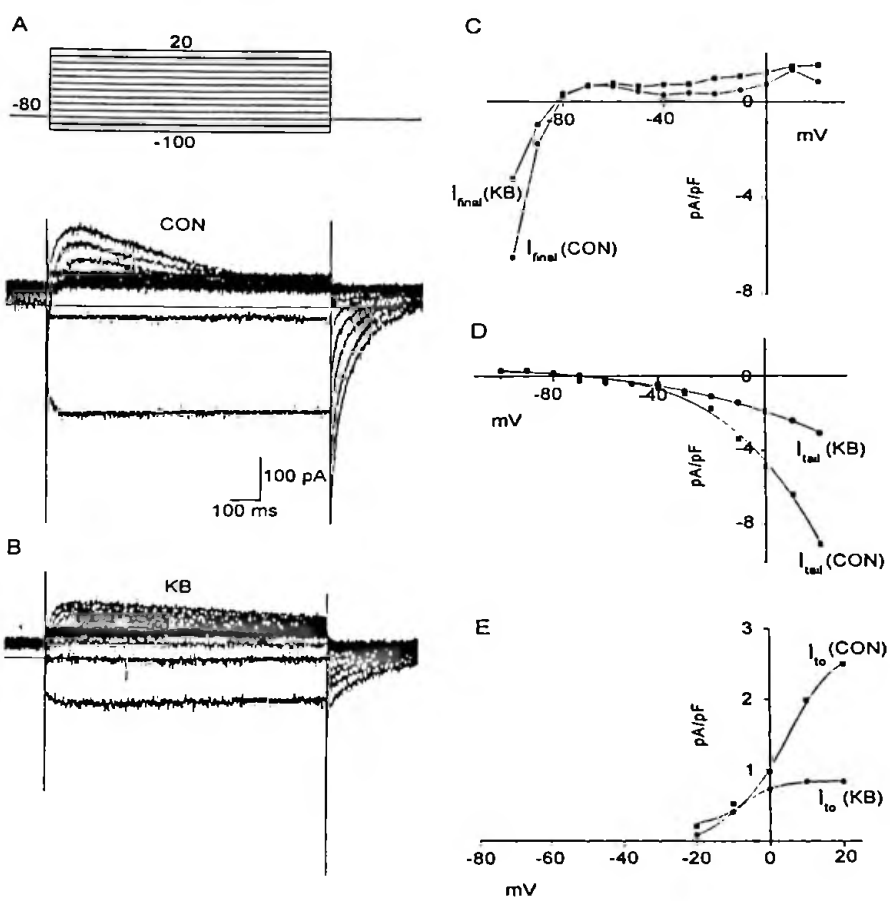

Figure 2. The effect of KB-R7943 on the currents recorded in shark ventricular myocyte. A, pulse protocol and whole cell currents in control solution (CON). B, currents in the presence of 10 μM KB-R7943. C, D, and E, current density-voltage relations of final currents (I_{final}), tail currents (I_{tail}), and transient outward currents (I_{to}) in A and B.