# CHARACTERIZATION OF A VOLTAGE-GATED CARDIAC POTASSIUM CHANNEL IN SQUALUS ACANTHIAS AND ITS EXPRESSION IN XENOPUS OOCYTES INJECTED WITH mRNA ISOLATED FROM HEARTS OF SQUALUS ACANTHIAS

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Action potentials recorded from dogfish heart are similar in voltage and duration to those recorded in mammalian species. A rapidly activating sodium current and a T-type calcium current are responsible for the upstroke of the action potentials and the L-type calcium current is responsible for maintenance of the action potential plateau. Activation of a potassium current during the plateau of the action potential contributes in part to initiation of repolarization. In this report we show measurements of a voltage-gated potassium current recorded in shark ventricular myocytes and in Xenopus oocytes injected with mRNA isolated from shark ventricle.

Shark ventricular myocytes isolated from Squalus acanthias (Mitra and Morad, Am. J. Physiol. 249:H1056-H1060, 1985) were studied with the whole cell patch clamp configuration. The patch pipette contained (mM): KCl 220, UREA 300, MgATP 5, HEPES 20, TMAO 70, $MgCl_2$ 1, EGTA 10, $CaCl_2$ 0.588, pH 7.2. The standard external solution bathing the cells was (mM): NaCl 270, $Na_2SO_4$ 0.5, KCl 4, $KH_2PO_4$ 0.5, $MgCl_2$ 1, $CaCl_2$ 5, UREA 350, HEPES 10, glucose 10, pH 7.2. Whole cell currents were measured with an EPC-9 (HEKA). For expression studies, poly $A^+$ mRNA from ventricle was isolated by poly(dT) chromatography with a FasTrack kit (Invitrogen). 50 nl of solution containing 50-70 ng of mRNA were injected into Xenopus oocytes and 2-7 days later the transmembrane currents were recorded with a double electrode voltage clamp (CA-1, Dagan). Oocytes were continuously superfused with a solution containing (mM) NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, Hepes 5, pH 7.5 at room temperature. Oocytes were prepared as described by Christie, et al., (Science 244: 221-224, 1989).

In shark ventricular myocytes, depolarizations to potentials greater than -20 mV activated an outward current superimposed on a transient sodium and calcium current (figure 1A). Analysis of tail current reversal showed that this current is carried by potassium. The current-voltage relation of the final outward current and the tail current measured on repolarization to -40 mV is shown in figure 1C. A Boltzmann relation applied to the tail currents yielded a potential for half maximal activation of 8.8 mV and a slope factor of 6.3 mV. The kinetics of activation evaluated at 40 and 60 mV following a 1 s prepulse to -40 mV to inactivate sodium and calcium channels could be described by a single exponential with time constants of 312 and 193 ms, respectively.

Most of the oocytes studied exhibited only a small and leak correctable endogenous chloride current that was not different from that observed in uninjected oocytes. In four out of approximately 40 oocytes studied, however, an additional current was recorded that activated at potentials greater than -10 mV (figure 1B). Analysis of tail currents showed that the deactivation of this current reversed at around -100 mV, close to the reversal potential for potassium in oocytes bathed in 2 mM $[K]_o$. We concluded that this current reflects the activation of a potassium channel expressed from the shark mRNA injected into the oocyte. The current-voltage relation of the final and tail current is shown in figure 1D. The voltage for half maximal activation of the tail current was 9.5 mV and its slope for activation was 6.9 mV. These values are very similar to those for potassium currents recorded in myocytes. The activation kinetics, however, were much faster in oocytes; 32 ms at 40 mV. This current was not observed in approximately 20 uninjected oocytes studied; sham-injected oocytes were not tested. Oocytes studied 2-7 days after injection showed no relationship in the appearance of this current with time after injection.

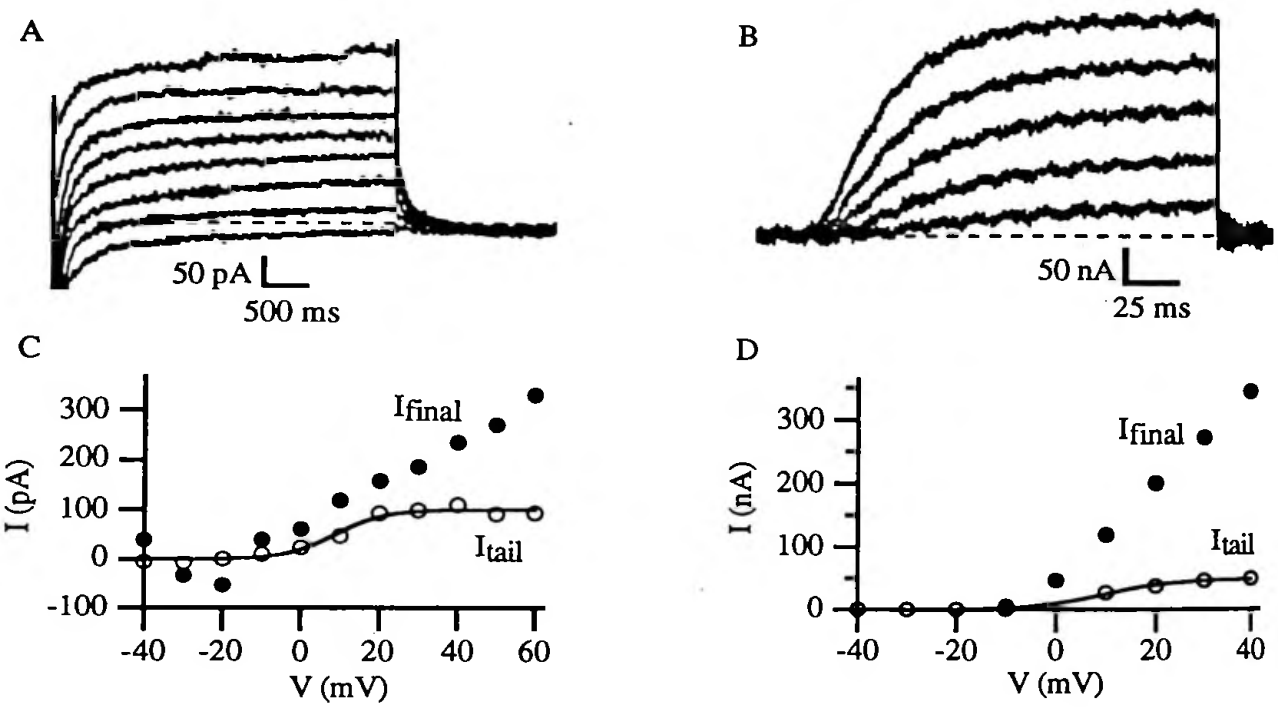


Figure 1. Activation of a potassium current in shark ventricular myocytes and Xenopus oocytes injected with shark mRNA. A.) Whole cell currents in a ventricular myocyte elicited by 4 s depolarizing pulses from -10 to 60 mV in 10 mV steps and then back to -40 mV. Holding potential was -90 mV. B.) Outward currents recorded in an oocyte elicited by 200 ms depolarizing pulses from 0 to 40 mV in 10 mV steps and then back to -60 mV. Holding potential was -80 mV. Currents were leak corrected. C.) Myocyte current-voltage relation of the final and tail currents from panel A. The continuous curve was drawn according to Boltzmann equation, $I_{max}/\left(1+e^{-(V_m-V_{1/2})/k}\right)$; $I_{max}$ = 100 pA, $V_{1/2}$ = 8.8 mV, k = 6.3 mV. D.) Oocyte current -voltage relation of final and tail current from panel B. The continuous curve was drawn according to Boltzmann relation; $I_{max}$ = 48 nA, $V_{1/2}$ = 9.5 mV, k = 6.9 mV.

In conclusion a voltage-gated potassium current has been identified in shark ventricular myocytes that may be responsible for initiation of repolarization of the cardiac action potential. This current has electrophysiological characteristics similar to the delayed outward potassium current in rabbit heart and to the rapidly activating component of the delayed outward current in guinea pig heart. The isolation and injection of mRNA from shark heart directed the expression of a potassium current with characteristics similar to those recorded in shark myocytes. The kinetics of activation were, however, much faster. It is not uncommon for the activation kinetics of channels expressed in oocytes to differ from those in native cells; though usually they are slower in oocytes. Several explanations are possible: 1) A regulatory factor which modulates the rate of activation was not expressed along with the channel in oocytes. 2) The high osmolarity and ionic strength of shark Ringer in some way affects the activation of this potassium channel. The low frequency of channel expression was disappointing and may be related to a low endogenous message level for this channel or the necessity for concomitant expression of an additional factor. We were also surprised that other channels were not observed. It is not known whether the structure of the potassium channel from dogfish is similar to the voltage-gated potassium channels of the shaker family. Such a comparison will have to wait until the shark delayed outward potassium channel is cloned.

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