EVALUATION OF Ca^{2+}-ACTIVATED TRANSIENT OUTWARD K^+ CURRENT IN SHARK VENTRICULAR MYOCYTES

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Action potentials recorded from dogfish heart are similar in voltage and duration to those recorded in mammalian species. Ca^{2+}-activated transient outward current in mammalian heart plays important roles in the repolarization of the action potential (Hiraoka & Kawano, J. Physiol. 410:187-212, 1989). A Ca^{2+}-activated transient outward current, carried by K^+, has been described previously in calf (Siegelbaum & Tsien, J. Physiol. 299:485-506, 1980), and a Ca^{2+}-activated transient outward Cl^- current has been identified in sheep Purkinje fibres (Carmeliet & Coraboeuf, Pflügers Archiv. 392:352-359, 1982) and rabbit ventricular and atrial myocytes (Zygmunt & Gibbons, Circ. Res. 68:424-437,1991 & J. Gen. Physiol. 99:391-411, 1992). Activation of this current is thought to be related to the release of Ca^{2+} from the intracellular pools (Maylie & Morad, J. Physiol. 357:267-292, 1984; Sipido et al., J. Physiol. 468:641-667, 1993). In this report we describe the presence of a Ca^{2+}-activated transient outward current in shark ventricular myocytes which is carried primarily by K^+ and is activated by Ca^{2+} current directly.

Single ventricular cells were isolated from dogfish heart (*Squalus acanthias*) using previously described procedures (Maylie & Morad, Am. J. Physiol. 269:H1695-H1703, 1995). Briefly, male dogfish (2~7 kg), were immobilized by complete spinal pithing. Hearts were removed and mounted in a Langendorff apparatus. The two major coronary vessels and aorta were cannulated and perfused with oxygenated Ca^{2+}-free elasmobranch solution containing (in mM) 270 NaCl, 4 KCl, 3 $MgCl_2$, 0.5 KH_2PO_4, 0.5 Na_2SO_4, 350 Urea, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 0.5 glucose, pH 7.2 at 30 °C for 10~15 min. The heart was then perfused for 15 min with Ca^{2+}-free elasmobranch solution containing 1 mg/ml collagenase (type A, Boehringer Mannheim) and 0.2 mg/ml protease (type XIV, Sigma), and was washed free of enzyme with 0.2 mM Ca^{2+}-containing elasmobranch solution for 10 min. The ventricle was then cut free of the cannula and gently agitated in 0.2 mM Ca^{2+}-containing solution to disperse the cells. Dissociated myocytes were placed in a chamber on the stage of an inverted microscope and superfused with 2 mM Ca^{2+}-containing elasmobranch solution. Whole-cell currents were measured using 3~5 MΩ pipette attached to the input of a patch clamp amplifier (model 8900, Dagan Instruments, Minneapolis). The pipette solution contained (in mM) 220 KCl, 60 NaCl, 300 Urea, 10 HEPES, 5 Mg-ATP, 10 TEA-Cl, 0.2 EGTA, pH 7.2 with KOH. In some experiment KCl was replaced with K-aspartate to make low chloride solution. To block K^+ current we used CsCl in the internal solution instead of KCl.

Depolarizing step pulses in the range of –45 to +60 mV from a holding potential of –80 mV activated a transient outward current (Fig. 1A). Detailed analysis of the current traces illustrated in Fig. 1A suggests that a transient outward current (I_{to}) is activated following the activation of Ca^{2+} current (I_{Ca}). Fig. 1B compares the voltage dependence of the transient outward current and I_{Ca}. Note that the current-voltage relation for I_{to} shows bell-shaped characteristics, similar to that of I_{Ca}. Whereas I_{Ca} activated around –40 mV and had a

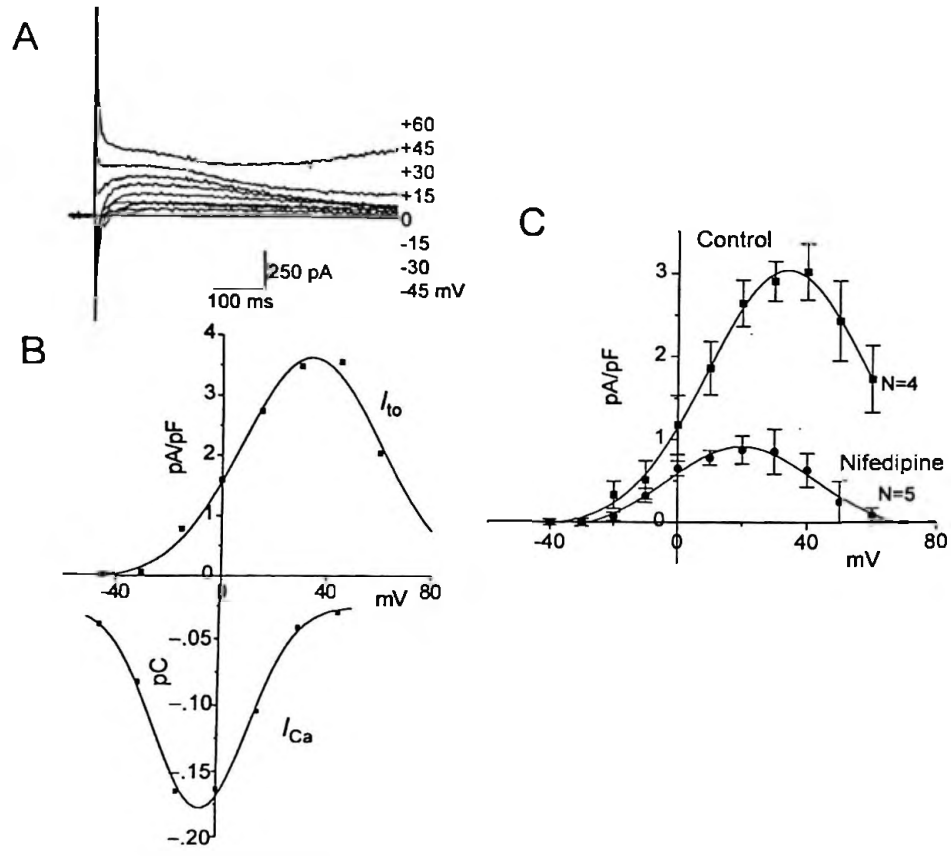


Figure 1. Activation of transient outward currents (I_{to}) in shark ventricular myocyte. (A) Whole cell currents during depolarizing steps from a holding potential of –80 mV to the indicated potentials. The zero level is indicated by a line at the traces. (B) Current-voltage relation of charge (Q) carried during Ca^{2+} currents (I_{Ca}) and I_{to}. The magnitude of I_{to} was measured as the peak outward current relative to the maintained component of outward current. (C) Current-voltage relations of I_{to} in the absence and presence of 10 μM nifedipine. Data were expressed as mean ± SEM.

maximum at about 0 mV, the transient outward current activated at potentials positive to –30 mV and reached a maximum between +30 and +40 mV. It should be noted that the amount of charge (Q, pC) carried by the activation of Ca^{2+} channel maybe underestimated at the voltages above 0 mV, because of the presence of outward currents, such as background Cl^- and monovalent currents through the Ca^{2+} channel at positive potentials. In experiments where such currents were blocked, I_{Ca} activated at about –40 mV, reached maximum values around 0 mV, and appeared to reach reversal values around +80 mV (Maylie & Morad, Am. J. Physiol. 269:H1695-H1703, 1995). Figure 1C compares the voltage dependence of I_{to} in the presence and absence of 10 μM nifedipine, a Ca^{2+} channel blocker. I_{to} was suppressed from 2.97 ± 0.24 pA/pF (mean ± SEM, n=4) at +30 mV in control solution to 0.86 ± 0.27 pA/pF (n=5) in the presence of 10 μM nifedipine. The suppression of I_{to} in the presence of nifedipine is consistent with the idea that the preceding I_{Ca} is inevitable for triggering I_{to}.

To examine the contribution of Ca^{2+} influx to the activation of I_{to} we also studied the effect of increasing or decreasing extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$). Activation of I_{to} was strongly dependent on $[Ca^{2+}]_o$ as it could be enhanced in 5 mM Ca^{2+}-contatining external solution (Fig. 2A and B) and completely suppressed in the zero Ca^{2+} solution (Fig. 2C and D).

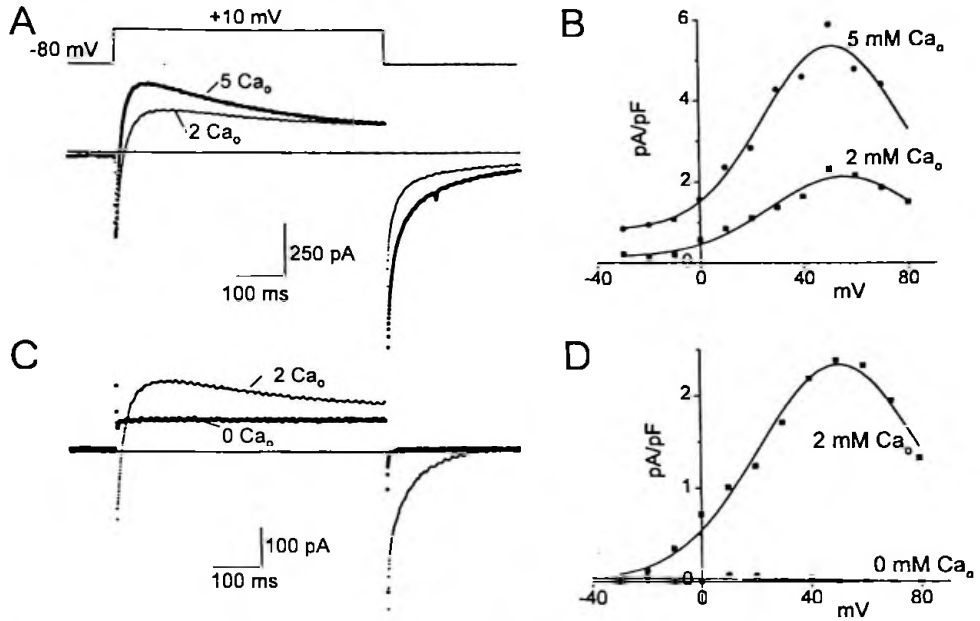
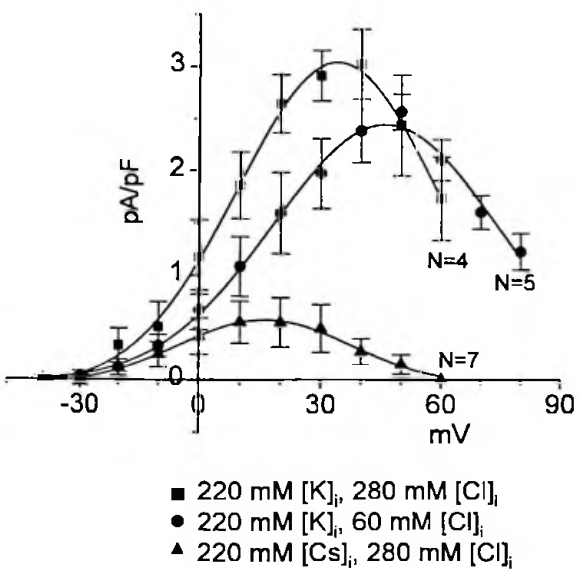


Figure 2. Effect of changes of $[Ca^{2+}]_o$ on transient outward currents (I_{to}) in shark ventricular myocytes. (A) Voltage pulse protocol and membrane currents in 2 and 5 mM Ca^{2+}-containing extracellular solutions. (B) Current-voltage relations in the presence of 2 and 5 mM $[Ca^{2+}]_o$. (C) Current traces in 2 mM Ca^{2+}-containing external solution and zero Ca^{2+} solution. (D) Current-voltage relations in 2 mM- and zero $[Ca^{2+}]_o$.

To identify the ionic nature of I_{to} we examined the effects of substituting Cl^- and K^+ by non-permeant cations in the internal solution. First, we carried out experiments with low Cl^- internal solution ($[Cl^-]_i$ = 60 mM) by substituting aspartate for Cl^- and compared the voltage dependence of I_{to} at different $[Cl^-]_i$ (Fig. 3). In five cells perfused with 60 mM $[Cl^-]_i$ internal solution (chloride equilibrium potential, E_{Cl}, = –38 mV) we could not observe a significant change in the magnitude of I_{to} or a shift of I_{to}-voltage relation toward more negative voltages (close to the apparent E_{Cl} value). Second, we replaced the internal K^+ with Cs^+. In seven cells tested the amplitude of I_{to} was significantly suppressed in Cs^+-containing internal solutions (0.47 ± 0.22 pA/pF).



We therefore concluded that, the transient outward current of shark ventricular myocytes, is carried primarily by potassium, and is activated by direct influx of calcium through the Ca^{2+} channel. Unlike some of the mammalian species it does not appear that the activation of this current is related to the release of Ca^{2+} from intracellular Ca^{2+} stores. (Supported by NIH #16152)

Figure 3. Effects of Cl^- and K^+ substitutions on the transient outward currents (I_{to}) in shark ventricular myocytes. Current-voltage relations of I_{to} in symmetric (280 mM) and low (60 mM) Cl^- internal solutions (Cl^- was substituted with aspartate.), and in Cs^+ containing internal solution (K^+ was replaced by Cs^+).