# IONIC CURRENTS UNDERLYING THE ACTION POTENTIAL OF ISOLATED CARDIAC MYOCYTES FROM SQUALUS ACANTHIAS

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We have attempted to characterize the membrane currents in single isolated ventricular myocytes using the giga-seal technique of O. Hamill et al.(Pflügers Arch., 391:85, 1981). Cells were prepared by cannulating one of the coronary arteries running along the length of the conus arteriosus. The initial perfusate was the standard extracellular solution without added $Ca^{2+}$ (Table 1). After 20 minutes, the perfusate was switched to an enzyme solution, containing 50 mg Collagenase I (Sigma) and 7 mg Protease XIV (Sigma) in 25 ml of $Ca^{2+}$ free Ringer for 20 minutes. The calcium activity of this solution was 0.220 mM. The solution used to wash out the enzyme also contained 0.2 mM $Ca^{2+}$. The cells were mechanically dispersed in this solution. The presence of 0.2 mM $Ca^{2+}$ in the enzyme and washout solutions appeared to prevent damage from $Ca^{2+}$-overload. Yields varied from 50% to 80% $Ca^{2+}$ tolerant cells. The temperature during perfusion was maintained at 30°C. Isolated cells were 3 - 6 μm in diameter, 150 - 300 μm in length and displayed clear striations (average sarcomere length of 1.9 μm). All cells were quiescent and studied at 22 - 25°C in 5 mM $Ca^{2+}$ Ringer solution. Glass pipettes with tip diameters between 3 - 4 μm and resistances between 0.5 - 2 mega ohms were used to achieve seal resistances of 5 - 15 giga ohms. The stimulation frequency was 0.1 Hz in all experiments unless specified otherwise. Table 1 lists the standard extracellular and intracellular solutions used in these experiments. We found that it was essential to maintain the $[Cl^-]_i$ below 20 mM to prevent loss of excitability; therefore, we used gluconate as the primary intracellular anion. It was also necessary to buffer the internal $Ca^{2+}$ between 10 - 100 nM with Ca-EGTA. Levels below this range resulted in gradual shortening of the action potential and loss of excitability over 5 - 15 minutes.

Figure 1, Panel A, shows a typical action potential with resting potential between -80 to -90 mV, and overshoot potential of +45mV. Currents that accompany a clamp pulse to zero mV from a holding potential of -80 mV are shown in Panel B. This panel shows that addition of 5 uM TTX eliminates the fast inward current but does not significantly affect the slower inward current. The addition of 0.25 mM $Cd^{2+}$ blocked the slower inward current as shown in Panel C. Like most cardiac preparations, an inwardly rectifying $K^+$ current was identified. Addition of 50mM TEA to the internal pipette solution not only suppressed the delayed time-dependent outward currents but also partially blocked the inwardly rectifying current. In most preparations the resting conductance generally ranged around 50 nS in control solutions. Addition of TEA to the internal solution reduced the resting conductance to about 4 nS. $Cs^+$ or $Ba^{2+}$ were more effective blockers of the inwardly rectifying $K^+$ channel when added to the extracellular space. Both $Ba^{2+}$ and $Cs^+$ blocked the $K^+$ channel in a voltage-dependent manner. $Ba^{2+}$ unlike $Cs^+$ showed time-dependent properties in blocking the inwardly rectifying channel.

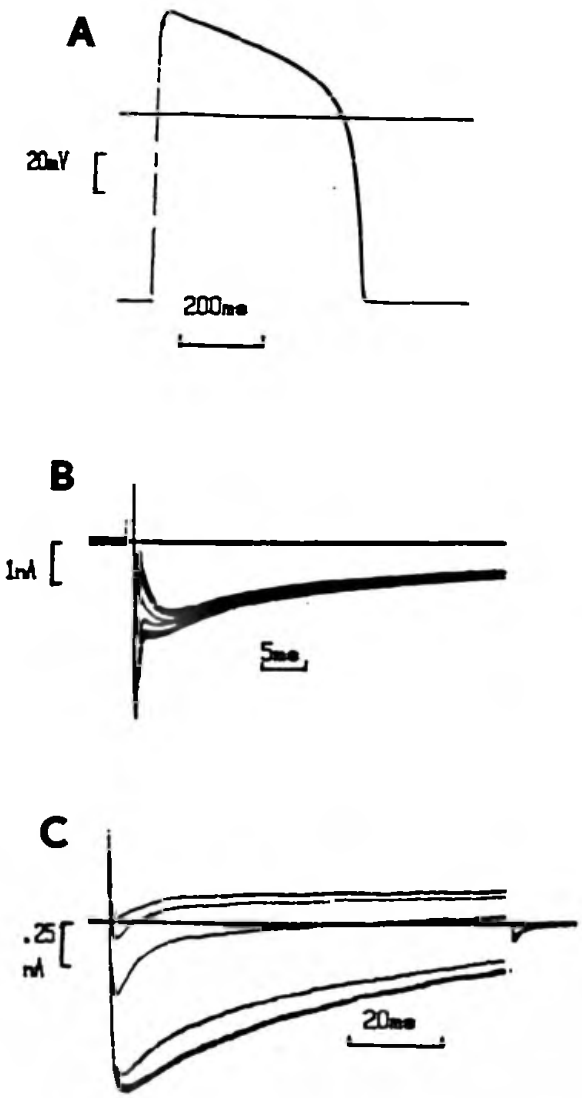


Figure 1

In some experiments we noticed an unexpected effect of $Cs^+$ on the $Ca^{2+}$ current. 20 mM $Cs^+$ greatly reduced the plateau height, duration of the action potential, and $Ca^{2+}$ current of myocytes in which $[Ca^{2+}]_i$ was reduced to the picomolar range by addition of 14 mM EGTA in patch pipette. This effect was reversible when $Cs^+$ was washed out. This effect of $Cs^+$ was not seen in the standard intracellular solutions where $[Ca]_i$ was in the nanomolar range. In both cases the inward rectifier was clearly blocked by $Cs^+$. These observations suggest possible alteration of the $Ca^{2+}$ channel selectivity in the presence of very low $[Ca^{2+}]_i$. The selectivity of the $Ca^{2+}$ channel in mammalian cardiac preparations has been shown to depend upon the external $Ca^{2+}$ (P. Hess & R. Tsien; Nature 309:453, 1984). It has been suggested that the selectivity of the channel to $Ca^{2+}$ is determined by $Ca^{2+}$ binding to a site within the channel thus preventing other cations from approaching and binding to the mouth of the channel through an electrostatic repulsion mechanism. One might suspect that lowering the internal $Ca^{2+}$ to the pico-molar range may remove the regulatory $Ca^{2+}$ ions within the channel thereby altering the channel selectivity and enabling a monovalent ion such as $Cs^+$ to block the channel.

We thank Dr. Michael Duffy for suggesting gluconate as a substitute for chloride.

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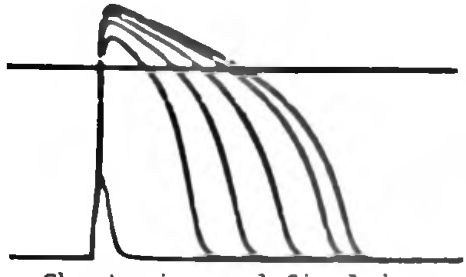
Table 1. Composition of Standard Solutions (mM)

| Internal | |
|---|---|
| K Gluconate | 150 |
| KCl | 25 |
| TMAO | 100 |
| $Na_2ATP$ | 10 |
| $MgCl_2$ | 10 |
| $CaCl_2$ | 1 |
| $K_2EGTA$ | 11 |
| Urea | 350 |
| HEPES | 20 |
| +KOH to pH=7.20 | |

| External | |
|---|---|
| NaCl | 280 |
| KCl | 4 |
| $MgCl_2$ | 3 |
| $NaH_2PO_4$ | 1 |
| $K_2SO_4$ | 0.5 |
| Urea | 300 |
| HEPES | 10 |
| +NaOH to pH=7.20 | |
| $CaCl_2$ | 5 |
| unless specified otherwise | |



Shortening and final inexcitability when K Gluconate is replaced by KCl