A FAST METHOD FOR ISOLATION OF SINGLE MYOCYTES FROM THE SPINY DOGFISH HEART (SQUALUS ACANTHIAS)

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A method is described for enzymatic isolation of ventricular myocytes from the elasmobranch Squalus acanthias modified from the technique described by Mitra and Morad (Am. J. Physiol. 249: H1056-1060, 1986). The cells obtained from this present method were identified by their spindle shapes and characteristically striated appearance. The cells were 6-8 um wide and 150-300 um long. This method of dissociation yields large numbers of calcium tolerant myocytes (80% viability) suitable for comparative electrophysiological studies.

Dogfish (2-7 kg) were heparinized (600 units/kg) before spinal pithing. After sacrifice, the heart was removed from the animal and placed in aerated Ca^{2+}-free elasmobranch physiological saline (270 mM NaCl, 4 mM KCl, 3 mM $MgCl_2$, 1 mM NaH_2PO_4, 350 mM urea, 10 mM Hepes, 0.5 mM dextrose; pH 7.2 with NaOH); this solution contained a few drops of heparin. It was imperative that the elasmobranch saline be made fresh daily. Next, the heart was mounted in a Langendorff apparatus and cannulated through the coronary vessels. Briefly, coronary vessels were carefully snipped and were cannulated bilaterally with polyethylene tubing (I.D. 0.38 mm, O.D. 1.09 mm) and were secured with surgical suture. Perfusion at 5 ml/min commenced with the introduction of Ca^{2+}-free elasmobranch buffer. The atria were removed and a cut was made at the apex of the ventricle to allow the drainage of blood. The perfusion solutions were oxygenated and maintained at 30° C. We found that successful enzymatic dispersion required monitoring the temperature of the heart closely. The initial perfusion was continued until all the blood was eliminated from the heart (approximately 5-10 minutes). The heart was the perfused with oxygenated enzymatic solution containing 1.2 mg/ml collagenase Type I (Sigma), 0.2 mg/ml protease Type XIV (Sigma). To prevent dilution of the enzymes, recycling was delayed 3 minutes. The enzymatic perfusion of the heart was performed for 30-35 minutes depending on the size of the heart. Finally, 25 ml of 200 uM Ca^{2+}-elasmobranch saline was perfused through the ventricle. The heart was removed from the cannulae and placed in 200 uM Ca^{2+}-elasmobranch buffer. The ventricle was cut and gently agitated in the buffer to release the dissociated myocytes into a volume of approximately 30 ml.

The physiological integrity of the myocytes isolated from this preparation is excellent since they tolerate electrophysiological experimentation. Figure 1 is a recording from a Squalus ventricular myocyte. The recording was taken using the "giga-seal" technique of Hamill et al. (Pfluegers Arch. 391: 85-100, 1981). Fig. 1 illustrates an action potential recorded under current clamp conditions. The cells obtained from this method of dissociation possess physiological and pharmacological properties of mammalian myocytes, making them an excellent model for comparative electrophysiological studies.

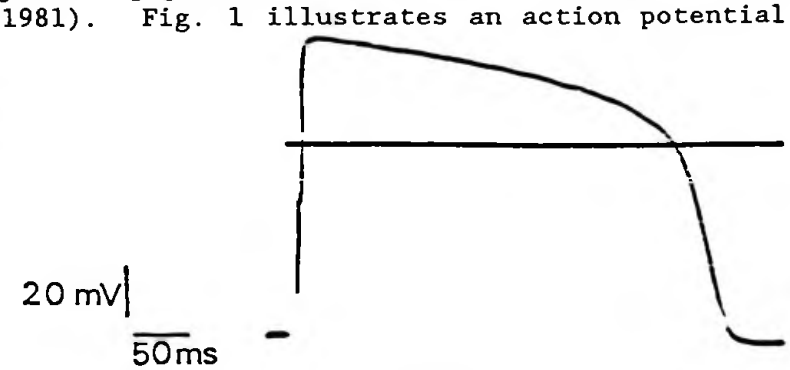

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Figure 1

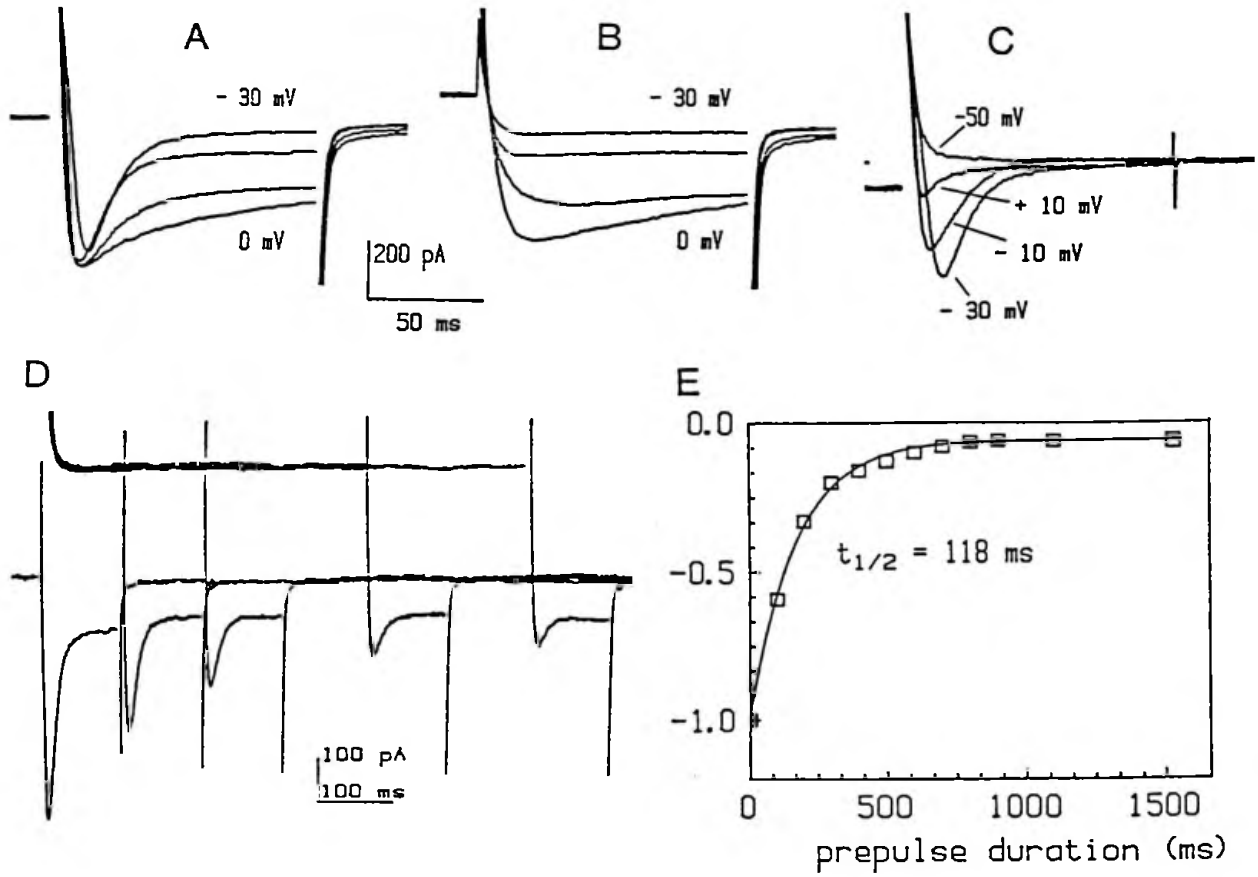

Figure 1. A-C: Voltage clamp records from single shark ventricular myocytes. Cells were dialysed with an internal solution containing (in mM) 200 N-Methyl-Glucamine, 60 NaCl, 10 MgATP, 300 Urea, 70 TMAO, 20 HEPES, 0.1 cAMP, 20 EGTA at pH=7.2, and superfused with a solution containing 270 NaCl, 4 KCl, 3 MgCl, 0.5 KH_2PO_4, 250 Urea, 10 HEPES and 3 CaCl at pH=7.2. In A, the membrane was held at a potential of -80mV and depolarized in 10 mV steps to voltages in the range of -30 to 0 mV. In B, the membrane was first pre-pulsed for 500 ms to a potential of -40 mV, and then depolarized to the same potentials in A. Notice that in contrast to A the rapidly inactivating (t) component of inward current is absent. C shows the difference current resulting from the subtraction of current records in B from A.

D-E: Time dependence of voltage-dependent inactivation of the t-channel. In D, the membrane was initially depolarized frlom -80mV to -20 mV to produce the leftmost inward current trace. Subsequent inward current traces were produced by pre-pulsing (from left to right) to -40 mV for 100, 200, 400, and 600 ms respectively before depolarizing to -20mV. The upper trace represents the current produced by the conditioning pulse. The decay of the rapidly inactivating component as a function of the duration of the pre-pulse is shown in D.