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L-Type Ca2+ Current Protocol

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This protocol is for patch-clamp recordings of L-type Ca2+ current and slow delayed rectifier K+ current responses to norepinephrine and acetylcholine.

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1 Membrane currents were recorded from isolated rat venticular myocytes using the whole-cell configuration of the patch clamp technique.

2 Isolated cells were placed in a perfusion chamber (Warner Instruments) on the stage of an inverted microscope (Olympus IX71) and bathed in a K^+-free extracellular solution containing (in mM) NaCl 137, CsCl 5.4, $MgCl_2$ 0.5, $CaCl_2$1.0, NaH_2PO_4 0.33, glucose 5.5, and HEPES 5 (pH 7.4), maintained at room temperature.

3 Cells were patched using microelectrodes with resistances between 1 and 2 MΩ. Access resistance was compensated to obtain series resistance errors of less than 5 mV.

4 Cells were dialyzed with a K^+-free microelectrode solution containing (in mM): CsCl 130, TEA-Cl 20, EGTA 5, MgATP 5, TrisGTP 0.06, and HEPES 5 (pH 7.2).

5 Whole cell currents were recorded under voltage-clamp conditions using a Multiclamp 700B voltage clamp amplifier, Digidata 1440A computer interface, and pClamp 11 data acquisition and analysis software (Molecular Devices). Data were lowpass filtered at 4 kHz, and sampled at 10 kHz.

6 The membrane potential was held at -80 mV. A 50 ms pre-pulse to -40 mV was used to inactivate Na^+channels. This was followed by a 100 ms test pulse to 0 mV to elicit the L-type Ca^{2+} current.

7 The time course of changes in the amplitude of the Ca^{2+} current was monitored by recording the amplitude of the peak inward current elicited during the test pulse to 0 mV applied once every 5 s.

8 A stable baseline (about 5 minutes) was obtained before application of test drugs.

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