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cAMP Regulated Cl- Current Protocol

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1 Membrane currents were recorded from isolated pig ventricular 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 140, CsCl 5.4, $CaCl_2$2.5, $MgCl_2$ 0.5, glucose 5.5, and HEPES 5 (pH 7.4), maintained at room temperature. Nisoldipine (1 μM) was added to the extracellular solution to block L-type Ca^{2+} channels.

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): Cs-glutamate 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 -30 mV. A 100 ms test pulse to +50 mV was used to measure the cAMP-regulated Cl^- current.

7 The time course of changes in the amplitude of the Cl^- current was monitored by measuring the amplitude of the current recorded during the test pulse to +50 mV applied once every 5 s.

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

9 The Cl^- current was defined as the cAMP-induced current obtained by subtracting the current recorded before application of test-drug(s) from those recorded after.

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