Cardiac Action Potential Protocol V.1

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1 Membrane potentials 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 on the stage of an inverted microscope and bathed in an extracellular solution containing (in mM) NaCl 140, KCl 5.4, $CaCl_2$2.5, $MgCl_2$ 0.5, glucose 5.5, and HEPES 5 (pH 7.4), maintained at 37^{o}C.

3 Cells were patched using microelectrodes with resistances between 1 and 2 MΩ.

4 Cells were dialyzed with an intracellular solution containing (in mM): K-aspartate 110, KCl 25, NaCl 5, MgATP 3, cAMP 0.002, phosphocreatine dipotassium 10, EGTA 0.01, and HEPES 10 (pH 7.2).

5 Membrane potential was recorded under current 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 Action potentials were elicited by applying a depolarizing stimulus (2 ms) once every 5 s. The amplitude of the stimulus was adjusted to approx. 1.2 times the minimum value needed to generate an action potential.

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

8 Cells were not used if the resting membrane potential was less than -75 mV, the stimulus artifact overlapped the upstroke of the action potential, or a stable baseline could not be established,

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