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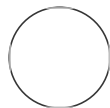
Cardiac Action Potential Restitution Protocol

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ABSTRACT

This protocol describes an approach that can be used to quantify the rate dependence of cardiac action potential duration.

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Protocol status: Working
We use this protocol and it's working

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75160

Keywords: cardiac action potential, restitution, action potential duration rate dependence

- 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, CaCl₂ 2.5, MgCl₂ 0.5, glucose 5.5, and HEPES 5 (pH 7.4), maintained at 37°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 (APs) were elicited by applying a depolarizing stimulus approximately 2 ms in duration. The amplitude of the stimulus was adjusted to approximately 1.2x the minimum value needed to trigger an AP.
- 7 Cells were not used if the resting membrane potential was less than -75 mV, the stimulus artifact overlapped the upstroke of the AP, or a stable baseline could not be established.
- 8 AP restitution was evaluated by triggering an AP once every 800 ms for 20 beats to establish a baseline. The stimulus interval was then reduced by 20 ms each subsequent beat for 10 beats, then by 10 ms each beat for 20 beats, and then by 5 ms each beat until we observed AP duration alternans or it was no longer possible to elicit an AP.
- 9 Myocytes were then exposed to test drug. During this period a new baseline was established by recording APs elicited once every 5 s for approximately 5 min.

- 10 Drug effects were then evaluated by repeating the restitution protocol outlined in step 8.
- 11 Data were analyzed by measuring the duration of each AP recorded during the restitution protocol and plotting it as a function of the preceding diastolic interval (time between 90% repolarization of the previous AP and upstroke of the current AP).
- 12 The slope of the restitution curve was then measured as the first derivative of the polynomial fit to the data at the shortest diastolic interval.