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Cardiac Action Potential Restitution Protocol

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We use this protocol and it's working

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Abstract

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

Troubleshooting

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, CaCl22.5, 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.