SUPPLEMENTAL MATERIAL

Methods

All experiments were carried out in compliance with the *Guide for Care and Use of Laboratory Animals published by the National Institutes of Health* (NIH publication No 85-23, Revised 1996) and approved by the Institutional Animal Care and Use Committee. We used mongrel dogs weighing 20–35 kg of either sex. The chest was opened via a left thoracotomy and the heart was excised. Transmural wedge preparations with dimensions of up to 32 x 20 x 15 mm were dissected from the LV wall. The preparations were cannulated via a distal diagonal branch of the left anterior descending coronary artery, or a left marginal branch of the circumflex artery, or a branch of the posterior descending artery and perfused with cardioplegic solution (Tyrode's containing 12 mmol/L KCl). Non-perfused regions of the tissue were removed using a razor blade. The preparations were then placed in a tissue bath and perfused with oxygenated Tyrode's solution (mM): NaCl 129, KCl 4, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 0.5, glucose 5.5, pH 7.4. The perfusate was delivered using a peristaltic pump (Masterflex peristaltic pump, Cole Parmer Instrument Co, Niles, Illinois) at a constant flow rate at 12-14 mL/min warmed to 37±0.5°C.

The preparations were equilibrated in the tissue bath until electrically stable, usually 1 hour. Pacing stimuli were delivered to the endocardial surface basic cycle length of 1000 ms using bipolar silver electrodes insulated except at the tips. The temperature of the perfusate was controlled by a heating bath associated with a glass condenser, a tube internally coiled within a wide cylindrical housing. The Tyrode's solution was warmed while passing through the heated coils to deliver the perfusate at 37°C. To simulate hypothermia, the solution was redirected to

two coiled-perfusion lines in series immersed in beakers filled with water, before reaching the tissues. We lowered the temperature of the perfusate to 32°C.

A transmural ECG was recorded using two electrodes consisting of AgCl half cells placed in the tissue bath, 1.0 to 1.5 cm from the Epi and Endo surfaces of the preparation, along the same axis as the transmembrane recordings (Epi electrode is connected to the positive input of the ECG amplifier). Transmembrane APs were simultaneously recorded from two Epi sites (Epi 1 and Epi 2; Epi1-Epi2 distance was approx. 10-20 mm) and one Endo site with the use of floating microelectrodes (DC resistance=10 to 20 MΩ) filled with 2.7 mol/L KCl, each connected to a high-input impedance amplifier. Impalements were obtained from the Epi and Endo surfaces of the preparation at positions approximating the transmural axis of the ECG recording. Spike 2 for Windows (Cambridge Electronic Design, Cambridge, UK) was used to record and analyze the ECG and the AP. NS 5806, cilostazol and milrinone were dissolved in dimethyl sulphoxide (DMSO); acetylcholine, verapamil HCl, quinidine, were dissolved in distilled water (10 mM stock). DMSO controls were performed to ensure the absence of an effect of the solvent.