Cardiac, Smooth, & Skeletal Muscle Electrophysiology II

1506-Pos Board B398

Remodelling of the Cardiac NCX1-TRPC3 Signaling Complex Promotes Angiotensin Ii-Induced Arrhythmogenesis

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TRPC3 has been demonstrated as a player in structural remodeling of the heart, while its proarrhythmogenic potential is incompletely understood. using a TRPC3 transgenic overexpression mouse model, we examined the impact of TRPC3 expression on cardiac actions of Angiotensin II (AngII) in whole hearts (Langendorff perfusion) and in isolated single ventricular myocytes. At low TRPC3 expression (WT mice) AngII elicited a previously described 3-phasic inotropic response without generating significant arrhythmias. By contrast, at high TRPC3 expression (TRPC3 transgenic mice; TRPC3-TG), AngII induced substantial impairment of cardiac contractile function associated with prominent bigeminal beats and ventricular tachycardia. Calcium imaging experiments revealed a dramatic increase in diastolic Ca^{2+} levels associated with spontaneous sarcomere shortenings during AngII exposure of TRPC3-TG myocytes. More detailed electrophysiological characterization demonstrated AngII-induced depolarization, distortion of action potential morphology and enhanced frequency of delayed afterdepolarizations (DADs) in TRPC3-TG. This phenotype was not observed in WT mice. Analysis of NCX-tail currents suggested that AngII-induced arrhythmias were based on profound NCX-1 forward mode-associated depolarization. On the contrary, our analysis of Ca^{2+} handling in TRPC3-TG myocytes at basal conditions clearly indicated suppressed forward-mode NCX-1 action as expected from enhanced Na^{+} entry at TRPC3- NCX-1 signalplexes. Immunocytochemistry demonstrated a partial disruption of the tight colocalization of TRPC3 and NCX-1 confined to the T-tubuli system during AngII stimulation of TRPC3-TG myocytes. Our results confirm the concept of a close physical and functional coupling between TRPC3 and NCX-1, which results in a digitalis-like modulation of cardiac function by TRPC3 overexression in non-stimulated myocardium. AngII is suggested to disrupt the organization of TRPC3- NCX-1 signalplexes, thereby enabling NCX-1-mediated distortion of AP morphology, DADs and arrhytmogenesis.

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The Importance of the Sarcoplasmic Reticulum for the Influence of I(F) Carried by Hyperpolarization-Activated Cyclic Nucleotide-Gated Ion Channels on Pacemaker Activity in the Sino-Atrial Node

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The mechanisms underlying pacemaker activity in the sino-atrial (SA) node remain controversial, with some giving greatest prominence to I(f), others emphasising a 'calcium clock', and some favouring membrane currents other than I(f). The aim of the present experiments was to explore the possible dependence of I(f) on cytosolic Ca^{2+}, including that released from the sarcoplasmic reticulum (SR). ZD7288 applied to block I(f) reduced spontaneous rate from 505 ± 24 to 228 ± 24 bpm (reduction 55 ± 4 % expressed as % resting rate). Ryanodine (to interfere with SR Ca^{2+} release) reduced beating rate, and with ryanodine present the effects of ZD7288 were smaller: 197 ± 40 bpm (ryanodine alone) to 148 ± 40 bpm (ryanodine and ZD7288), a reduction of 27 ± 12%. Cyclopiazonic acid (CPA) to inhibit Ca^{2+} re-uptake by the SR reduced spontaneous rate, and ZD7288 effects were also smaller in the presence of CPA: 213 ± 13 bpm (CPA alone) to 147 ± 9 bpm (CPA and ZD7288), a reduction of 19 ± 4%. In addition, ZD7288, ryanodine and CPA in various combinations reduced the slope and maximum response of the log(concentration)-response curve for effects of isoprenaline on spontaneous rate.

These observations are consistent with a role for Ca^{2+} released from the SR in regulating I(f) and therefore the rate of spontaneous beating of the SA node, although there appears to be an additional contribution of SR-derived Ca^{2+} to the effects of β-adrenoceptor stimulation on spontaneous rate that is independent of I(f).

1508-Pos Board B400

Acute Effect of Bacterial Lipopolysaccharide on Cardiac Sodium Current

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Inflammation, sepsis and septic shock are correlated with cardiac arrhythmias like atrial fibrillation. In these conditions, levels of endotoxins such as lipopolysaccharides (LPS) from the outer membrane of bacteria are increased. LPS activates the human immunomodulatory defense system and contributes to the pathophysiology of sepsis. It is hypothesized that endotoxins like LPS play a role in cardiac arrhythmogenesis during sepsis, but a modulatory role of LPS on cardiac ion channels is studied less intensely. We hypothesized that LPS had a direct effect on cardiac sodium channels. To investigate this, we studied the acute effects of LPS on the kinetic properties of cardiac Na+ channel. Methods: HEK293 cells expressing the LPS receptor complex, consisting of cluster of differentiation 14 (CD14) and toll-like receptor 4 (TLR4) were transiently transfected with a a vector containing wild-type SCN5A sodium channel + GFP and a vector containing the sodium channel beta1-subunit. Successfully transfected cells, identified by fluorescence of GFP, were used in whole-cell voltage clamp experiments. Voltage dependence of activation and inactivation, recovery from inactivation, and development of slow inactivation of sodium current were determined immediately before and after application of LPS. Results: LPS acutely shifts both activation and inactivation to more negative voltages and slows recovery from inactivation, which is expected to decrease sodium current and thereby conduction velocity in vivo, which predisposes for re-entrant arrhythmias.

1509-Pos Board B401

TTX Converts Polymorphic VT to Monomorphic VT in Transgenic Rabbit Model of LQT1

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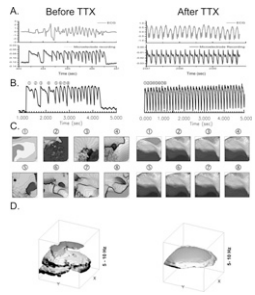
Long QT syndrome Type 1 (LQT1) is a congenital disease defined by loss of function mutations in KCNQ1, and associated with syncope and sudden death preceded by polymorphic ventricular tachycardia (pVT). We hypothesized that inhibition of sodium channel function suppresses pVT formation. using a transgenic rabbit model of LQT1 (n=4), we simultaneously mapped activation patterns from a voltage sensitive dye and microelectrode. The AV node was ablated to slow intrinsic heart rate, isoproterenol (140nM) was injected to mimic sympathetic stimulation, and pVTs were subsequently observed (avg = 3-10 s). The associated activation patterns revealed multifocal activities and complex waveform propagation varying in spatial origin and direction. Subsequent perfusion of a sodium channel blocker, TTX (2 μM), notably converted pVTs toward monomorphic activation patterns. We also report evidence of fully rotating spiral waves, and their role in maintaining monomorphic VTs. Taken together, these results strongly suggest that 1) early afterdepolarizations (EADs) play an important role in maintaining pVTs complexity, 2) sodium currents significantly influence EAD regeneration by their continuous firing during pVTs, 3) sodium channel inhibition by TTX reduces EAD generation and stabilizes reentry into monomorphic VTs.



1510-Pos Board B402

Different Ventricular Fibrillation (VF) Dynamics in Long QT Syndrome Type 1 vs. 2 in a Transgenic Rabbit Model

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Long QT syndrome (LQTS) is a congenital disease characterized by APD prolongation and associated with sudden cardiac death. LQT1 and LQT2, the most common types of LQTS, lack delayed rectifying potassium currents, I_{Ks} and I_{Kr}, respectively. We hypothesize that the differential kinetic properties of I_{Ks} and I_{Kr} can influence vulnerability to ventricular fibrillation (VF) through genotype-specific enhancement of VF maintenance. We investigated VF