Cx43 hemichannels in ventricular cardiomyocytes can be activated by an elevation of cytoplasmic Ca\(^{2+}\) through a CaM-dependent signaling cascade and are a potent contributor to cardiac arrhythmogenesis

Authors:
M De Smet\(^1\), A Lissoni\(^1\), N Wang\(^1\), L Leybaert\(^1\), \(^1\)Ghent University, Department of Basic Medical Sciences - Physiology Group - Ghent - Belgium,

Topic(s):
Ion channels, ion exchangers and cellular electrophysiology - Heart

Citation:
Cardiovascular Research Supplements (2016) 111 (S1), S54

Introduction and purpose: Cx43, most abundantly expressed in the ventricles, is engaged in forming both gap junctions that electrically couple the cardiac tissue and unapposed hemichannels (HCs) that function as a non-selective transmembrane conduit. The latter are typically closed but open in response to electrical or chemical triggers such as pro-inflammatory cytokines or elevation of cytoplasmic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)). Evidence for hemichannel opening by [Ca\(^{2+}\)]\(_i\) elevation is based on indirect ATP/dye uptake measurements, but conclusive electrophysiological evidence is lacking. Here, we studied [Ca\(^{2+}\)]\(_i\)-dependent activation of cardiac Cx43 HCs with the use of single-channel recordings and further proposed their arrhythmogenic potential.

Methods: Left ventricular cardiomyocytes were acutely isolated from C57/Bl6 and inducible Cx43 knockdown mice (Cx43Cre-ER(T)/Fl). We provoked Ca\(^{2+}\) release from the sarcoplasmic reticulum by local application of caffeine (10 mM) under voltage-clamp conditions (Vm = -70 mV) and studied the effect on HC unitary currents using whole-cell patch-clamp techniques. Involvement of Cx43 HCs was verified by the biophysical properties, intracellular application of Gap19 (100 µM) and CT9 (100 µM) (a selective Cx43 HC blocker and positive modulator respectively) and Cx43 knockdown. Involvement of [Ca\(^{2+}\)]\(_i\) was assessed by applying the Ca\(^{2+}\)-chelator EGTA (10 mM) intracellularly. Calmodulin (CaM) antagonist W7 (50 µM) was used to investigate the contribution of CaM signaling.

Results and conclusions: Applying caffeine pulses (8 s) at resting membrane potential activated an inward current carried by the Na\(^+)/Ca\(^{2+}\) exchanger (NCX). Superimposed on this macroscopic current, there appeared microscopic current spiking activity characterized by a unitary conductance of ~210 pS. Unitary currents were inhibited by EGTA, by conditional Cx43 knockdown (Cx43Cre-ER(T)/Fl) and by the Cx43 HC blocking peptide Gap19. Additionally, CT9 peptide promoted the HC currents activated by caffeine-induced Ca\(^{2+}\)-release while delivery of W7 into the cell completely abolished HC currents. The results indicate that Cx43 HCs can be opened by Ca\(^{2+}\)-stimulation without any associated electrical stimulus through a CaM-dependent signaling cascade. The kinetics of Ca\(^{2+}\)-triggered opening activity were very different from voltage-triggered activity (fast spiking versus prolonged opening respectively) indicating a distinct gating mechanism. Computational modeling of the impact of electrically/chemically-triggered Cx43 HC opening on cardiomyocyte excitability indicates that this may contribute to arrhythmogenesis in the heart by providing depolarizing inward currents with potential for generating delayed afterdepolarizations and subsequent focal excitation.