Overexpression of HCN-encoded pacemaker current silences bioartificial pacemakers

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DK Lieu[1,2], YC Chan[2,3], CP Lau[3], HF Tse[1,3], CW Siu[1,2,3], RA Li[1,2,3]
[1]Stem Cell & Regenerative Medicine Program, Department of Medicine & HBHA, The University of Hong Kong, Hong Kong
[2]Stem Cell Program, UC Davis, California, United States
[3]Division of Cardiology, Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Current strategies of engineering bioartificial pacemakers from otherwise silent yet excitable adult atrial and ventricular cardiomyocytes primarily rely on either maximising the hyperpolarisation-activated I_f or on minimising its presumptive opponent, the inwardly rectifying potassium current I_{K1}. The purpose of this study was to determine quantitatively the relative current densities of I_f and I_{K1} necessary to induce automaticity in adult atrial cardiomyocytes.

Methods: Automaticity of adult guinea pig atrial cardiomyocytes was induced by adenovirus (Ad)-mediated overexpression of the gating-engineered HCN1 construct HCN1-DeltaDeltaDelta with the S3-S4 linker residues EVY235-7 deleted to favour channel opening.

Results: Whereas control atrial cardiomyocytes remained electrically quiescent and had no I_f, 18% of Ad-CMV-GFP-IRES-HCN1-DeltaDeltaDelta (Ad-CGI-HCN1-DeltaDeltaDelta)–transduced cells demonstrated automaticity (240±14 bpm) with gradual phase-4 depolarisation (143±28 mV/s), a depolarised maximal diastolic potential (-45.3 ±2.2 mV), and substantial I(f) at -140 mV ($I_{f,-140\ mV}$= –9.32±1.84 pA/pF). In the remaining quiescent Ad-CGI-HCN1-DeltaDeltaDelta–transduced atrial cardiomyocytes, two distinct immediate phenotypes were observed: (1) 13% had a hyperpolarised resting membrane potential (-56.7±1.3 mV) with $I_{f,-140\ mV}$ of -4.85±0.97 pA/pF; and (2) the remaining 69% displayed a depolarised resting membrane potential (-27.6±1.3 mV) with I(f,-140 mV) of -23.0± 3.71 pA/pF. Upon electrical stimulation, both quiescent groups elicited a single action potential with incomplete phase-4 depolarisation that was never seen in controls. Further electrophysiologic analysis indicates that an intricate balance of I_{K1} and I_f is necessary for induction of atrial automaticity.

Conclusion: Optimised pacing induction and modulation can be better achieved by engineering the I_f/I_{K1} ratio rather than the individual currents.

Toll-like receptor 4 mediates tubular inflammation in diabetic nephropathy

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M Lin, AWH Chan, LYY Chan, JCK Leung, KN Lai, SCW Tang
Division of Nephrology, Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Toll-like receptor 4 (TLR4) has been implicated in the regulation of immune responses and inflammatory disease. Diabetic nephropathy is being increasingly recognised to comprise a heavy inflammatory element that results in tubulointerstitial lesions, which govern overall renal prognosis. However, the role of TLR4 in tubulointerstitial injury during diabetic nephropathy is still unknown.

Methods: Human proximal tubular epithelial cells (PTEC) were employed to examine the effect of high glucose (HG) on TLR4 expression. The Ikappa B/NF-kappa B activation was examined by western blot and ELISA. TLR4 content was detected by immunohistochemistry in nine human renal biopsies with histologically proven diabetic nephropathy.

Results: HG induced TLR4 overexpression in PTEC in a time- and dose-dependent manner, resulting in upregulation of IL-6 mRNA via Ikappa B/NF-kappa B activation. Blockade of TLR4 in PTEC by pre-incubation with a neutralising antibody resulted in a significant decrease in HG-induced Ikappa B/NF-kappa B activation, and the associated downstream IL-6 synthesis. Immunohistochemical analyses of human renal biopsies revealed that TLR4 were expressed in proximal and distal tubules, with more intense staining in kidneys with histologically proven diabetic nephropathy compared with normal controls.

Conclusion: Our findings suggest a novel TLR4-mediated pathway through which hyperglycaemia may contribute to tubular inflammation in the diabetic kidney.