P^{21} activated kinase-1 as a key regulator of cardiac automaticity and excitability

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Cardiac electrical function is highly regulated on a beat to beat basis by multiple extra- and intra-cellular signalling pathways that operate through the modulation of the activity and properties of the ion channels across the membrane of the heart cells. How such regulation is designed to maintain cardiac electrical function under physiological conditions, and how it comes into play in the face of disease conditions are important issues to be addressed. Our recent studies suggest a novel role of P^{21} activated kinase-1 (Pak1) in regulating cardiac electrical and contractile functions (Ke *et al.*, 2007; Liu *et al.*, 2011).

The present study thereby aims at further clarifying the mechanisms underlying the critical roles of Pak1 in regulating cardiac electrical function and its therapeutic potential in cardiac arrhythmogenesis. We developed a mouse model carrying a cardiomyocyte-restricted deletion of Pak1 ($Pak1^{CKO}$). $Pak1^{CKO}$ mice displayed relative high basal heart rate with generally normal electrocardiography (ECG) parameters such as P-R, QRS and QT intervals compared with their control littermates $Pak1^{f/f}$ mice. To evaluate ventricular arrhythmic vulnerability, both $Pak1^{CKO}$ and $Pak1^{f/f}$ (control) mice were subjected to acute stress by either treating the mice with isoprenaline (ISO) *in vivo* (1 mg/kg, i.p), or an *ex vivo* heart preparation (10-50 nM ISO), or cardiac hypertrophy induced by chronic treatment of ISO (at concentration of 100 mg/ml/g bodyweight for two weeks delivered by mini-osmotic pump) (Charles river labs, UK). $Pak1^{CKO}$ mice showed significantly higher incidence of ventricular arrhythmias than $Pak1^{f/f}$ mice in both acute and chronic stress conditions. Ventricular myocytes isolated from $Pak1^{CKO}$ mice displayed disturbances in their rhythmic Ca^{2+} oscillations, and occurrence of delayed afterdepolarizations. We investigated sarcoplasmic reticulum (SR) and sarco/endoplasmic reticulum calcium ATPase (SERCA) function. The myocytes were stimulated constantly to reach a steady state prior to 10mM caffeine application to empty the SR, then the SR was refilled by constant stimulation. The SR refill rate in $Pak1^{cko}$ myocytes was significantly prolonged, particularly in chronic ISO treatment. The function of SERCA of $Pak1^{f/f}$ & $Pak1^{cko}$ were calculated and a significant reduction in function of SERCA in $Pak1^{cko}$ myocytes was detected under both basal and chronic ISO conditions. Moreover, we found that the Pak1 activator FTY720, a sphingosine-like analog, was able to attenuate ventricular arrhythmias associated with ISO-induced hypertrophy in wild-type mice but failed to exert such an effect on $Pak1^{cko}$ mice, suggesting that the antiarrhyhmic effect of FTY720 likely acts through Pak1 activation.

Our results thus indicate that Pak1 is a key regulator of cardiac automaticity and excitability. As a novel anti-arrhythmic regulator, Pak1 may be a potential therapeutic target for the treatment of cardiac tachyarrhythmias associated with stress conditions.

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