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Dissertations-Kurzfassung

Alpha 1-adrenoceptor signaling contributes to toxic effects of catecholamine on electrical properties in human-induced stem cell-derived cardiomyocytes

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Background: Takotsubo cardiomyopathy (TTC) is characterized by a transient regional wall motion abnormality with clinical manifestations similar to that of an acute coronary syndrome (ACS), including chest pain, dyspnea and T-wave inversion or S-T segment elevation in ECG. TTC can present severe complications including long QT syndrome (LQTs) and life-threatening arrhythmias. It is well-known that TTC is a stress-associated cardiomyopathy, but the mechanisms are not clearly clarified. Catecholamine excess and beta adrenoceptor signaling are implicated in the pathogenesis of TTC. β-blockers are also prescribed for some TTC-patients. Surprisingly, some clinical studies reported that application of β-blocker showed no benefits regarding the in-hospital mortality and the mortality after 1 year of follow-up. Furthermore, no benefit of β-blockers in preventing recurrence of TTC was detected in two meta-analyses. Animal studies showed that alpha-adrenoceptor blocker helped ameliorate phenotypic changes of TTC. All of these evidences suggest that mechanisms other than beta-receptor mediated signaling contribute also to the pathogenesis of TTC. However, studies on non-beta-adrenoceptor signaling for pathogenesis of TTC remain spars. Experimental data about the importance of alpha-adrenoceptor signaling for LQTs and occurrence of arrhythmias under circumstances of TTC are lacking.

Objectives: We aimed to study possible roles and underlying mechanisms of alpha-adrenoceptor activation for ion channel dysfunctions, abnormal action potentials and the occurrence of arrhythmias in high concentration of catecholamine mimicking the setting of TTC.

Methods: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), which were generated from human skin fibroblasts of three healthy donors, were treated with toxic concentration of epinephrine (Epi, 0.5 mM for 1h) or phenylephrine (Pheny, 0.1 mM for 1 h) to mimic the setting of TTC. Patch clamp, qPCR, immunostaining and FACS techniques were employed for the study.

Results: High concentration Epi suppressed the depolarization velocity and prolonged the duration (APD) of action potentials (APs) and induced arrhythmic events in iPSC-CMs. The Epi effects were attenuated by an alpha-adrenoceptor blocker (Phentolamine). An alpha 1-agonist (Pheny) but not an alpha 2-agonist (clonidine) mimicked Epi-effects, suggesting an involvement of alpha 1-adrenoceptor signaling in APD-prolongation and arrhythmogenesis in the setting of TTC. Epi enhanced ROS-production, which could be attenuated by the alpha-adrenoceptor blocker. Treatment of cells with H_2O_2 (100 µM) mimicked the effects of Epi and Pheny on APs and a ROS-blocker (NAC, 1 mM) prevented the Epi and Pheny effects, indicating that the ROS-signaling is involved in the alpha 1-adrenoceptor actions. DPI, an inhibitor of NADPH oxidases, blocked the Pheny effects, implying that NADPH oxidases were involved in alpha 1-adrenoceptor signaling. A PKC-blocker (chelerythrine) suppressed the effects of Epi and Pheny, whereas a PKC activator (PMA) mimicked the effects, implying that PKC participated in alpha 1-receptor signaling. The PKC blocker blocked the effects of H_2O_2, suggesting that PKC acted as a downstream factor of ROS. Epi or Pheny suppressed the peak sodium channel currents and rapidly activating rectifier K currents and enhanced the L-type Ca channel currents, which can explain the abnormal APs induced by alpha-adrenoceptor activation.

Conclusions: The alpha-adrenoceptor signaling plays important roles for ion channel dysfunction, long QT interval and arrhythmogenesis in the setting of catecholamine excess via alpha1-adrenoceptor-NADPH-ROS-PKC related signaling. The application of alpha-blocker may be helpful for treating arrhythmias in patients with TTC- or other cardiac disorders associated with toxic catecholamine effects.