



Title	Roles of Functional Ion Channels In Human Cardiac C-Kit+ Progenitor Cells
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assay were applied to observe the effect of the TRP channels on cell migration. Cell proliferation assay was made with MTT and ^3H -thymidine incorporation approaches.

Results: A small background current was inhibited by the TRPC channel blocker La^{3+} . Removal of Mg^{2+} of pipette solution or bath solution induced a Mg^{2+} -sensitive TRPM7 current, and the current was suppressed by the TRP channel blocker 2-aminoethoxydiphenyl borate. RT-PCR revealed significant mRNA expression of TRPC1, TRPC3, TRPC4, TRPV2, TRPV4, and TRPM7 channels in human preadipocytes. Western blot analysis confirmed the protein expression of these TRP channels. ShRNAs targeting TRPV2, TRPV4 and TRPM7 suppressed the corresponding gene and protein expression. Interestingly, TRPV2-shRNA and TRPM7-shRNA significantly reduced proliferation of human cardiac c-kit⁺ cells. Migration of human cardiac c-kit⁺ cells was reduced by TRPV2-shRNA, TRPV4-shRNA.

Conclusion: Our results demonstrate for the first time that multiple TRP channels, TRPC1/3/4, TRPV1/2/4, and TRPM7, are present in human cardiac c-kit⁺ cells. TRPV2, TRPV4 and TRPM7 channels participate in regulating migration and proliferation in human cardiac c-kit⁺ progenitor cells.

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PATCH-CLAMP STUDY OF SINGLE RYANODINE RECEPTOR CHANNELS IN THE OUTER NUCLEAR MEMBRANE

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Modulation of cytoplasmic free calcium (Ca^{2+}) concentration is a universal signaling pathway that regulates numerous cellular processes. Ubiquitous intracellular Ca^{2+} release channels – inositol 1,4,5-trisphosphate receptor (InsP_3R) and ryanodine receptor (RyR) channels – localized in the sarco/endoplasmic reticulum (ER) play a central role in this pathway in all animal cells. Electrophysiological study of the single-channel conductance and gating properties of these Ca^{2+} release channels with conventional patch-clamp approach has been hindered by their intracellular localization. To overcome this limitation, patch-clamp electrophysiology has been applied on isolated nuclei where these Ca^{2+} release channels are found abundantly in the outer nuclear envelope. We have successfully utilized this nuclear membrane electrophysiology to study the gating properties of single InsP_3R channels in several cellular systems. Whereas, all the current single channel data, including channel conductance, permeation properties, and ligand regulation, of the RyR channels were done exclusively by reconstituting the channels into artificial planar lipid bilayers. To gain insights into the single channel properties of the RyR in its native membrane milieu, we applied nuclear membrane electrophysiological study on isolated nuclei from stable-inducible mouse RyR2 HEK-293 cells. Using potassium as charge carrier, caffeine activated single channel current with conductance of ~ 750 pS in isolated nuclei. This caffeine activated current showed a linear current/voltage relationship under symmetrical ionic conditions and was sensitive to non-specific RyR inhibitor, ruthenium red. Furthermore, the single RyR channels recorded from the outer nuclear membrane exhibited bi-phasic Ca^{2+} regulation. In conclusion, we demonstrated, for the first time, that single RyR channels recordings from isolated nuclei and our results suggested that the nuclear membrane electrophysiology could be a sensitive and robust technique to study the gating properties of intracellular channels, including the InsP_3R and RyR.

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ROLES OF FUNCTIONAL ION CHANNELS IN HUMAN CARDIAC C-KIT+ PROGENITOR CELLS

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Background and objectives: Cardiac progenitor cells play an important role in cardiac repair and regeneration; however, cellular biology and electrophysiology are not understood. The present study was to investigate the functional ion channel expression in human cardiac c-kit⁺ progenitor cells and the

potential roles of these ion channels in regulating proliferation and migration.

Methods: Multiple experimental approaches were employed in this study, including whole-cell patch voltage-clamp, RT-PCR, Western blots, cell proliferation and migration assays, etc.

Results: Several ionic currents were heterogeneously expressed in human cardiac c-kit⁺ progenitor cells, including a large conductance Ca²⁺-activated K⁺ current (BKCa) in most (93%) of cells, an inwardly-rectifying K⁺ current (I_{Kir}) in 87% of cells, a transient outward K⁺ current (I_{to}) in 39% of cells, a voltage-gated tetrodotoxin-sensitive Na⁺ currents (I_{Na,TTX}) in 76% of cells. Molecular identities of these ionic currents were determined with RT-PCR and Western blot analysis. KCa.1.1 (for BKCa), Kir2.1 (for I_{Kir}), Kv4.2, Kv4.3 (for I_{to}), NaV1.2, NaV1.3, NaV1.6, NaV1.7 (for I_{Na,TTX}) were expressed in human cardiac progenitor cells. Inhibition of BK_{Ca} with paxilline, I_{to} with 4-aminopyridine, but not I_{Na,TTX} with TTX and I_{Kir} with Ba²⁺, decreased cell proliferation. Silencing of KCa.1.1, Kv4.2 or Kv4.3, but not Kir2.1, with siRNA targeting corresponding channels reduced proliferation. Inhibition of KCa.1.1 or Kv4.2 or Kv4.3 channels accumulated cells at G0/G1 phase. Interestingly, down regulation of KCa.1.1, Kv4.2 or Kv4.3 channels decreased, while of Kir2.1 channels increased migration in human c-kit⁺ progenitor cells.

Conclusions: These results demonstrate for the first time that multiple ion channels are expressed in human cardiac c-kit⁺ cells. KCa.1.1, Kv4.2, and Kv4.3 channels, but not Na⁺ channels and Kir 2.1 channels, participate in regulating proliferation. KCa.1.1, Kv4.2 or Kv4.3 channels promote, while Kir2.1 channels reduce cell migration in human cardiac c-kit⁺ progenitor cells.

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CHRONIC INTERMITTENT HYPOXIA INDUCES OXIDATIVE STRESS AND INFLAMMATION VIA ANGIOTENSIN II RECEPTOR 1 IN RAT LIVER

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Chronic intermittent hypoxia (IH) associated with obstructive sleep apnea (OSA) is characterized by repetitive cycles of hypoxia and reoxygenation, leading to excessive production of reactive oxygen species and oxidative stress in tissues and organs. However the mechanistic effects of chronic IH on the liver are not clear at present. We hypothesized that renin-angiotensin system (RAS) plays a role in the IH-induced oxidative stress and tissue inflammation in the rat liver.

Adult Sprague-Dawley rats were exposed to air (normoxic (Nx) control) or IH treatment (with inspired oxygen fraction in the normobaric chamber cyclic between 5-21% ± 0.5% per min, 8 hours per day) for 14 days. Rats were fed with an angiotensin II type 1 (AT1) receptors blocker telmisartan (10mg/kg body weight), or vehicle daily before the IH treatment. Hepatic expression levels of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β were detected with ELISA assay; serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were examined for liver injury; also the level of oxidative stress with malondialdehyde (MDA) in the liver.

Our results showed that the protein expression of IL-6, TNF-α and IL-1β were significantly higher in the hypoxic group than that of the Nx control and telmisartan-treated hypoxic (TIH) groups, suggesting that inhibition of the binding of angiotensin II to AT1 receptors attenuates IH-induced tissue inflammation in the rat liver. In addition, the MDA level was significantly elevated in the hypoxic group but was normalized by the telmisartan treatment. Furthermore, the serum ALT to AST ratio was increased significantly in the hypoxic group when compared to the Nx and TIH groups.

In conclusion, blockade of the AT1 receptor mitigates oxidative stress, tissue inflammation and cellular injury in the liver of rats exposed to chronic IH mimicking a severe OSA condition, thus supporting a pathogenic role of RAS in the IH-induced hepatic injury.

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NADPH OXIDASE UPREGULATED BY AT1 RECEPTOR MEDIATES CHRONIC INTERMITTENT HYPOXIA-INDUCED OXIDATIVE STRESS AND INFLAMMATION IN RAT ADRENAL MEDULLA

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