Al collegio docenti del Dottorato in Medicina Molecolare

Dr.ssa Valentina Balducci

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Tutor Prof.ssa Elisabetta Cerbai

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Electrophysiological characterization of novel blockers of hyperpolarization-activated cyclic nucleotide-gated channels

Introduction. Hyperpolarization-activated cyclic-nucleotide-gated (HCN) channels are voltage-dependent ion channels that are activated by hyperpolarization. They comprise four isoforms (HCN1–4) mediating a Na_+/K_+ current (I_{HCN}) and regulated by cyclic nucleotides. HCN channels are involved in several physiological functions including rhythmic electrogenesis of pacemaker cells. Ivabradine, a zatebradine derivative in clinical use as bradycardic agent, blocks I_{HCN} without selectivity toward HCN isoforms. Therefore, identification of HCN selective blockers could be important to identify pharmacological tools to study the role of HCN channels in physiological and pathological processes and develop drugs to prevent or treat diseases involving changes of HCN function.
Methods. Five new zatebradine derivatives (GG10R, GG9R, PK15R, PK19 and PK9) were tested by single-cell patch-clamp recordings on HEK293 cells expressing recombinant HCN1, 2 and 4. Derivative effects were analysed in term of percent current blockade, potency and channel state-dependency.
Results. All derivatives were effective to inhibit I_{HCN1-4} at all potentials tested with different selectivity towards single HCN isoforms. At physiological potential (-80 mV) 5μM GG10R, GG9R and PK15R were most effective on I_{HCN4} that was reduced by 84%, 74% and 64%, respectively. They also suppressed I_{HCN1} by 70% to 49% and I_{HCN2} by 70% to 20%. PK19 and PK9 were most effective on I_{HCN2} that was reduced by 86% and 100%. They also blocked I_{HCN1} by 62% and 58% and I_{HCN4} by 84%, and 48%, respectively. State dependency study showed all derivatives act as use-dependent blockers.
Future investigations will thoroughly study PK19 properties that appear of particular interest for isoform selectivity and fast kinetics of HCN2 isoform blockade. To this aim experimental approach will be implemented using human pluripotent-derived sinoatrial nodal cells that similarly to the native tissue has elevate levels of HCN channel expression.