in the channels composed by V65M (5.5 ± 0.03 μM) and G343D (8.4 ± 0.04 μM) Kir6.1 + SUR2AWT subunits. Pinacidil-induced increase of Kir6.2WT+SUR2A H60Y and Kir6.2WT+SUR2A S1020P was similar to that of Kir6.2WT+SUR2AWT channels. Unexpectedly, pinacidil failed to increase I_{KATP} generated by Kir6.2WT+SUR2A S1054Y channels. Glibenclamide inhibited currents generated by Kir6.1WT+SUR2AWT and Kir6.2WT+SUR2AWT in a similar extent, yielding inhibitory concentration 50 (IC_{50}) values (0.3 ± 0.1 and 0.22 ± 0.06 μM, respectively) within the range of therapeutic concentrations of glibenclamide. The presence of the V65M mutation in Kir6.1 significantly reduced the glibenclamide potency (IC_{50}=1.6 ± 0.04 μM), while the rest of the mutations in Kir6.1 (G343D) or SUR2A (H60Y and S1020P) did not affect the glibenclamide-induced block. These results demonstrate that most of the CS-associated mutations reduced channel response to pinacidil, whereas only the V65M mutation in Kir6.1 affected channel sensitivity to glibenclamide.

Keywords: Kir6.x, SUR2A, glibenclamide, Cantu Syndrome, cardiac

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THE PARKINSON'S DISEASE-ASSOCIATED GPR37 RECEPTOR IS AN ADENOSINE A_{2A} RECEPTOR REPRESSOR IN MICE

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GPR37, also known as parkin-associated endothelin-like receptor (Pael-R), is an orphan G protein-coupled receptor that has been related with Parkinson's disease (PD) neuropathology. Interestingly, the genetic blockade of GPR37 enhances cell surface expression of striatal dopamine transporter (DAT), resulting in reduced dopamine content in the striatum. In addition, it has been shown that deletion of GPR37 triggers anxiolytic-like effects and sensitizes mice to adenosine A_{2A} receptors ($A_{2A}R$)-mediated signaling. Here we report that GPR37 and $A_{2A}R$ physically and functionally interact both in living cells and in native tissue. Thus, by using biochemical techniques (i.e. co-immunoprecipitation and proximity ligation assay) we demonstrated a physical interaction between these two receptors in the striatum. Also, by means of post-*embedding immunogold-electron microscopy* (EM) *techniques* detection it was demonstrated a close proximity at the postsynaptic level of striatal synapses. On the other hand, GPR37 deletion promoted striatal $A_{2A}R$ cell surface expression, which correlated well with an increased $A_{2A}R$ agonist-mediate cAMP accumulation both in primary striatal neurons and synaptosomes from striatum. Furthermore, GPR37-KO mice showed enhanced catalepsy induced by an $A_{2A}R$ agonist and an increased response to $A_{2A}R$ antagonist-mediated locomotor activity. Overall, these results demonstrate for the first time an important role for GPR37 controlling $A_{2A}R$ expression and function in the striatum, which may be of interest for the treatment of PD.

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CELLULAR BIOLOGY OF HUMAN CARDIAC NAV1.5-KIR2.1 CHANNEL COMPLEXES

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Cardiac Kir2.1 and Nav1.5 channels generate the inward rectifier K^+ (I_{K1}) and the Na^+ (I_{Na}) currents, respectively. Nav1.5 and Kir2.1 channels exhibit positive reciprocal modulation and the augmented presence of Nav1.5 and Kir2.1 channels at the membrane could be the result of an increase in the protein synthesis and forward trafficking and/or to a decrease in channel internalization. Here we compared the differential characteristics of these processes when Nav1.5 and Kir2.1 channels are expressed together or separately. The proximity ligation assays demonstrated that Nav1.5 and Kir2.1 proteins are in close proximity to each other (<40 nm apart) in the membrane of ventricular myocytes, suggesting that they form complexes. Patch-clamp experiments in heterologous transfection systems demonstrated that the inhibition of endoplasmic reticulum (ER) to Golgi transport with Brefeldin A did not abolish the positive reciprocal modulation between Kir2.1 and Nav1.5 channels and that the internalization time constants of Kir2.1 (5.1 ± 0.5 h) and Nav1.5 (4.9 ± 0.4 h) channels were not modify when they were coexpressed with Nav1.5 and Kir2.1 channels, respectively. Either when they were coexpressed or not, the inhibition of dynamin-dependent endocytosis similarly reduced the internalization of Nav1.5 and Kir2.1 channels. Inhibition of the dynein/dynactin motor suggested that it is involved in the backward and forward traffic of Kir2.1 and Nav1.5, respectively. Conversely, Nav1.5-Kir2.1 complexes were forwarded by the dynein/dynactin motor. Nav1.5 but not Kir2.1 were ubiquitinated by Nedd4-2 ubiquitin-protein ligase and degraded by the proteasome. Nav1.5-Kir2.1 complexes were also degraded following this route as demonstrated by the overexpression of Nedd4-2 and the inhibition of the proteasome with MG132. We concluded that there is a pool of Kir2.1 and Nav1.5 channels that form complexes whose biology is similar to that of the Nav1.5 channels.

Keywords: Nav1.5 channel, Kir2.1 channel, cardiac, intracellular traffic

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A METHOD FOR QUANTIFYING BIASED SIGNALLING OF INVERSE AGONISTS

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Biased agonism, the ability of an agonist to selectively trigger specific signalling pathways when they act at a given receptor, is an increasingly frequently described pharmacological phenomenon. Several methods to quantify the bias of agonists have been proposed, for example the ΔΔlog(τ/Ka) parameter of Kenakin and co-workers (Kenakin et al, 2011, ACS Chem. Neurosci. 3, 193–203). However, all of the methods used for the analysis of biased signalling focus on the analysis of agonists as they are based on the use of the Operational model. However, the classical Operational model does not account for constitutive receptor signalling and cannot be used to analyse the properties of inverse agonists. Here we report a method for the analysis of ligand bias based on the Operational model of Slack & Hall (Slack & Hall, 2012, Br. J. Pharmacol. 166, 1774–1792) which accounts for receptor constitutive activity and hence can be used to analyse biased signalling in response to inverse agonists as well as agonists. This method also has the advantage of providing a measure of absolute rather relative bias since it is derived from estimates of ligand intrinsic