efficacy and is not, therefore, influenced by the efficiency of the signal transduction cascades involved.
Keywords: biased agonism, constitutive activity, inverse agonism, Operational model

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A MUTATION IN THE TBX5 TRANSCRIPTION FACTOR DECREASES THE HUMAN CARDIAC SODIUM CURRENT AND IS ASSOCIATED WITH THE BRUGADA SYNDROME

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In a proband diagnosed with Brugada syndrome (BrS), in whom screening for mutations in all described BrS genes was negative, we found a missense mutation in the Tbx5 transcription factor (p.F206L) that was predicted as pathogenic. It has been described that Tbx5, besides its effects on cardiac development, drives the expression of Nav1.5 channels in the adult mouse heart. Since BrS is associated to loss-of-function Nav1.5 mutations, here we analyzed the effects of p.F206L Tbx5 on the cardiac sodium current (I_{Na}) generated by Nav1.5 channels to unravel whether the mutation can underlie the BrS. Human native (WT) and mutated Tbx5 tagged with GFP were transfected in HL-1 cells or included in lentiviral particles for infecting human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). Tbx5 WT doubled the peak I_{Na} density recorded in HL-1 cells (from -37.5 ± 5.1 to -62.6 ± 8.2 pA/pF, $n \geq 6$, $P < 0.05$), whereas p.F206L Tbx5 strongly reduced the peak I_{Na} density (-6.7 ± 0.2 pA/pF; $n = 6$; $P < 0.01$). Importantly, in hiPSC-CM Tbx5 WT and p.F206L significantly increased (-27.6 ± 1.9 pA/pF; $n = 7$) and decreased (-9.5 ± 1.9 pA/pF), respectively, the I_{Na} peak density compared to non-infected cells (-19.4 ± 2.8 pA/pF; $n = 10$; $P < 0.05$). Both in HL-1 cells and hiPSC-CM neither WT nor mutated Tbx5 modified the voltage dependence of Nav1.5 channels activation and inactivation. Luciferase reporter assays using the human minimal promoter of the gene encoding Nav1.5 channels (*SCN5A*) demonstrated that p.F206L Tbx5 completely abolished the Tbx5 pro-transcriptional activity produced by WT Tbx5. We concluded that the p.F206L mutation markedly decreases the I_{Na} density by suppressing the remarkable Tbx5 pro-transcriptional activity over the human *SCN5A* gene and thus, it could be associated with the BrS.
Keywords: sodium current, Tbx5, Brugada Syndrome, cardiac

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THE LARGE CONDUCTANCE, CA^{2+}-ACTIVATED K^+ CHANNEL OPENER VSN16R MODULATES HIPPOCAMPAL CA1 PYRAMIDAL NEURON FIRING

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Ion channels are critical regulators of neuronal excitability and are also implicated in a variety of pathologies such as epilepsy. Large conductance, Ca^{2+}-activated K^+ (BK_{Ca}) channels play an important functionally modulate neuronal firing and have a known role in seizure aetiology. BK_{Ca} are widely expressed in the central nervous system, regulate action potential duration, firing frequency and consequential neurotransmitter release, and are activated by membrane depolarization and increased intracellular Ca^{2+}. The unique coupling of Ca^{2+} signalling to membrane depolarization associated with BK_{Ca} plays a crucial role in controlling neuronal hyperexcitability, since K^+ efflux via BK_{Ca} causes neuronal hyperpolarisation. Moreover, loss-of-function mutations to, or reduced expression of, BK_{Ca} contribute to neuronal hyperexcitability that has been associated with temporal lobe epilepsy, tonic-clonic seizures and alcohol withdrawal-induced seizures. Conversely, there is evidence to show that that BK_{Ca} can facilitate high-frequency neuronal firing in some neuronal populations (e.g. hippocampal CA1 pyramidal neurons) and some gain of function mutations to BK_{Ca} subunits have been associated with the development of idiopathic epilepsy (primarily absence epilepsy). Thus, both loss of function and gain of function of BK_{Ca} channels can differentially subserve modulation of seizure phenotypes such as temporal lobe seizures and absence seizures, respectively.
Here, we examined the effects of the BK_{ca} channel opener, VSN16R (50 μM), on the firing properties of hippocampal CA1 pyramidal neurons in acute hippocampal slices obtained from healthy male C57BL/6 mice (P30–P40) using whole-cell current clamp electrophysiological recording. Neuronal outputs in response to a variety of hyperpolarizing and depolarizing stimulation protocols were assessed. Modulate of hippocampal CA1 pyramidal neuron firing via VSN16R effects on BK_{Ca} channels results will be presented and discussed.
Keywords: BK_{ca} channels; Hippocampal pyramidal neurons

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MODELING HUMAN BIPOLAR DISORDER: THE HINT1-DEFICIENT MICE

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Human bipolar disorder (BPD) is a chronic and debilitating illness with alternating periods of mania and depression. Several currently available animal models reproduce select behavioral facets of human mania and depression and can be used to reliably detect novel drugs. However, in the case of BPD, there is no single valid animal model for reproducing the fluctuating moods of affected patients. Mice with histidine triad nucleotide-binding protein 1 (HINT1) deletion exhibit manic-like symptoms that evolve into depressive-like behavior in response to stressful paradigms. Recent studies have indicated that HINT1−/− mice exhibit molecular changes similar to those found in BPD patients, such as increased PKC, PKA, and GSK3β activities, as well as glutamate N-methyl-D-aspartate receptor (NMDAR)/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptor (AMPAR) and NR2B/NR2A subunit ratios[1,2]. Pharmacological interventions stabilized their behavior. GSK3β inhibitors and valproate directly attenuated the expression of the manic-like symptoms, whereas PKC inhibition, lamotrigine, or risperidone promoted NMDAR-mediated depressive-like behaviors that counterbalanced the preexisting manic-like symptoms. Naïve HINT1−/− mice exposed to stressful paradigms rapidly manifested depressive-like behaviors in subsequent stressful situations that persisted for a couple of weeks thereafter. During the depressive-like phase, citalopram, amitriptyline and MK801 precipitated manic-like behaviors in stressed HINT1−/− mice. Notably, as observed in BPD patients, the antagonism of NMDARs prevented HINT1−/− mice from alternating behaviors in response to stress[3]. In HINT1−/− mice, PKC supports manic like symptoms and reduces the expression of depressive-like behaviors via activation of GSK3β and regulation of NR2B-enriched NMDARs. Our data show that HINT1−/− mice represent a suitable model for studying human BPD and may facilitate the identification of novel targets and drugs to treat this mental disorder.(Supported by Plan Nacional de Drogas 2014-012 and MINECO, SAF-2015-65420R)
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