Network: Systematic Gene Identification and Functional Analyses in Common CNS Disorders

Project: Pathophysiological Mechanisms of Neurodegeneration and Epilepsy in a Mouse Model of KCNQ/M-channel Deficiency

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Introduction

The aim of our project is to investigate the pathophysiological mechanisms underlying neurodegeneration and epilepsy in transgenic mouse models of KCNQ/M-channel deficiency. By combining genome-wide transcriptional profiling in affected mouse brain tissue with conditional transgenic techniques, electrophysiological, morphological, and behavioral methods, we seek to identify pathways and genes that are involved in epileptogenesis and neurodegeneration and, therefore, might represent targets for therapeutic intervention.

Mutations in the human KCNQ2 and KCNQ3 genes are associated with a dominantly inherited form of generalized epilepsy called *Benign Familial Neonatal Convulsions* (*BFNC*)[1]. The *BFNC* phenotype is characterized by frequent epileptic seizures in the first weeks of life and by a substantially increased risk of seizures after infancy[2]. Recently, severe mental impairment and therapy-resistent epilepsy have been found in a number of *BFNC* patients.
Members of the KCNQ gene family, namely KCNQ2, KCNQ3 and KCNQ5, encode M-channel subunits that may form homo- or heteromeric M-channels (KCNQ/M-channels). M-channels are voltage-gated potassium (K^+) channels underlying the non-inactivating M-current (I_M) in neurons. These channels are of particular interest because they activate already at membrane potentials negative to the action potential threshold where few other ionic channels are active, and, therefore, may exert pivotal control over neuronal excitability and response patterns. During repetitive neuronal discharge, M-channel activity is thought to cause early spike frequency adaptation and to generate afterhyperpolarizations of medium duration (mAHPs). M-channel activity stabilizes the membrane potential and therefore tends to prevent spiking. Immunohistochemistical studies revealed overlapping KCNQ2 and KCNQ3 protein expression patterns on somata and dendrites of pyramidal and polymorphic neurons in hippocampus and cerebral cortex. In addition, KCNQ2-containing channels are prominently expressed on axon initial segments, axons, and pre-synaptic terminals in CNS and PNS, where they may control neurotransmitter release.
Functional analyses of BFNC-associated mutations in heterologous expression systems have revealed impairment of KCNQ channel function, although an unambiguous genotype-phenotype correlation could not be established. The disease mechanisms are not well understood and remain to be investigated.
Besides the causal involvement of KCNQ subunits in human epilepsy, the recent finding that three types of drugs in development for treatment of Alzheimer's disease, epilepsy, and stroke exert their effects partly via modulation of brain KCNQ/M-channels further underlines the importance of M-channels.

Results

The physiological role of KCNQ/M-channels in the nervous system and the pathophysiology underlying the epilepsy phenotype associated with KCNQ2/3 gene mutations is not well understood and cannot be investigated in humans. In addition, spontaneous mutations or targeted disruption of the KCNQ2 gene in mice have not yielded a useful mouse model due to neonatal mortality caused by lung failure.
To gain insight into cellular, network, and behavioral consequences of M-channel deficiency, we suppressed M-currents in mouse brain. Since genetic KCNQ2 gene deletions were lethal, we used a different strategy. We generated mice expressing a KCNQ2 subunit with a dominant-negative (DN) pore mutation that can suppress M-channel activity by co-assembling with native KCNQ subunits. By using the Tet-Off system and the prion protein promoter, we gained temporal control over transgene expression and restricted it to the nervous system. This strategy yielded viable M-current-deficient mice[3]. Depending on the period of transgene expression, the mice showed epilepsy, behavioral hyperactivity, cognitive deficits, and/or changes in brain morphology. M-channel-deficient mice showed striking electrophysiological changes in their hippocampal CA1 pyramidal neurons: substantially increased excitability, reduced spike frequency adaptation, attenuated afterhyperpolarizations (mAHPs), and reduced intrinsic subthreshold *theta* resonance. Furthermore, these mice had markedly impaired spatial memory in the Morris water maze. Our results strongly support the notion that M-channels are critical determinants of cellular and neuronal network excitability, and that attenuation of the M-current had profound consequences for behavior and cognitive performance.

Outlook

Since we thoroughly characterized the time course of neurodegeneration and onset of epilepsy in the transgenic mice, we were able to perform transcriptional profiling experiments at different time points/disease stages. These experiments identified a number of differentially regulated genes including some of those that were also found in pharmacological models of temporal lobe epilepsy. Our present work aims at validating these data and making functional sense out of them.
In addition to temporal control of transgene expression, we now can restrict expression spatially in mouse brain using the Tet-system, and, thus, investigate the phenotypic consequences and physiological functions of KCNQ/M-channels in a defined subset of neurons.

*Lit.: **1.** Jentsch, T. J. Neuronal KCNQ potassium channels: physiology and role in disease. Nat Rev Neurosci 1, 21-30. (2000). **2.** Leppert, M. F. & Singh, N. Susceptibility genes in human epilepsy. Semin Neurol 19, 397-405 (1999). **3.** Peters, H.C., Hu, H., Pongs, O., Storm, J.F. & Isbrandt, D. Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. Nat Neurosci 8, 51-60 (2005).*