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

Project: CNS Hyperexcitability in Mouse Models Affecting Ion Transport Diseases

Thomas Jentsch - **University Hamburg – jentsch@zmnh.uni-hamburg.de**

Introduction

Signal transduction in the nervous system is critically dependent on electrical signals that are generated by nerve cells. These signals are generated by the opening, closing and modulation of ion channels. The flow of ions through these channels, and hence the electrical current, depends on the electrochemical gradient of the respective ions. These gradient are generated by active pumps (in particular the Na.K-ATPase) and cotransporters such as K-Cl cotransporters. Action potentials, the elementary signal in the nervous system, are generated by a depolarizing influx of Na^+ through voltage-dependent Na^+ channels. These channels close (inactive) by themselves, leading to a termination of action potentials. Further, K^+ channels, many of which are activated by the depolarizing voltages of action potentials, repolarize action potentials. Modulating the activity of potassium channels that are open at the resting potential can modulate the excitability of neurons.

Neurons signal to other neurons mostly through synapses, where the presynaptic neuron releases a chemical neurotransmitter through exocytosis into the synaptic cleft. These activate neurotransmitter receptors – ligand-gated ion channels – at the postsynaptic membrane. The resulting currents depolarize or hyperpolarize the postsynaptic neuron, resulting in excitation or inhibition, respectively. Whereas excitation occurs through Na^+ influx, which is favoured by a large inwardly-directed Na^+ gradient, inhibition uses ligand-gated GABA- and glycine receptor chloride channels. In contrast to the Na^+ gradient, the electrochemical chloride gradient may have an inward- or an outward direction. During development, there is indeed a change from an excitatory fetal or neonatal response to an inhibitory GABA-response in the adult. Thus, transporters affecting intracellular chloride have an important impact on the inhibition in the CNS and thus on neuronal excitability.

Given the pivotal role of ion channels in neuronal excitability, it is not surprising that mutations in ion channels and transporters can cause an hyperexcitability in the central nervous system, i.e. epilepsy (1,2). Together with Ortrud Steinlein, we could show that mutations in the KCNQ2 potassium channel underlie a form of epilepsy (3). The same rare form of neonatal epilepsy (BFNC) can also be caused by mutations in KCNQ3, which can form a heteromeric complex with KCNQ2 (4). Indeed, a small loss of the M-current mediated by these heteromers suffices to cause epilepsy (4). The stimulatory effect of KCNQ3 on KCNQ2 currents could be explained by an increase in surface expression (5). In addition to KCNQ2/KCNQ3, also KCNQ4 (6) and KCNQ5 (7) can mediate M-currents. Whereas the expression of KCNQ4, mutations in which cause deafness (6), is restricted to the inner ear and only some nuclei in the brainstem, KCNQ5 is broadly expressed in the CNS and may therefore also play a role in epilepsy (7). As KCNQ2/3 are also expressed in the spinal cord, a specific mutation in KCNQ2 not only caused epilepsy, but also myokymia (8).

The pivotal role of the neuron-specific K-Cl contransporter KCC2 in determining intraneuronal chloride became apparent from our knock-out mouse model (10) which died shortly after birth because of a failure to breathe. We showed that this was due to an excitatory GABA-response and thus to an hyperexcitability (10). The disruption of KCC3 also led to an increase in the intraneuronal chloride concentration, which, however, was less pronounced (11). Their EEG, however, showed clear epileptifom signs. In addition, there was a strong neurodegeneration in the CNS and PNS that resembled that of patients with Andermann syndrome, which is caused by mutations in KCC3.

By contrast, the knock-out in mice of ClC-2, a chloride channel that had been speculated to also co-determine intraneuronal chloride concentration, did not lead to epilepsy (9).

Results/Project Status

We have re-investigated the excitability of our ClC-2 KO mouse (9), confirming that there is no hyperxcitability. Furthermore, we have investigated the electrophysiological effects of ClC-2 mutations found by Haug et al in some families with epilepsy. Except for the truncation, which of course leads to non-functional channels, we detected no effects. In addition, the truncated ClC-2 clearly lacked a dominant negative phenotype, in contrast to the data presented by Haug et al..

We are currently in the process of generating and analysing several other sophisticated mouse models of ion channels that may have effects on neuronal excitability. In some cases, we are generating knock-ins of dominant negative mutants, and in other cases we are creating conditional knock-outs that will allow a selective disruption of these genes.

Results from these ongoing studies will be reported in due time in the scientific literature. Such studies typically take several years.

Outlook

Ion channel diseases affect many organs, and in particular the CNS. The combination of human molecular genetics and mouse models has strengthened considerably our insight into these diseases. We expect significant progress from the models we are currently generating and analysing.

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