Michael Thomas Barbe
Dr. med.

The Physiological Characterization of Pannexin Hemichannels

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Doktormutter: Frau Prof. Dr. med. H. Monyer

In 2000, a database search led to the identification of a small family of three proteins named pannexins which are expressed in the genome of vertebrates and some invertebrates. Pannexins share the same membrane topology with innexins and connexins as well as regularly spaced cysteine residues in the two extracellular loops connecting the transmembrane domains. Two of the three pannexin genes, Px1 and Px2, are abundantly expressed in brain.
This initial information gave the impetus for more focused investigations, primarily because of the interest to decipher their role in the formation of gap junctions, which are the morphological correlate of electrical synapses. Based on our working hypothesis, pannexin gap junctions could contribute to high frequency oscillation in brain, which has been proposed to underlie a variety of cognitive processes, such as perception, memory, and learning. The purpose of my study was to investigate the functional characteristics of pannexins in terms of their ability to form hemichannels in Xenopus laevis oocytes.
I could demonstrate for the first time that Px1 forms hemichannels, whereas Px2 and Px3 were not able to form hemichannels by themselves. Coinjection of Px1 and Px2 into the same cell led to the formation of channels that differed in current amplitude, channel opening time, and kinetics of channel closure, suggesting that Px1 and Px2 are able to form heteromeric hemichannels with different biophysical properties. A finding that has been verified since it could be confirmed with co-immunoprecipitation of tagged pannexin proteins in HEK cells.
In a second series of experiments, the sensitivity of pannexin homo- and heteromeric hemichannels to well known connexin blockers was tested. Hemichannels exhibited a remarkable sensitivity to blockade by carbenoxolone (with an IC50 of ≈5 μM) which showed complete reversibility after wash-out, whereas flufenamic acid exerted only a modest inhibitory effect. In contrast to most of the connexin hemichannels, extracellular calcium did not close pannexin hemichannels.
Tagged pannexin constructs (Px1EGFP and Px1myc) were investigated electrophysiologically. A comparison between channel kinetics was performed indicating a significant difference between Px1 and Px1EGFP in current amplitude, opening time, and channel closure which could have an impact on future studies in in vitro cell cultures or transgenic animals.
Besides the ability to form hemichannels, other experiments from our group could show that pannexins form intercellular channels in Xenopus oocytes as well. Therefore, pannexins have left the role of candidate gap junction genes, as their ability to assemble active hemichannels and intercellular channels (inboth homo- and heteromeric configurations) has been verified.
As a new family of gap junction forming genes, pannexin hemi- and intercellular channels could be involved in a variety of physiological and pathological processes. According to our working hypothesis, pannexin gap junctions could be the molecular correlate for high frequency oscillation in brain. As discussed in this thesis, pannexins are very good candidates for the

molecular substrate of hemichannel-mediated calcium waves. Pathologically, pannexin intercellular channels could play a role in epileptogenesis and psychotic disorders.

These initial studies on pannexin hemi- and intercellular channels have started a quest to understand their functional contribution to cell communication in vivo and thereby have smoothed the way for further research on this novel family of gap junction genes.