Prion protein as a mediator of synaptic transmission

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Neurodegenerative disorders are characterized by synaptic and neuronal dysfunction which precedes general neuronal loss and subsequent cognitive or behavioral anomalies. Although the exact early cellular signaling mechanisms involved in neurodegenerative diseases are largely unknown, a view is emerging that compromised synaptic function may underlie the initial steps in disease progression. Much recent research has been aimed at understanding these early underlying processes leading to dysfunctional synaptic signaling, as this knowledge could identify putative sites of interventions, which could potentially slow progression and delay onset of disease. We have recently reported that synaptic function in a Drosophila melanogaster model can be modulated by the presence of native mouse prion protein and this modulation is negatively affected by a mutation within the protein which is associated with the Gerstmann-Sträussler-Scheinker syndrome, a human form of prion disease. Indeed, wild-type prion protein facilitates synaptic release, whereas the mutated form induced diminished phenotypes. It is believed that together with the gain-of-function of neurotoxic misfolded prion signaling, the lack of prion protein contributes to the pathology in prion diseases. Therefore, our study investigated a potential endogenous role of prion protein in synaptic signaling, the lack of which could resemble a lack-of-function phenotype in prion disease.

It is of great importance to understand the signaling pathways involved in neurodegenerative processes as the average lifespan continues to increase worldwide and with it the incidence of neurodegenerative disorders (ND) such as Parkinson and Alzheimer disease. Much research is now focused on unravelling the molecular mechanisms which lead to dysregulation of synaptic transmission by studying several synaptic proteins involved in neurotransmission. The neuronal network relies on plasticity mechanisms where reversible formation and disassembling of synaptic connections occurs in a controlled manner. It is generally accepted that in neurodegenerative conditions there is an early onset dysfunction at the synapse, opening up the possibility of intervention to manipulate neuroprotective pathways to balance between degenerative and survival signaling. It is now widely established, that the loss of presynaptic termini is a key event in the process, which initiates further axonal dysfunction and neuronal cell soma loss, resulting in cell death as a hallmark of many ND

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Addendum to: Prion protein facilitates synaptic vesicle release by enhancing release probability. Robinson SW, Nugent ML, Dinsdale D, Steinert JR. Hum Mol Genet. 2014 Sep 1;23(17):4581-96. doi: 10.1093/hmg/ddu171. Epub 2014 Apr 9.
human. The unique feature of these conditions is that it can be caused by either sporadic mutations or inherited variants of the prion protein but it can also be transmitted by the scrapie isoform of PrP according to the ‘protein only’ hypothesis. The early onset of the disease before manifestation of neuronal cell death may be caused by loss-of-function of the prion protein and/or by a gain-of-function of the cytosolic PrP. It is therefore important to recognize the functions of PrP, especially in the synaptic context.

In order to distinguish between these 2, not exclusive, possibilities it is crucial to investigate the endogenous functions of PrP itself. PrP is ubiquitously expressed in the body, reaching the highest levels in the nervous system. Morphological studies suggest that PrP is preferentially located along axons and in presynaptic terminals but post-synaptic localization and signaling has also been reported. Evidence demonstrates that neuroprotective roles of PrP are essential as loss-of-function of Prnp animals or mutations in PrPC lead to neuronal dysfunction. Interestingly, Prnp animals exhibit phenotypes with impaired long-term potentiation, abnormal circadian rhythm and glutamatergic synaptic signaling. In addition, compromised dopaminergic transmission but also more severe characteristics such as Purkinje cell degeneration and demyelination of peripheral nerves leading to ataxia have been reported in Prnp animals. Comparisons of wild-type with Prnp animals have revealed that PrP expression at synapses contributes to hippocampal synaptic function and exerts neuroprotection by modulating neuronal excitability. In particular, PrP has been shown to inhibit N-methyl-D-aspartate receptors (NMDAR) containing the NR2D subunit, the activation of which has direct links to the general neurotoxic signaling mediated by the NMDAR-nitric oxide pathway. However, the above studies have investigated prion protein functions in neuronal networks, which include pre- and postsynaptic compartments making it difficult to unambiguously define specific roles of PrP.

In order to study the functions of the prion protein in more detail and to isolate pre- and postsynaptic mechanisms, model systems other than mouse have been recently utilised. In particular, non-mammalian neurodegeneration models have been employed with expression of wild-type or mutant prion proteins in Drosophila melanogaster or C. elegans allowing investigations of prion protein function in host organisms that do not have a direct prion protein ortholog.

We recently showed that presynaptic expression of a wild-type mouse prion protein at a glutamatergic synapse, the Drosophila neuromuscular junction (NMJ), leads to an enhanced release of synaptic vesicles as a result of larger functional vesicle pool sizes. This positive modulation of transmitter release was accompanied by increased presynaptic vesicle sizes leading to an overall augmentation of transmission. We did not observe any effects on the NMJ morphology, including numbers of release sites following prion protein expression, consistent with previous data. We hypothesized in this study that expression of wild-type prion protein has a gain-of-function effect at presynaptic signaling, which corroborates previous findings in neurons of the mammalian central nervous system.

In conclusion, given the fact that PrP interacts with proteins involved in synaptic release and additionally with various metabotropic and ionotropic neurotransmitter receptors and reminiscent of the GSS syndrome. Other data also indicate that expression of hamster and mouse (although to a lesser degree) PrP in Drosophila causes neurodegeneration in aged flies suggesting an age- and species-dependent difference in prion protein signaling. However, our study provided important evidence that prion protein signaling, in the absence of misfolding and aggregation, has fundamental effects on synaptic transmission. It further suggests that in prion disease, due to the conversion of unfolded native PrP into PrP, neurons face both, a continuously diminishing prion protein function as well as an increasingly additional cytotoxic PrP function. So how can prion protein contribute to synaptic function? There are several lines of evidence suggesting that endogenous prion protein can modulate transmitter release via multiple pathways. Studies in mouse NMJs and hippocampal CA1 neurons showed that PrP potentiates synaptic release consistent with PrP expression at presynaptic terminals. PrP has been reported to interact with synapsin (Fig. 1) and its internalisation is mediated via clathrin-coated pits in a dynamin-dependent process. So it is conceivable to suggest that PrP may play a role in endocytosis, vesicle replenishment and release, which is likely to impact on vesicle pool availabilities. This interaction offers a new functional explanation of how PrP can modulate transmitter release and how a consequent conversion of PrP into PrP could lead to synaptic dysfunction. An essential part of synaptic transmission is synaptic Ca2+ homeostasis in which Ca2+ influx through Ca2+ channels determines the release of neurotransmitter. Reports showed that a mutation in PrPC leads to impaired membrane delivery of the α8δ-1 subunit of voltage-gated Ca2+ channels (VGCC) in cerebellar granule neurons. This caused reduced Ca2+ currents and a defective glutamate release suggesting that PrP function is directly required for synaptic Ca2+ signaling and vesicular release.

In conclusion, given the fact that PrP...
ion channels\(^{23}\) (Fig. 1), our data provides further evidence for a direct functional role of presynaptic prion protein signaling.\(^{46}\) This study highlights the ability of prion protein to modulate vesicles and release properties leading to enhanced synaptic strength and transmission thereby corroborating and extending information gained from mouse models.\(^{28-30,32}\) The use of the Drosophila NMJ system allows detailed investigations of presynaptic PrP functions to support studies in other model systems. Thus, our data point toward a physiological role of prion protein in synaptic function and will thereby help understanding the fundamental signaling pathways of prion proteins and their involvement in prion pathogeneses.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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