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Role of prions in neuroprotection and neurodegeneration

A mechanism involving glutamate receptors?

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There is increasing evidence that cellular prion protein plays important roles in neurodegeneration and neuroprotection. One of the possible mechanism by which this may occur is a functional inhibition of ionotropic glutamate receptors, including N-Methyl-D-Aspartate (NMDA) receptors. Here we review recent evidence implicating a possible interplay between NMDA receptors and prions in the context of neurodegenerative disorders. Such is a functional link between NMDA receptors and normal prion protein, and therefore possibly between these receptors and pathological prion isoforms, raises interesting therapeutic possibilities for prion diseases.

Prions are most often discussed in the context of transmissible spongiform encephalopathies (TSEs) which encompass a range of neurological disorders that include human Creutzfeldt-Jakob disease (among others), sheep scrapie and bovine spongiform encephalopathy.[1,2] It is well established that these disorders arise from a progressive conversion of the normal, mainly helical form of cellular prion protein (PrP^{C}) into a different PrP^{Sc} protein conformation with a high beta sheet content.[3] In their PrP^{Sc} form, prions act as templates that catalyze misfolding of PrP^{C} to produce increasing levels of PrP^{Sc}, which likely represents several or even many different conformational states of the same source protein, resulting in diverse clinical phenotypes. This in turn leads to accumulation of PrP^{Sc} deposits in the brain that can appear as aggregates and amyloid-like plaques[4] and which disrupt normal neurophysiology.[5] While the neuropathology of TSE's has been explored in great detail dating back to the 1920s,[6] less effort has perhaps been expended on understanding the cellular and physiological function of PrP^{C} which is ubiquitously expressed, and found even in simple organisms.[5,7,8] A number of mouse lines either lacking PrP^{C} or overexpressing PrP^{C} have been created, including the widely used Zurich I PrP^{C} knockout strain.[9,10] Despite the wide distribution of PrP^{C} in the mammalian CNS, it perhaps surprisingly has only a relatively mild behavioral phenotype that appears to include some deficits in spatial learning at the behavioral level[11,12] as well as alterations in long term potentiation at the cellular level.[13-17] In addition, it has been shown that these mice show an increased excitability of hippocampal neurons.[13,18-20] In contrast, deletion of certain parts of the PrP^{C} protein in vivo can have serious physiological consequences. For example, deletion of a stretch of amino acids between just upstream of the octarepeat copper binding motifs produces a lethal phenotype, that can be rescued by overexpression of increasing levels of normal PrP^{C}.[21,22] Of particular note, these deletion mutants show degeneration of axons and myelin, both in the CNS and in peripheral nerves; indeed some mutants show a predilection for axomyelinic degeneration with little neuronal pathology,[21] suggesting that certain mutated forms of PrP have a direct toxic effect on oligodendrocytes and/or myelin.[23] Moreover, activation of the Dpl1 gene in mice lacking PrP^{C} leads to an ataxic phenotype, that is not observed in the presence of PrP^{C}.[24] Collectively, this indicates that PrP^{C} may act in a protective capacity and in contrast, certain abnormal forms of PrP are "toxic", promoting much more injury to various elements of the CNS and PNS than outright absence of wild-type PrP^{C}.

This notion is further corroborated by a number of studies in PrP^{C} knockout mice, both in vivo and in cell culture models. Cultured hippocampal neurons from PrP^{C} null mice display greater apoptosis during oxidative stress.[25] Moreover, overexpression of PrP^{C} in rats protects them from neuronal damage during ischemic stroke, whereas PrP^{C} null mice show greater damage.[27-29] When PrP^{C} null mice are subjected to different types of seizure paradigms, they showed increased mortality and increased numbers of seizures.[30] This increased neuronal damage can be diminished by the NMDA receptor blocker MK-801,[31] potentially implicating glutamate receptors in this process. Finally, it was recently shown that the absence of PrP^{C} protein protects neurons from the deleterious effects of beta amyloid, a protein involved in Alzheimer disease.[32] It is important to note that NMDA receptors have been implicated in seizure disorders and in cell death during ischemic stroke.[33-35] Indeed, our own work has shown that NMDA receptors expressed endogenously in myelin contribute to myelin damage and may be one of the first steps leading to demyelination.[36] Furthermore, the NMDA receptor blocker memantine is used to treat Alzheimer disease, implicating NMDA receptors. The observations above suggest that there may be an interplay between NMDA receptor activity and the physiological

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function of PrP^C. In support of this hypothesis, our recent work has directly identified a common functional and molecular link between NMDA receptors and PrP^C.[37] Brain slices obtained from Zurich I PrP^C null mice showed an increased excitability of hippocampal slices, which could be ablated by blocking NMDA receptor activity with amino-5-phosphonovaleric acid. Removal of extracellular magnesium ions to enhance NMDA receptor activity resulted in stronger pro-excitatory effects in slices and cultured neurons from PrP^C null mice compared with those from normal animals. Synaptic recordings indicate that the amplitude and duration of NMDA mediated miniature synaptic currents is increased in PrP^C null mouse neurons, and evoked NMDA receptor currents show a dramatic slowing of deactivation kinetics in PrP^C null mouse neurons. The NMDA current kinetics observed in these neurons were qualitatively consistent with NMDA receptors containing the NR2D subunit.[38] Consistent with a possible involvement of NR2D containing receptors, siRNA knockdown of NR2D normalized current kinetics in PrP-null mouse neurons. Furthermore, a selective co-immunoprecipitation between PrP^C and the NR2D, but not NR2B subunits, was observed. This then may suggest the possibility that under normal circumstances, PrP^C serves to suppress NR2D function, but when PrP^C is absent, NR2D containing receptors become active, and because of their slow kinetics, may contribute to calcium overload under circumstances where excessive (or even normal) levels of glutamate are present. This would include conditions such as epileptic seizures, ischemia and Alzheimer disease, thus providing a possible molecular explanation for the link between PrP^C and neuroprotection under pathophysiological conditions. Indeed, NMDA promoted greater toxicity in PrP^C null mouse neurons, and upon injection into brains of PrP^C null mice. It is interesting to note that one of the major NMDA receptor subtypes expressed in myelin is NR2D, thus bridging the observations of Micu et al.[36] of NMDA receptor mediated cell death in ischemic white matter, and those of Baumann and colleagues[21] showing that PrP^C deletion mutants can cause damage to myelin.

How might PrP^C deletion mutants affect neuronal survival? One possibility may be that these deletion mutants compete with normal PrP^C for NMDA receptors, but are unable to functionally inhibit them. Alternatively, it is possible that the PrP^C deletion mutants, by virtue of binding to the receptors, may in fact increase receptor activity, thus causing increased cell death. In both cases, increasing the expression of normal PrP^C would be expected to outcompete the deletion variants, thus reestablishing the protective function. A similar mechanism could perhaps apply to TSEs. It is possible that the PrP^{SC} form, perhaps in a manner reminiscent of the PrP^C deletion mutants, may be unable to inhibit NMDAR function, or perhaps would even enhance it. Any excess glutamate that may be released as a result of cell damage due to PrP^{Sc} aggregates, or even normally released amounts glutamate during the course of physiological neuronal signaling, could be sufficient to cause NMDAR mediated cell death and neuronal degeneration. In this context, it is interesting to note that chronic administration of the weakly NR2D selective inhibitor memantine delays death as a consequence of scrapie infection in mice.[39] In the context of Alzheimer disease, binding of PrP^C to beta amyloid may prevent the inhibitory action of PrP^C on NMDA receptor function, thus increasing NMDA receptor activity and promoting cell death. This then may perhaps explain the beneficial effects of memantine in the treatment of Alzheimer disease.

In summary, despite the fact that PrP^C is one of the most abundantly expressed proteins in the mammalian CNS, its physiological role is uncertain. Recent observations from our labs have established an unequivocal functional link between normal prion protein and the ubiquitous excitatory NMDA receptor. Thus, one of the key physiological roles of PrP^C may be regulation of NMDA receptor activity. The presence of abnormal species of prion protein, whether acquired via "infection", spontaneous conformational conversion or genetically inherited, may in turn alter normal function and regulation of NMDA receptors, leading to chronic "cytodegeneration" of elements in both gray and white matter regions of the CNS. This key functional link between PrP and glutamate receptors may provide our first opportunity for rational therapeutic design against the devastating spongiform encephalopathies and potentially other neurodegenerative disorders not traditionally considered as TSE's.

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