The prion protein as a receptor for amyloid-β

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Increased levels of brain amyloid-β, a secreted peptide cleavage product of amyloid precursor protein (APP), is believed to be critical in the aetiology of Alzheimer's disease[1]. Increased amyloid-β can cause synaptic depression[2,3], reduce the number of spine protrusions (that is, sites of synaptic contacts)[4,5] and block long-term synaptic potentiation (LTP)[6,7], a form of synaptic plasticity; however, the receptor through which amyloid-β produces these synaptic perturbations has remained elusive. Laurén *et al.*[8] suggested that binding between oligomeric amyloid-β (a form of amyloid-β thought to be most active[5,6,9–11]) and the cellular prion protein (PrP^C)[8] is necessary for synaptic perturbations. Here we show that PrP^C is not required for amyloid-β-induced synaptic depression, reduction in spine density, or blockade of LTP; our results indicate that amyloid-β-mediated synaptic defects do not require PrP^c.

To test whether PrP^c is required for amyloid-β-induced synaptic depression we infected organotypic hippocampal slice neurons with a Sindbis virus driving expression of APPct100, a precursor of amyloid-β, which leads to increased neuronal production and secretion of amyloid-β that does not perturb the health of the neuron, as assessed electrophysiologically[3]. Synaptic transmission was depressed in neurons expressing APPct100, in both wild-type and *Prnp*$^{-/-}$ mouse slices (where *Prnp* is the gene that encodes PrP^C) 24 h after infection (Fig. 1a). As previously shown for wild-type tissue[3], this depression was blocked if *Prnp*$^{-/-}$ slices were maintained during the APPct100 expression period with an inhibitor of NMDA (*N*-methyl-D-aspartate) receptors (Fig. 1a). Thus, synaptic depression after expression of APPct100, an effect of elevated amyloid-β[3], is intact in animals lacking PrP^C.

We next examined the effects of amyloid-β on dendritic spines, sites of excitatory synapses. Overexpression of APP[4] (or exposure to oligomeric amyloid-β[5]) leads to loss of dendritic spines in organotypic slices prepared from wild-type animals, which can be visualized by co-expression of a cytoplasmic marker. The same APP-induced decrease of spines was seen in slices made from mice lacking PrP^C (Fig. 1b). We next determined which species of amyloid-β is responsible for the observed effects. Synthetic amyloid-β(1−40) (Aβ40) peptides remain predominantly in monomeric form, and did not affect spine density (Fig. 1c). Amyloid-β(1−42) peptides (Aβ42) form oligomers, and when exogenously applied they produced a similar loss of dendritic spines in slices prepared from either wild-type or PrP^C-lacking mice (Fig. 1c). Thus, the loss of dendritic spines produced by oligomeric Aβ42 exposure does not depend on PrP^C.

We repeated the experiments as described in Laurén *et al.*[8] that studied PrP^C's role in the Aβ-mediated blockade of LTP. Acutely prepared hippocampal slices from mature wild-type and *Prnp*$^{-/-}$ mice were exposed to either species of amyloid-β and LTP was induced by theta burst stimulation. We found that Aβ42 (but not Aβ40) blocked LTP irrespective of whether PrP^C is present or absent (Fig. 1d).

We show that amyloid-β-induced synaptic depression, loss of dendritic spines and blockade of LTP are present in hippocampal slices prepared from PrP^C-deficient animals. In line with our results,

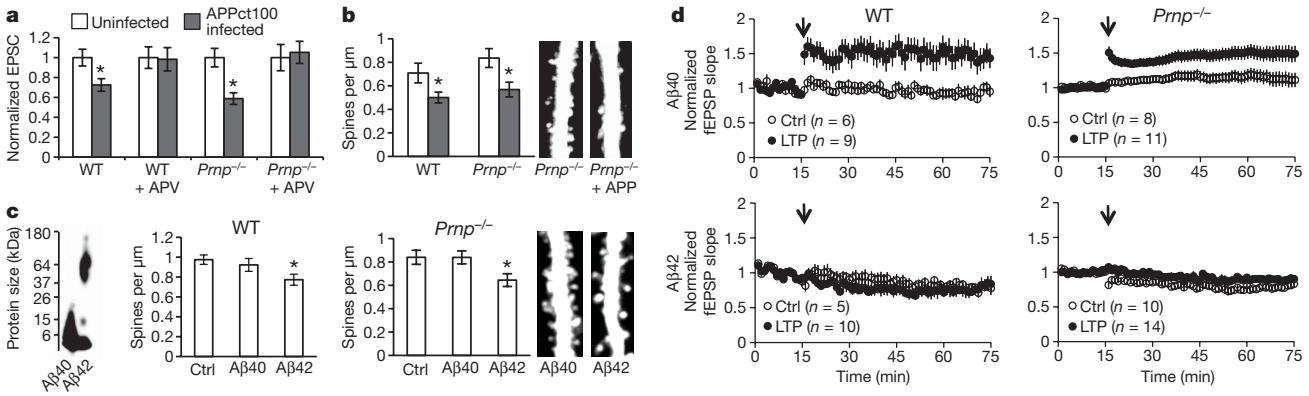

Figure 1 | PrP^C is not required for amyloid-β-induced synaptic deficits. **a**, Depression of whole-cell recorded synaptic AMPA receptor currents in a CA1 hippocampal neuron infected with Sindbis virus expressing APPct100 (grey bars) compared to a simultaneously recorded non-infected neuron (white bars) in wild-type (WT; 73 ± 8%, $n = 18$, $P = 0.02$) and *Prnp*$^{-/-}$ slices (59 ± 9%, $n = 25$, $P = 0.002$). Incubation of slices with 100 µM D-amino-phosphono-valeric acid (D-APV) during APPct100 expression abolished synaptic depression in WT (98 ± 11%, $n = 12$, $P = 0.6$) and *Prnp*$^{-/-}$ slices (105 ± 13%, $n = 13$, $P = 0.6$). EPSC, excitatory postsynaptic current. **b**, Decreased spine density by APP expression in WT ($n = 14$ APP expressing, $n = 9$ control dendrites, $P = 0.03$) and *Prnp*$^{-/-}$ slices ($n = 13$ APP expressing, $n = 9$ control dendrites, $P = 0.01$). Representative images of a dendrite in conditions as indicated. **c**, Spine loss in PrP^C-deficient hippocampal slices is specific to incubation with oligomeric amyloid-β. Aβ42, but not Aβ40, preparation produced high molecular mass oligomers, as indicated by western blot analysis. WT and *Prnp*$^{-/-}$ slices were incubated with no peptide (ctrl: WT, $n = 22$; *Prnp*$^{-/-}$, $n = 22$), with Aβ40 (WT, $n = 22$; *Prnp*$^{-/-}$, $n = 20$), or with Aβ42 (WT, $n = 22$; *Prnp*$^{-/-}$, $n = 22$) at 1 µM peptide concentration for 24 h, and spine densities were analysed blind to the experimenter. Spine densities were unaffected by monomeric amyloid-β incubation (WT, $P = 0.4$; *Prnp*$^{-/-}$, $P = 0.9$), but significantly reduced by incubation with oligomeric amyloid-β (WT, $P = 0.02$; *Prnp*$^{-/-}$, $P = 0.03$). Representative images of a dendrite in conditions as indicated. **d**, Oligomeric Aβ42 blocks LTP independent of the PrP^C. Hippocampal slices were isolated from 2–3-month-old WT or *Prnp*$^{-/-}$ mice. Field excitatory postsynaptic potentials (fEPSPs) were measured on two independent pathways in the presence of either 500 nM Aβ40 or 500 nM Aβ42. A control pathway (open circles) was recorded while theta burst stimulation (ten 100-Hz four shock bursts with 200-ms interburst intervals, arrow) was delivered to the second pathway (filled circles) after recording a 15 min baseline. LTP was significantly depressed by Aβ42 compared with Aβ40 in both WT ($P < 0.001$) and *Prnp*$^{-/-}$ slices ($P < 0.001$). When compared to their corresponding control pathway, LTP was successfully induced in WT ($P < 0.05$) and *Prnp*$^{-/-}$ slices ($P < 0.05$) treated with monomers. Block of LTP was found in both WT ($P > 0.05$) and *Prnp*$^{-/-}$ ($P > 0.05$) oligomer-treated slices when compared to their corresponding control pathway. Statistical comparisons (P) were performed using paired (**a**) or non-paired (**b**–**d**) two-tailed Student's *t*-test of log-transformed data. All error bars, s.e.m.; asterisk indicates $P < 0.05$.

amyloid-β oligomers were shown to impair long-term memory equally in PrP^C-lacking and PrP^C-expressing mice[12]. Thus, although oligomeric amyloid-β can bind PrP^C, it does not seem to be the receptor responsible for synaptic perturbations caused by oligomeric amyloid-β. Elucidation of the molecular mechanisms by which amyloid-β produces synaptic perturbations remains as a major goal in finding therapeutic treatments of Alzheimer's disease.

METHODS

C57/BL10 and C57/BL10 *Prnp*$^{-/-}$ mice were provided by M. Oldstone (Scripps Research Institute, grant AG04342). Organotypic slice cultures were infected at 7–12 days *in vitro* with Sindbis virus expressing APPct100[3]. After 24 h simultaneous whole-cell paired recordings were obtained as described previously[13]. At 12–17 days *in vitro* slices were infected with Sindbis virus expressing tdTomato or APP plus tdTomato for 48 h, or infected with eGFP and incubated with 1 μM monomeric Aβ40 or oligomerized Aβ42[14] for 24 h. Two-photon laser scanning images were taken of dendrites at the site of primary apical dendrite bifurcation. Spine densities were counted as described previously[4]. The protocol for the LTP experiments was as described in Laurén *et al.*[8]. This work was supported by NIH grant AG032132 and the Shiley-Marcos Endowment for Alzheimer's Disease Research to R.M.

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Amyloid-β oligomers are correlated with Alzheimer's disease progression and suppress synaptic plasticity[1–3]. Through unbiased expression cloning, we identified cellular prion protein (PrP^C) as an amyloid-β oligomer binding protein[4]. PrP^C was necessary for acute amyloid-β(1−42) (Aβ42) oligomer suppression of synaptic plasticity[4]; thus, it becomes critical to explore the importance of PrP^C in a range of Alzheimer's-disease-related deficits. Transgenic Alzheimer's disease model mice show deficits of spatial learning and memory[5], so the most direct assessment of PrP^C will monitor memory in transgenic Alzheimer's disease model mice deficient for PrP^C. In this paradigm, amyloid-β species are produced endogenously and the brain is exposed chronically over months. Recently, we have found that deletion of PrP^C from APPswe/PSen1ΔE9 transgenic mice restores spatial learning and memory without altering amyloid-β[6]. Furthermore, the early death, synapse loss and serotonin axonal degeneration of transgenic Alzheimer's disease mice require PrP^C (ref. 6). Kessels *et al.*[7] examine PrP^C in alternative paradigms.

First, Kessels *et al.*[7] overexpressed carboxy-terminal amyloid precursor protein (APP) in cultured neurons. Consistent with their previous findings, expression of this APP fragment led to α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) internalization and reduced dendritic spine density[8,9]. Viral expression is likely to involve monomeric amyloid-β and physiological APP. To our knowledge, there is no evidence that local viral expression produces pathological amyloid-β oligomers. Because PrP^C shows little affinity for amyloid-β monomer[4], it follows that the effects of viral APP will be unaltered in *Prnp*$^{-/-}$ neurons. Indeed, decreases in AMPAR and dendritic spines were unchanged by the absence of PrP^C.

Next, Kessels *et al.*[7] exposed neurons to synthetic amyloid-β(1−40) (Aβ40) or Aβ42 (monomeric or oligomeric in SDS, respectively). PrP^C was not required for Aβ42-induced dendritic spine loss over 24 h. As we[4] and others[10] have observed that the PrP^C accounts for approximately half of neuronal Aβ42 oligomer binding, other sites may replace PrP^C function in spine morphology. Of note, Kessels *et al.*[7] used oligomer concentrations more than an order of magnitude greater than those reported for amyloid-β–PrP^C interaction[4,11], which might engage additional PrP^C-independent mechanisms.

A third experiment by Kessels *et al.*[7] was similar to that described in our own Fig. 4[4]. In distinction to our own findings and those of others (see http://www.alzforum.org/pap/annotation.asp?powID=98973 for comment on ref. 11), Kessels *et al.*[7] observed Aβ42 suppression of CA1 plasticity in *Prnp*$^{-/-}$ slices. A possible explanation for this outcome of Kessels *et al.*[7] is provided by the fact that plasticity suppression by amyloid-β oligomer was total (100%) at the earliest time points. In contrast, previous studies demonstrated limited decrement of short-term potentiation by amyloid-β oligomer[1,3,4,12–14]. Rather, the maintenance/consolidation of potentiation is impaired by amyloid-β[1,3,4,12–17]. The Aβ42 preparation of Kessels *et al.*[7] has distinct effects on plasticity, and these are PrP^C-independent. This Aβ42 sample also suppresses baseline transmission by ~25%, which is indicative of more general or non-specific toxicity. Native Aβ42 biochemical analysis of this preparation without SDS would be helpful. Potentially, amyloid-β suppression of baseline transmission and immediate plasticity by this

particular preparation are PrP^C-independent, whereas LTP maintenance/consolidation is PrP^C-dependent.

PrP^C dependency may also vary with dosing. PrP^C is not a transmembrane protein, so its primary function may be in binding and concentrating amyloid-β oligomers at the neuronal surface. Local accumulation may then permit action at a transmembrane co-receptor when amyloid-β oligomer is dose-limited, rather than saturated. Thus, PrP^C may be essential for amyloid-β oligomer action at low concentrations but dispensable at higher concentrations. For oligomers, effective concentrations are difficult to standardize. It will be valuable to examine various amyloid-β species in multiple assays and doses to confirm the full extent of PrP^C participation.

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