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Synaptic plasticity in animal models of early Alzheimer's disease

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Amyloid β-protein (Aβ) is believed to be a primary cause of Alzheimer's disease (AD). Recent research has examined the potential importance of soluble species of Aβ in synaptic dysfunction, long before fibrillary Aβ is deposited and neurodegenerative changes occur. Hippocampal excitatory synaptic transmission and plasticity are disrupted in transgenic mice overexpressing human amyloid precursor protein with early onset familial AD mutations, and in rats after exogenous application of synthetic Aβ both *in vitro* and *in vivo*. Recently, naturally produced soluble Aβ was shown to block the persistence of long-term potentiation (LTP) in the intact hippocampus. Sub-nanomolar concentrations of oligomeric Aβ were sufficient to inhibit late LTP, pointing to a possible reason for the sensitivity of hippocampus-dependent memory to impairment in the early preclinical stages of AD. Having identified the active species of Aβ that can play havoc with synaptic plasticity, it is hoped that new ways of targeting early AD can be developed.

Keywords: long-term potentiation; β-amyloid; excitatory synaptic transmission; glutamate; NMDA receptors; acetylcholine

1. INTRODUCTION

A major goal of current research on AD is to determine the causes of the mild cognitive impairment that usually presages the insidious onset of clinical dementia. Mild cognitive impairment of preclinical AD is characterized by deficits in the forms of memory that are known to be dependent on the function of the medial temporal lobe, including the hippocampus and related structures (Laakso 2002). It is argued here that potentially reversible impairments of synaptic memory mechanisms in these brain regions are likely to precede neurodegenerative changes that are characteristic of clinical AD.

Other contributors to this issue (Bear 2003; Bozon *et al.* 2003; Hédou & Mansuy 2003; Lisman 2003; Morris 2003; Pittenger & Kandel 2003; Tonegawa *et al.* 2003) give cogent reasons why studies of synaptic plasticity, in particular LTP of fast excitatory synaptic transmission, are particularly suited to the elucidation of mechanisms of memory function. Our hope is that investigations of how putative causes of AD affect excitatory synaptic transmission and plasticity in the hippocampus will aid the discovery of the processes underlying the early memory deficits of preclinical AD. Such an approach should help to provide a fertile basis, for probing not only the causes of the synaptic dysfunction underlying memory impairment, but also possible targets for therapeutic intervention very early in the disease process.

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One contribution of 30 to a Theme Issue 'Long-term potentiation: enhancing neuroscience for 30 years'.

2. SYNAPTIC PLASTICITY AND THE Aβ HYPOTHESIS OF ALZHEIMER'S DISEASE

AD has been suggested to be a form of neuroplasticity failure (Mesulam 1999; Selkoe 2002). Consistent with this, the potential for neuroplasticity in the adult brain occurs unevenly in different regions, with synaptic plasticity, axonal and dendritic remodelling and synaptogenesis being particularly high in areas affected early in AD (Arendt 2001). For example, plasticity-related increases in the length and branching of the dendritic tree during adulthood (Arendt *et al.* 1998), expression of the growth associated protein GAP 43 (a marker for axonal sprouting; Lin *et al.* 1992) and the expression levels of mRNAs for brain-derived neurotrophic factor and TrkB receptors (Okuno *et al.* 1999) are all relatively high in the hippocampus and entorhinal cortex. This indicates that the processes underlying experience-dependent remodelling and synaptic turnover in the adult are particularly vulnerable to the primary causes of AD.

The Aβ hypothesis of AD proposes that Aβ is a primary cause (Hardy & Selkoe 2002). The APP is axonally transported to presynaptic axon terminals where it is a transmembrane protein with a large extracellular amino terminus and a short cytoplasmic carboxy terminus. Aβ is a 4 kDa peptide of 39–43 amino acids located at the part of APP that spans the transmembrane domain, lying partly outside and partly within the membrane (Glenner & Wong 1984; Kang *et al.* 1987). APP is cleaved in the middle of the Aβ between sites 16 and 17 by α-secretase to yield a secreted form of APP, sAPPα (a large 90 kDa N-terminal portion; Esch *et al.* 1990), which is secreted into the extracellular space and can act as a neuronal survival-promoting factor. Cleavage at the N-terminal site by β-secretase (Seubert *et al.* 1993) results in an approxi-

mately 99-amino-acid transmembrane fragment, C-99. This is further cleaved inside the membrane by α-secretase (which includes PS1) to yield APP intracellular domain, a fragment with a putative nuclear signalling role, and Aβ (predominantly Aβ1–40 and Aβ1–42). The latter species of Aβ is highly hydrophobic and has been particularly implicated in causing AD (Pike *et al.* 1995).

3. TRANSGENIC ANIMAL MODELS

(a) *Familial Alzheimer's disease APP mutations*

Strong support for the Aβ hypothesis came from a small proportion of familial AD clusters that are caused by mutations of APP, which lead to increased Aβ levels and the relatively early onset of dementia. Many groups have developed transgenic mice that overexpress these mutant forms of human APP. Most currently studied models show cognitive deficits and age-related disruption of synaptic markers and amyloid plaque deposition, but few strains show evidence of significant cell death (Janus *et al.* 2000; Ashe 2001; Chapman *et al.* 2001; Richardson & Burns 2002).

The neurophysiological consequences of such mutations have been examined in the hippocampus of these mutant mice. This has allowed the investigation of the role of age-related factors such as plaque deposition and synaptic loss in functional deficits. Most studies have reported, principally, either inhibition of LTP or reduction in baseline fast excitatory transmission prior to plaque deposition. The relative importance of these changes and apparent discrepancies still need to be resolved (see also § 5b). For example, transgenic mice (called PDAPP mice) overexpressing hAPP mutated at valine to phenylalanine at amino acid 717 (V717F, Indiana mutation) were studied initially at age 4–5 months, prior to the deposition of Aβ. These mice had a small (less than 20%) reduction in basal synaptic transmission in the CA1 area *in vitro*. LTP induced by theta-burst conditioning stimulation was inhibited, and was associated with a change in the synaptic response during burst stimulation. In aged mice (27 months), which had Aβ plaque formation, baseline transmission was reduced by 70%, but LTP was normal (Larson *et al.* 1999). Complete inhibition of LTP of the population spike with no significant effect on baseline amplitude was detected in the CA1 area *in vivo* in PDAPP mice both at ages 3–4.5 and 24–27 months. No changes were detected in the dentate gyrus (Giacchino *et al.* 2000). In a further study on a separate line with the same mutation, greatly reduced (*ca.* 40%) basal synaptic transmission was observed *in vitro* at 1–4 months, probably owing to a decrease in the number of functional synapses even though no amyloid plaques were present at this age. LTP induction in response to strong HFS was unchanged in these animals or at a later stage (8–10 months) when amyloid plaques had been deposited (Hsia *et al.* 1999).

Transgenic mice overexpressing hAPP 695 mutated with both K670N and M671L (Swedish mutation, called Tg2576 mice) also show age-dependent plaque deposition but no major disruption of synaptic markers or cell viability. At 15–17 months, such mice had normal excitatory synaptic transmission in CA1 and dentate gyrus, but were severely impaired in LTP induction in these areas when assessed both *in vitro* and *in vivo*. Young mice of 2–8 months had no such deficits (Chapman *et al.* 1999). However, further *in vitro* studies by other researchers failed to observe a reduction in LTP in the same transgenic line at 12 or 18 months despite compromised baseline transmission (Fitzjohn *et al.* 2001). A further transgenic mouse line, with both the Swedish and Indiana mutations present, had a major loss of basal transmission *in vitro* at 2–4 months. Aβ in these animals was expressed at a high level, whereas hAPP levels remained relatively low, strongly suggesting that the loss in synapses and in synaptic transmission is caused by Aβ (Hsia *et al.* 1999).

Another mutant hAPP line (V642I, London mutation, termed TgAPP/Ld/2 mice) had a deficit in the persistence of LTP of the EPSP induced by strong HFS in CA1 at age 5–7 months, even though amyloid plaque formation was only detected in animals older than 12 months (Moechars *et al.* 1999). Recently, the inhibition of LTP was confirmed in similar mice (V717I, London mutation) but was absent in double transgenic mice that also had a conditional knockout of PS1. Since Aβ production was blocked in the double transgenics, the inhibition of LTP in V717I mice was attributed to Aβ (Dewachter *et al.* 2002). Neither of these reports included detailed analysis of baseline transmission.

In summary, hippocampal transmission and plasticity at excitatory glutamatergic synapses have been found to be sensitive markers of early dysfunction, often being reduced in young animals long before Aβ is found deposited in neuropathological plaques. This indicates that soluble Aβ (i.e. Aβ that remains in aqueous solution after high-speed centrifugation, composed of monomeric, oligomeric and protofibrillary species) may be a critical player in producing functional synaptic deficits in the absence of fibrillar Aβ or cell death.

(b) *Other APP and PS mutants*

Several other related transgenic models have examined the effects of modifying APP and PS expression on synaptic transmission and plasticity and therefore may help to elucidate their normal and/or pathological roles. Overall, mice with loss of APP had reduced LTP in response only to certain conditioning protocols. Generally, transgenic mice expressing human familial AD PS mutations had facilitated LTP, possibly owing to alterations in Ca buffering, whereas transgenic mice underexpressing PSs had decreased LTP induction, possibly owing to increased GABAergic transmission. None of these mutations was reported to affect basal fast excitatory synaptic transmission.

Transgenic mice lacking the APP gene (APP null) at age 8–12 months or 20–24 months had normal LTP induced by a weak HFS (10 stimuli at 100 Hz) but had reduced LTP induced by a subsequent (30 min later) stronger theta-burst HFS (4×10 stimuli at 100 Hz), with the level of LTP being reduced immediately following the HFS (Seabrook *et al.* 1999; see also Dawson *et al.* 1999). By contrast, LTP induced by a strong HFS of 100 Hz for 1 s was not reduced, either in control media or in the presence of picrotoxin (Seabrook *et al.* 1999; Fitzjohn *et al.* 2000). GABAergic inhibition was also reduced in these mice, the inhibitory postsynaptic current amplitude being reduced (Seabrook *et al.* 1999).

Transgenic mice expressing human PS mutations (familial AD-linked mutants PS1[A246E] or PS2[N141I]) did not display any pathological deficiencies. They had normal LTP induction in CA1 in response to strong HFS, but LTP induction was enhanced in response to a weak HFS, with controls showing LTP declining to baseline over 2 h, but mutants having non-declining LTP. Glutamate application led to a higher than normal Ca^{2+} elevation in transgenic mice (Schneider *et al.* 2001). Further studies in mice with familial AD-linked mutations in PS1 (deletion of exon 9 variant, M146L and A246E mutations) also showed normal basal transmission and enhanced LTP (larger amplitude and more persistent) in response to theta burst and HFS (Parent *et al.* 1999; Barrow *et al.* 2000; Zaman *et al.* 2000) and also showed enhanced inhibitory transmission (Zaman *et al.* 2000).

In transgenic mice in which PS1 conditional knockout was restricted to the postnatal forebrain (PS1 expression was progressively eliminated from the third postnatal week), levels of Aβ1–40 and Aβ1–42 were reduced compared with controls, while APP C-terminal fragments increased. Basal transmission and LTP induction in response to theta burst or HFS was normal in such mice (Yu *et al.* 2001). Somewhat similarly, mutant mice underexpressing PS1 (PS1 ± mice) from birth had normal basal transmission in CA1. Although LTP induction in response to a single theta burst or a single tetanus was normal in these mice, LTP induction in response to multiple tetanus was reduced, with LTP declining more rapidly than in controls (Morton *et al.* 2002). In view of the role of PS in the metabolism of the APP C-terminal, it is interesting that transgenic mice expressing an amyloidogenic C-terminal fragment of APP (C104 mice) exhibited age-related amyloid deposition and decrease in CA1 cell number. Although basal synaptic transmission was not reported, 8–10-month-old transgenic mice showed deficient LTP induction, with significant inhibition at times greater than 10 min post-HFS (Nalbantoglu *et al.* 1997).

4. EXOGENOUSLY APPLIED Aβ

Overall, although transgenic animals offer many advantages in the study of possible causes of AD, it has not been possible to disentangle clearly the role of APP *per se* or its breakdown products, including the different soluble and fibrillar Aβ species. Direct exogenous application of Aβ provides an alternative approach to determine whether Aβ can cause deficits in hippocampal excitatory synaptic transmission/plasticity and the necessity for amyloid plaque formation and neurodegeneration.

(a) *Effect of Aβ on LTP* in vitro

Several studies have shown that synthetic Aβ inhibits LTP induction *in vitro*. Thus, in hippocampal slices prepared from 20–30-day-old rats, soluble Aβ1–42 (500 nM) was found to inhibit LTP induction by strong HFS of the medial perforant path in the dentate gyrus both of the population spike (Lambert *et al.* 1998) and EPSPs (Wang *et al.* 2002). Both early- and late-phase LTP were strongly inhibited in these studies, whereas basal AMPA receptor-mediated synaptic transmission was not altered, although there was a reduction in paired-pulse depression at a short (20 ms) inter-pulse interval (Wang *et al.* 2002). In these studies, Aβ1–42 was specially prepared to contain large metastable Aβ oligomeric assemblies (termed ADDLs), providing evidence that non-fibrillar Aβ can selectively disrupt both short-term and long-term synaptic plasticity.

Similarly, LTP of field EPSPs in rat CA1 and the medial perforant path of the dentate gyrus was inhibited by Aβ1–40, Aβ1–42 and the truncated Aβ fragment 25–35 at concentrations of 200 nM or 1 μM. The N-terminal sequence of Aβ25–35 was found to be necessary for inhibition of LTP induction (Chen *et al.* 2000). In contrast to these studies showing an inhibition of LTP induction, synthetic Aβ1–40 (200 nM) enhanced LTP induction in the associational–commissural pathway of the dentate gyrus of 30–50-day-old rats (Wu *et al.* 1995*a*). Basal AMPA receptor-mediated synaptic transmission was not affected in this study.

In summary, the inhibitory effects of Aβ on LTP *in vitro* occurred in the absence of changes in baseline transmission and thus do not appear to be caused by a toxic action of the Aβ resulting in rapid neurodegeneration. Moreover, truncated Aβ variants that were not lethal to cultured neurons also blocked LTP induction (Chen *et al.* 2000). Intriguingly, non-fibrillar Aβ 1–42 (Wang *et al.* 2002) and Aβ variants that did not form fibrils *in vitro* (Chen *et al.* 2000) inhibited LTP, pointing to a critical role for soluble peptide.

(b) *Effect of Aβ on LTP* in vivo

Consistent with most *in vitro* studies, synthetic Aβ also inhibits LTP in the intact hippocampus. Thus late-phase LTP of field EPSPs in the CA1 area was strongly inhibited at doses that had no acute effect on baseline excitatory transmission in adult rats by intracerebroventricular (i.c.v.) injection of Aβ1–40 (0.4 and 3.5 nmol, but not 0.1 nmol), Aβ 1–42 (0.01 nmol) and the Aβ-containing C-terminal fragment CT105 (0.05 nmol). In these studies, LTP was only significantly inhibited at a time greater than 2 h post-HFS, and the LTP was completely blocked by Aβ1–40 and Aβ 1–42 at 3 h post-HFS (Cullen *et al.* 1997*a*), implicating late LTP. Somewhat similarly, LTP of EPSPs in the CA1 area was inhibited by the truncated fragments Aβ25–35 (10 nmol, 100 nmol) and Aβ35–25, but not Aβ 15–25, at times greater than 30 min post HFS (Freir *et al.* 2001).

Other studies have examined the delayed neurophysiological effects of Aβ *in vivo*. In contrast to the acute effect of Aβ1–40 (3.5 nmol), there was a small reduction in baseline transmission in the CA1 area 24 h after a single i.c.v. injection. The reduction was present for at least 5 days, whereas LTP was not affected at this time (Cullen *et al.* 1996).

In another study, induction of LTP of field EPSPs in the dentate gyrus by strong HFS was inhibited after direct intrahippocampal injection of Aβ1–43 or a combination of Aβ1–40 and Aβ1–43 in adult rats. Late-phase LTP of the EPSP was most sensitive to disruption, whereas EPSP-spike LTP was largely intact. The effect of the Aβ was examined *ca.* 7–16 weeks after the injections, a time when focal amyloid deposits and cell atrophy were detected. A reduction in baseline synaptic transmission and deficits in working memory were also present (Stéphan *et al.* 2001).

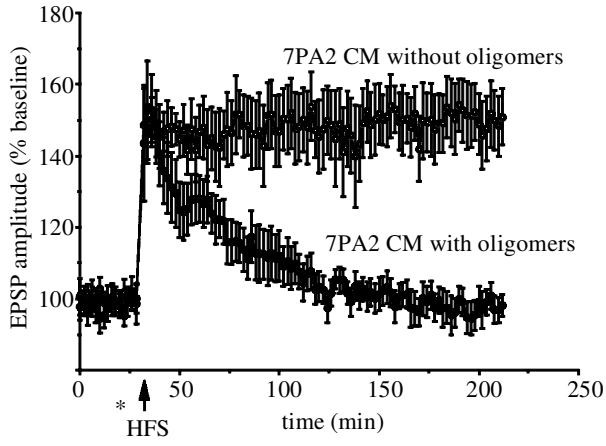

Figure 1. Naturally secreted oligomers of Aβ inhibit LTP of excitatory synaptic transmission in the intact hippocampus of anaesthetized rats (adapted from Walsh *et al.* 2002). i.c.v. (asterisk) injection of conditioned medium from cells transfected with mutant APP that release soluble Aβ oligomers (7PA2 CM with oligomers, closed circles) completely blocked LTP measured at 3 h after HFS (arrow), whereas conditioned medium from cells pretreated with a γ-secretase inhibitor, at a concentration that reduced Aβ oligomer production relatively selectively, prevented the block (7PA2 CM without oligomers, open circles).

Somewhat analogous to the *in vitro* studies on transgenic mice, two studies examined the effects of *in vivo* Aβ exposure on synaptic function in the hippocampal slice of adult rats. Whereas acute single i.c.v. injection of synthetic Aβ1–40 (0.4 or 3.5 but not 0.1 nmol) caused a reduction in baseline transmission and no change in LTP in the dentate gyrus 48 h later (Cullen *et al.* 1996), continuous i.c.v. infusion of Aβ1–40 (300 pmol day^{-1}) for 10–11 days inhibited LTP of the population spike in the CA1 area (Itoh *et al.* 1999). In the latter study, there was a tendency to require a greater current to evoke equivalent-sized spikes.

(c) *Effects of naturally produced Aβ on synaptic plasticity*

The brains of transgenic animals accumulate a variety of Aβ species, both soluble and insoluble, the relative importance of which in the disruption of synaptic transmission and plasticity is difficult if not impossible to separate. One of the problems with most published studies of exogenously applied Aβ is that the synthetic Aβ peptides are usually present in several different undefined states of association/aggregation. Some of these states may never occur naturally. We therefore believe that it is necessary to study the effects of naturally produced Aβ species as well as synthetic peptides.

By using medium collected from cells transfected with mutant hAPP (V717F) that secrete nanomolar concentrations of soluble Aβ, in the absence of fibrillar Aβ, we were able to examine the contribution of soluble monomers and oligomers of variable length (including Aβ1–42) to the effects of Aβ on synaptic function in the intact hippocampus (Walsh *et al.* 2002; figure 1). Although basal glutamatergic transmission was unaffected by such low amounts of Aβ, the persistence of LTP was reduced to less than 3 h when i.c.v. injected shortly before HFS. Remarkably, when all of the monomers were selectively cleared from the medium using the protease insulin-degrading enzyme, leaving only oligomers, the block was unaffected. This means that the oligomers are by far the most active species even though they were present only in sub-nanomolar concentrations. By contrast, medium from cells that were exposed to low doses of a γ-secretase inhibitor DAPM (N-[N-3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine methyl ester) to partially block the production of Aβ, thereby preferentially reducing oligomer formation but leaving monomers relatively unaffected, did not block LTP. This finding thus confirms the role of soluble Aβ oligomers and also gives support to the use of relatively low doses of γ- and possibly β-secretase inhibitors as a viable pharmacological approach to the therapy of AD.

Recently we have confirmed that medium containing sub-nanomolar concentrations of naturally produced Aβ oligomers also inhibits LTP in the rat dentate gyrus *in vitro* (H.-W. Wang, D. M. Walsh, M. J. Rowan, D. J. Selkoe and R. Anwyl, unpublished observations).

In view of the apparently limited ability of exogenously applied Aβ to penetrate into cells (Bi *et al.* 2002), this approach does not directly address the potentially important role of raised intracellular Aβ. In this context, it will be interesting to examine the effect of viral application of Aβ.

5. CELLULAR MECHANISMS OF Aβ ACTION

Many cellular mechanisms have been proposed to explain the toxic effects of high concentrations of fibrillar Aβ. Relatively little is known about the mechanisms of action of low concentrations of soluble Aβ. We focus on possible cholinergic and NMDA receptor-mediated mechanisms given the current, albeit limited, pharmacotherapy of AD with cholinesterase inhibitors such as donepezil and the NMDA receptor antagonist memantine.

(a) *Role of acetylcholine and related signalling*

There is extensive support for the involvement of cholinergic mechanisms in the biochemical and behavioural effects of soluble Aβ (Auld *et al.* 2002). Recent studies have found that alterations in acetylcholine and related signalling can reverse the inhibitory effect of Aβ on LTP, thereby providing a mechanistic basis for this important therapeutic target. Thus co-administration of an acetycholinesterase inhibitor, huperzine A, at a concentration that did not by itself enhance LTP induction completely prevented the suppression of LTP of the population spike by acute application of truncated Aβ fragments 25–35 and 31–35 in the CA1 area of the rat (Ye & Qiao 1999). These authors suggested that the inhibitory effect of Aβ on LTP induction may be via inhibiting cholinergic transmission, perhaps suppressing synthesis and/or release of acetylcholine (Hoshi *et al.* 1997) or blocking acetylcholine receptors (Kelly *et al.* 1996). Similarly, the block of LTP persistence by Aβ1–40 (3.5 nmol, i.c.v.) *in vivo* was completely prevented by pretreatment with the cholinesterase inhibitor physostigmine (0.1 mg kg^{-1}, i.p.). This dose of physostigmine on its own did not have a significant effect on baseline transmission or on the magnitude of LTP induced by HFS (Cullen *et al.* 1997*b*). Recently, Sun & Alkon (2002)

reported that the truncated Aβ 25–35 (but not Aβ 35–25) injected i.c.v. 4–6 days before recording *in vitro* greatly reduced a long-term synaptic modification of $GABA_A$ receptor-mediated synaptic potentials that depends on the associative activation of cholinergic and GABAergic inputs. Cholinergic (muscarinic)-induced theta in CA1 pyramidal cells was inhibited, but LTP was unaffected.

Much interest has been generated in the possible role of nicotinic receptors in the actions of Aβ. The α7 subunit-containing nicotinic receptor (α7nAChR) that has been suggested to be a high-affinity receptor for Aβ1–42. Aβ1–42, at very low concentration (0.1–100 nM), activates the ERK2 MAPK cascade acutely in hippocampal slices, an effect that is blocked by selective α7nAChR antagonists and prevents external Ca^{2+} influx (Dineley *et al.* 2001). It was suggested that Ca^{2+} influx via α7nAChR and α7nAChR-dependent depolarization led to MAPK activation. Subchronic exposure of organotypic hippocampal slice cultures for 6 days to Aβ1–42 upregulated α7nAChR. In addition, hAPP transgenic mice (Tg2576) had pronounced age-dependent upregulation of α7nAChR, which was accompanied by biphasic alterations in downstream ERK2 and CREB protein activation (Dineley *et al.* 2001). Whether or not these changes are related to the previously described disruption of synaptic function in these mice (Chapman *et al.* 1999; Fitzjohn *et al.* 2001) remains to be determined. Itoh *et al.* (1999) reported that nicotine failed to affect potentiation of the population spike in slices from animals treated with subchronic infusion of Aβ1–40 *in vivo*, and LTP was blocked. Since nicotine enhanced short-term potentiation in controls, this finding is consistent with a loss of nicotinic regulation of glutamatergic mechanisms. The involvement of GABAergic mechanisms needs to be addressed as α7nAChRs are located primarily on inhibitory interneurons (Frazier *et al.* 1998; Pettit *et al.* 2001). Clearly, any involvement of ERK/MAPK/CREB also potentially implicates many other transmitter pathways, including glutamatergic directly (see below).

(b) *Role of NMDA receptor-mediated synaptic transmission*

Since many forms of hippocampal synaptic plasticity and toxicity are NMDA receptor-dependent, alterations in NMDA receptor-mediated synaptic transmission and related mechanisms may contribute to the effects of Aβ. Overall, although there is some evidence of increased NMDA receptor-mediated function and excitotoxicity under defined *in vivo* and *in vitro* experimental conditions, it will be important to evaluate their general significance. Thus, Aβ1–40 (200 nM) can selectively elicit a rapid and persistent increase in NMDA receptor-mediated, but not AMPA receptor-mediated, transmission in the dentate gyrus *in vitro* (Wu *et al.* 1995*b*). Moreover, the delayed reduction in baseline synaptic transmission in the CA1 area *in vivo* caused by Aβ1–40 (3.5 nmol) can be prevented by treatment with the NMDA receptor antagonist CPP (7 mg kg^{-1} x2, i.p.) (Cullen *et al.* 1996).

Transgenic mice overexpressing hAPP (V717F) had a relative upregulation of NMDA receptor-mediated synaptic transmission at a time when AMPA receptor-mediated transmission was reduced (Hsia *et al.* 1999). Consistent with an age-related increased potential for NMDA receptor-dependent excitotoxicity, Fitzjohn *et al.* (2001) reported that the non-selective glutamate receptor antagonist kynurenic acid (1 mM), when present at the anoxic period of slice preparation, prevented the reduction in baseline transmission at 12 months in hAPP K670N/M671L mice. However, this strategy was not effective at a later age (18 months) or at 8–9 months in V717F mice (Hsia *et al.* 1999; see also Chapman *et al.* 2001). In this context, it is interesting that glutamate can potentiate the inhibitory effect of Aβ1–42 on LTP (Nakagami & Oda 2002). In view of the putative involvement of the p38 MAPK pathway in excitotoxicity, it is of interest that the acute reduction in baseline synaptic transmission and block of LTP in the dentate gyrus of young rats by the truncated fragment Aβ25–35 (1 μM) was prevented by perfusion with the inhibitor SB203580 (1 μM) (Saleshando & O'Connor 2000).

By contrast, Aβ 1–42 (200 nM and 1 μM) has recently been reported to reduce NMDA receptor-mediated synaptic currents in the dentate gyrus (Chen *et al.* 2002). Aβ1–42 and ADDLs at the sublethal concentrations of 5 μM and 100 nM, respectively, also strongly suppressed a NMDA-evoked/depolarization-induced increase in CREB phosphorylation in cultured cortical neurons, whereas Aβ25–35 (10 μM) was inactive (Tong *et al.* 2001). CREB phosphorylation has been implicated in late LTP. Remarkably, in a recent study rolipram and forskolin, agents that enhance the cAMP-signalling pathway can reverse inhibition of LTP by Aβ1–42. This reversal was blocked by H89, an inhibitor of protein kinase A (Vitolo *et al.* 2002).

An intriguing corollary to the block of LTP by Aβ is the facilitation of LTD induction by low-frequency stimulation and time-dependent LTP reversal in the CA1 area by very low-dose Aβ1–42 (1 pmol i.c.v.) and CT105 (1–2 pmol), respectively, in the adult rat. Both effects were blocked by the NMDA receptor antagonist D-AP5 (100 nmol), indicating their NMDA receptor dependence (Kim *et al.* 2001). Somewhat similarly, Aβ1–42 applied in the first hour after HFS inhibited LTP, and inhibition of calcineurin activity with FK506 or cyclosporin A completely prevented this effect (Chen *et al.* 2002). By contrast, ADDLs (500 nM) failed to affect a large, apparently NMDA receptor-independent form of LTD in the dentate gyrus of young (14–19-day-old) rats (Wang *et al.* 2002).

6. CONCLUSION

Extracellular deposition of fibrillar Aβ and cell death are not required for the development of functional deficits in AMPA receptor-mediated hippocampal synaptic transmission and plasticity in transgenic mutant hAPP mouse and acute Aβ rat models. Good evidence that the disruption of LTP is caused by highly mobile Aβ oligomers is provided by experiments using exogenously applied natural and synthetic Aβ. Further studies are required to determine the roles of recently discovered specific receptor/signalling mechanisms and facilitated LTD/depotentiation in either the block of LTP or reductions in baseline transmission.

This research may provide insight into the likely causes and possible treatments of mild cognitive impairment in preclinical AD (see figure 2 for a schematic view of possible mechanisms and targets). Clearly, the mechanisms

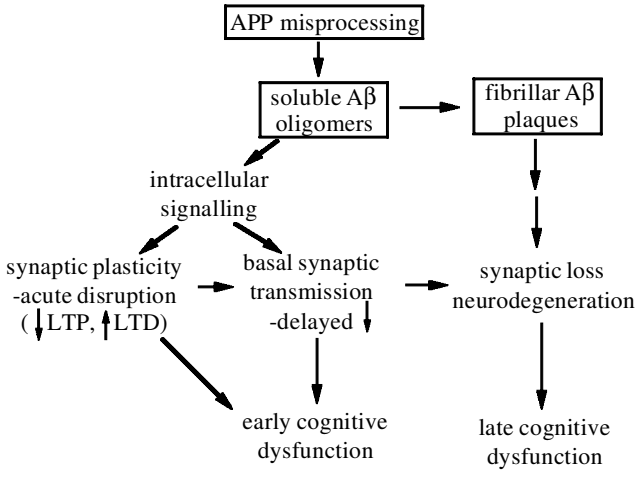

Figure 2. Speculative overview of the actions of Aβ oligomers on hippocampal synaptic transmission and plasticity that may underlie the mild cognitive impairment of early AD. Soluble Aβ oligomers released into the extracellular space consequent to APP misprocessing may, in the absence of adequate clearance, diffuse to neighbouring cells. The presence of sub-nanomolar concentrations of Aβ oligomers would rapidly block LTP and promote LTD of fast excitatory transmission in a use-dependent manner, as a result of disruption of intracellular signalling, NMDA receptor-mediated transmission and cholinergic transmission. A delayed reduction in basal AMPA receptor-mediated transmission would be caused possibly by the shift from LTP-like to LTD-like synaptic plasticity and increased vulnerability to excitotoxicity. The rapid disruption of synaptic plasticity and delayed reduction in basal transmission may lead to the insidious but intermittent mild cognitive impairment of preclinical AD. In clinical AD, such mechanisms may be superimposed on the irreversible neurodegeneration and synaptic loss that may be caused primarily by (proto-) fibrillary Aβ and thereby contribute to progressive dementia.

that are described here are probably more relevant to the very early rather than late symptoms of AD. Progressively higher concentrations of fibrillar Aβ associated with major neurodegenerative changes are found in clinical AD and are considered to be significant factors in the later course of the disease. However, it is possible that a disruption of synaptic plasticity-related mechanisms by soluble Aβ also contributes to clinical symptoms (Lue *et al.* 1999; McLean *et al.* 1999). Indeed, if the changes in synaptic function described here occur in the early preclinical stages they may provide the necessary trigger that interacts with age, oxidative status, energy supply and other major vulnerability factors to precipitate the onset of clinical disease.

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GLOSSARY

Aβ: amyloid β-protein
AD: Alzheimer's disease
ADDL: Aβ-derived diffusible ligand
APP: β-amyloid precursor protein
CREB: cAMP-regulatory element binding
EPSP: excitatory postsynaptic potential
ERK: extracellular signal-regulated kinase
hAPP: human β-amyloid precursor protein
HFS: high-frequency stimulation
LTD: long-term depression
LTP: long-term potentiation
MAPK: mitogen-activated protein kinase
NMDA: *N*-methyl-D-aspartate
PS: presenilin