Neurotransmitter receptor and time
dependence of the synaptic plasticity
disrupting actions of Alzheimer’s
disease Aβ in vivo

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Many endogenous factors influence the time course and extent of the
detrimental effects of amyloid β-protein (Aβ) on synaptic function. Here, we
assessed the impact of varying endogenous glutamatergic and cholinergic
transmission by pharmacological means on the disruption of plasticity at
hippocampal CA3-to-CA1 synapses in the anaesthetized rat. NMDA receptors
(NMDARs) are considered critical in mediating Aβ-induced inhibition of long-
term potentiation (LTP). However, intracerebroventricular injection of Aβ1–42
inhibited not only NMDAR-dependent LTP but also voltage-activated Ca2+-
dependent LTP induced by strong conditioning stimulation during NMDAR
blockade. On the other hand, another form of NMDAR-independent synaptic
plasticity, endogenous acetylcholine-induced muscarinic receptor-dependent
long-term enhancement, was not hindered by Aβ1–42. Interestingly, augment-
ing endogenous acetylcholine activation of nicotinic receptors prior to the
injection of Aβ1–42 prevented the inhibition of NMDAR-dependent LTP,
whereas the same intervention when introduced after the infusion of Aβ
was ineffective. We also examined the duration of action of Aβ, including
water soluble Aβ from Alzheimer’s disease (AD) brain. Remarkably, the
inhibition of LTP induction caused by a single injection of sodium dodecyl
sulfate-stable Aβ dimer-containing AD brain extract persisted for at least a
week. These findings highlight the need to increase our understanding of
non-NMDAR mechanisms and of developing novel means of overcoming,
rather than just preventing, the deleterious synaptic actions of Aβ.

1. Introduction

The last 10 years has seen little progress in the pharmacotherapy of Alzheimer’s
disease (AD), although there is a small number of ongoing promising clinical
trials, especially those focusing on treating patients at an early stage [1,2]. Current
approved pharmacotherapy is based on two main strategies. First, antichol-
inesterase drugs, which augment ongoing cholinergic transmission, thereby
activating both nicotinic and muscarinic acetylcholine receptors. Second, meman-
tine, which is a weak NMDA-type glutamate receptor (NMDAR) channel
blocker, thereby preventing presumed inappropriate activation of these receptors.
Neither class of drug treatment is clinically very effective, with a subset of patients
obtaining modest benefit at best, over a relatively brief period [3]. Rather than
modifying the insidious progress of the disease the drugs are considered to act
as symptomatic treatments primarily targeting cognitive impairment.

There is a growing consensus, given the available data, that the amyloid
β-protein (Aβ) ‘hypothesis’ of AD has reached ‘theory’ status [4]. Although
the neuropathological hallmark plaques are composed largely of fibrillar Aβ,
most attention has been devoted to investigating the pathophysiological role

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of non-fibrillar, water-soluble forms of Aβ [5,6]. Considerable progress has been achieved in elucidating the disruptive actions of soluble Aβ on synaptic transmission and plasticity of that transmission [7–9]. Extensive studies have examined how endogenous factors influence the time course and extent of the disruption of synaptic plasticity by soluble Aβ, with particular emphasis on the actions of putative ‘synaptotoxic’ assemblies ranging from small oligomers to relatively large protofibrils. Here, we focus on the role of NMDARs and cholinergic mechanisms in dysregulation of hippocampal synaptic plasticity caused by acute administration of Aβ in vitro. We found evidence that extends or differs from previously reported in vitro findings. We also address a topic that has potentially important clinical ramifications: the reversibility, be it spontaneous or intervention-based, of the synaptic plasticity disruptive effects of Aβ.

2. Aβ-mediated disruption of NMDAR-dependent and -independent forms of long-term potentiation

There are two related aspects to the involvement of NMDARs: how does Aβ affect NMDAR-independent forms of synaptic plasticity, and what is the role of NMDAR-independent mechanisms in the disruptive actions of Aβ? Some years ago, we reported that, in vitro, NMDAR-independent LTP was resistant to inhibition by Aβ1–42 [10]. Moreover, we found strong evidence that the inhibition of NMDAR-dependent LTP by Aβ is prevented by selective block of GluN2B subunit-containing NMDARs in vivo [11], which was confirmed in vitro [12–14]. However, not all NMDAR-dependent LTP is susceptible to inhibition by Aβ, and Aβ can facilitate NMDAR-independent long-term depression [11,12,15].

In order to compare the effect of Aβ on NMDAR-dependent and -independent forms of LTP in vivo, we have used two different high-frequency conditioning stimulation (HFS) protocols. Our standard HFS at 200 Hz triggers robust LTP that is completely blocked by injection of different NMDAR antagonists, including D-AP5 [16,17]. By contrast, i.c.v. injection of D-AP5 fails to fully block LTP induced by repeated strong HFS at 400 Hz [17]. The additional, D-AP5 resistant, LTP is fully inhibited by the R- and T-type voltage-activated Ca2+-channel blocker mibefradil [17–20]. We found that both forms of LTP were strongly inhibited by Aβ1–42 in vivo. Thus, after the i.c.v. injection of Aβ1–42, the standard HFS induced a decremental LTP that decayed back to baseline over a 2–3 h period (figure 1a). The same dose of Aβ1–42 completely inhibited all phases of LTP induced by the strong HFS protocol applied in the presence of D-AP5 (figure 1b).

The strong inhibition of NMDAR-independent LTP in the same pathway in D-AP5-injected rats in vivo was surprising. Previously, as noted above, we reported that NMDAR-independent LTP induced by strong HFS in the presence of D-AP5 in the same pathway was resistant to inhibition in rat hippocampal slices [10]. One likely explanation for the apparent discrepancy is that the mechanisms of NMDAR-independent LTP induced by strong HFS are different in vivo and in vitro. Indeed, strong HFS-induced NMDAR-independent LTP in vivo is known to require Ca2+ entry through dihydropyridine-sensitive L-type voltage-activated channels [21] rather than R-/T-type channels as found here in vivo. Although the relatively strong inhibition of NMDAR-independent LTP may be due to its relatively small magnitude, an alternative explanation is that Aβ interferes with the function of voltage-activated Ca2+ channels [22,23]. Aβ can boost Ni2+-sensitive Ca2+-channels in hippocampal pyramidal cells [24,25], which could lead to excessive or inappropriate Ca2+-entry into the cell. On the other hand, Aβ did not inhibit the function of Ni2+-sensitive Ca2+-channels under the same in vitro conditions in which it inhibited hippocampal NMDAR-dependent LTP [26]. Interestingly, R-type channels appear to be important in mediating a slow afterdepolarization that regulates hippocampal LTP induction by certain firing patterns [27,28]. It will be important in future studies to determine whether Aβ directly affects the function of R- or T-type channels.

Our finding that both voltage-activated Ca2+-channel and NMDAR-dependent LTP in vivo are strongly inhibited by Aβ indicates that the inhibitory action of Aβ is independent of the initial source of Ca2+-entry that triggers LTP. Furthermore, the ability of Aβ to inhibit LTP in the presence of D-AP5 indicates that NMDARs are not involved in the disruption of certain forms of synaptic plasticity. This contrasts with the necessity for GluN2B subunit-containing NMDARs in the inhibition of NMDAR-dependent LTP in the same pathway under similar experimental conditions, as reported by us previously [11].

3. Endogenous acetylcholine-dependent mechanisms

Anticholinesterase drugs increase the magnitude and time course of cholinergic transmission via activation of either muscarinic or nicotinic receptors. In the absence of Aβ, these agents have been found to enhance LTP, for example by lowering the induction threshold [8]. Indeed, Gu & Yakel [29] recently reported that pairing of electrically evoked release of acetylcholine (ACh) and stimulation of excitatory synaptic transmission in the Schaffer-collateral/commissural pathway induced LTP that was mediated through either muscarinic or nicotinic receptors, depending on the temporal sequence. Nicotinic receptor-dependent LTP was mediated through α7-receptor facilitation of Ca2+ entry through NMDARs on dendritic spines. Muscarinic receptor-mediated LTP was much less sensitive than nicotinic receptor-mediated LTP to inhibition by Aβ [29]. Somewhat similarly, in the medial perforant pathway of the dentate gyrus, anticholinesterases and nicotine activation of α7-receptors enabled the induction of additional NMDAR-dependent LTP that has different properties from LTP induced in the absence of cholinergic enhancement [30–32]. However, nicotinic receptor-dependent LTP was resistant to inhibition in this pathway in vitro [33].

(a) Resistance of muscarinic-dependent long-term synaptic enhancement to interference by Aβ

We investigated the effects of Aβ on muscarinic receptor-mediated synaptic plasticity in vitro using a well-characterized pharmacologically induced persistent enhancement of transmission at CA3-to-CA1 synapses in anaesthetized rats [34,35]. By blocking cholinergic autoreceptors, the M2-selective antagonist methoctramine disinhibits the release of ACh, which, in turn, activates M1/M5 receptors to trigger a long-term
Long-term enhancement was not inhibited by Aβ in vivo. Injection of the selective M2 muscarinic receptor antagonist methoctramine (Meth, asterisk (*), 34 nmol, 5 µl i.c.v.) triggered a rapid onset (less than 8 min) and persistent (more than 2 h) enhancement of synaptic transmission (120.3 ± 3.3% baseline at 2 h post-Meth, n = 5; p < 0.05 compared with pre-Meth baseline) in vehicle-injected (hash (#), 5 µl i.c.v.) animals in the CA1 area of anaesthetized rats. Similarly, in animals pre-injected with Aβ1–42 (15 pmol), methoctramine triggered a robust enhancement of transmission (132.1 ± 9.9%, n = 5, p > 0.05 compared with vehicle). Insets show traces of the field EPSPs recorded at times indicated. Horizontal bar, 5 ms; vertical bar, 1 mV; filled circles, vehicle + Meth; open circles, Aβ + Meth.

(b) Prevention, but not reversal, of the plasticity disrupting action of Aβ by augmenting endogenous ACh activation of nicotinic receptors

Pretreatment with anticholinesterase drugs has been found to prevent the inhibition of LTP by Aβ both in vitro and in vivo [32,41] but the underlying mechanisms are uncertain. Given the ability of anticholinesterases to enhance control LTP, as outlined above, it might be expected that post-Aβ treatment should be as effective as pretreatment in ameliorating synaptic plasticity deficits. We therefore compared the effectiveness of pre- and post-Aβ treatment with donepezil on the inhibition of LTP.

Consistent with our previous studies with the anticholinesterase physostigmine [41], systemic pretreatment with donepezil at a dose that did not affect baseline synaptic transmission (102 ± 0.7% pre-injection baseline at 2.5 h
post-injection, \( n = 5 \), data not shown) or control LTP completely prevented the inhibition of LTP by i.c.v. injection of A\( \beta \) (3rd) (figure 3a). We investigated the mechanism of this ameliorative action of donepezil using nicotinic receptor antagonists. In animals pre-administered with the general nicotinic receptor antagonist mecamylamine, at a dose that had no obvious effect on control LTP (123.6 ± 0.8% at 3 h post-HFS, \( n = 3 \), data not shown), treatment with donepezil no longer prevented the inhibition of LTP by A\( \beta \). Likewise, pre-administration of the more \( \alpha_7 \)-selective antagonist methyllycaconitine (MLA) prevented the ameliorative effect of donepezil. Neither mecamylamine nor MLA in combination with donepezil, at the doses used in these studies, appeared to affect LTP (127.0 ± 1.9 and 131.3 ± 8.5%, respectively, \( n = 3 \) per group, data not shown).

Next, we tested the ability of donepezil to ‘rescue’ A\( \beta \)-mediated disruption of synaptic plasticity. When administered after the i.c.v. injection of A\( \beta \), at the same dose level that was effective in the pretreatment protocol, donepezil did not abrogate the inhibition of LTP (figure 3b). As a control, we determined that this schedule of donepezil treatment also did not affect LTP in the absence of A\( \beta \) injection (136.6 ± 9.1 and 131.4 ± 2.8% in donepezil or vehicle groups, \( n = 3 \) and 4, respectively, data not shown). We also made sure that this dose of donepezil when given 1 h before HFS-prevented LTP inhibition by A\( \beta \) 30 min later (126.8 ± 5.1 and 96.2 ± 8%, donepezil + A\( \beta \) and vehicle + A\( \beta \), respectively, \( n = 3 \) per group, data not shown).

These experiments provide strong evidence that endogenous ACh, via activation of nicotinic receptors, can prevent the synaptic plasticity disrupting action of A\( \beta \) in vivo.

The presumed involvement of \( \alpha_7 \)-nicotinic receptors in mediating the beneficial action of donepezil is consistent with the ability of selective \( \alpha_7 \)-nicotinic acetylcholine receptor activators to enhance hippocampal LTP [42,43]. Indeed, their high Ca\(^{2+} \) permeability [44] makes \( \alpha_7 \)-nicotinic receptors well suited to trigger intracellular Ca\(^{2+} \)-dependent signalling pathways essential for the induction of LTP [45]. As noted above, \( \alpha_7 \)-nicotinic receptor activation can lower the threshold for NMDAR-dependent LTP induction [29] or facilitate an additional, A\( \beta \)-resistant, NMDAR-dependent novel form of LTP [33].

The apparent resistance of A\( \beta \)-mediated inhibition of LTP to ‘rescue’ by post-A\( \beta \)-treatment with donepezil in the present studies may be due to the ability of A\( \beta \) to block \( \alpha_7 \)-nicotinic receptors [46]. Indeed A\( \beta_1-42 \) has a high affinity for these receptors [47], and A\( \beta_1-42 \) potently inhibits \( \alpha_7 \)-nicotinic receptor-mediated, NMDAR-dependent LTP in hippocampal slices [29]. Resistance to anticholinesterase treatment given after the infusion of A\( \beta \) may help to explain the poor therapeutic efficacy when these agents are administered to many patients with AD.

In contrast to the present findings, we and others previously reported that selective activation of \( \alpha_7 \)-nicotinic receptors via exogenously applied agonist completely abrogated the inhibition of LTP in hippocampal slices when administered after either \textit{in vivo} [48] or \textit{in vitro} [43] exposure to A\( \beta \). However, when the dose-dependence of the effect was examined in the \textit{ex vivo} study, the relative potency of the agonist was found to be reduced several fold [43]. Future studies comparing the actions of more selective nicotinic agonists or modulators \textit{in vivo} will hopefully elucidate the apparent discrepancies and the potential implications of these findings for future drug development.

**Figure 3.** Endogenous ACh-mediated nicotinic receptor-dependent prevention but not reversal of the inhibition of LTP by A\( \beta \). (a) Pretreatment with the acetylcholinesterase inhibitor donepezil, by augmenting cholinergic activation of nicotinic receptors, prevented the inhibition of LTP by A\( \beta \). (i) Systemic treatment (hash (#)) with the anticholinesterase donepezil (1 mg kg\(^{-1} \), s.c.), followed by vehicle injection i.c.v. (asterisk (*), 5 \( \mu \)l), did not significantly affect the magnitude of LTP (Don + Veh, 127.7 ± 5.3% at 3 h post-HFS, \( n = 4 \); \( p < 0.05 \) compared with 131.4 ± 2.8%, \( n = 4 \), in animals that received a vehicle injection i.c.v. at −90 min, Veh + Veh, ANOVA). By contrast, injection of water-soluble A\( \beta_1-42 \) (80 pmol, i.c.v.) after a systemic vehicle injection strongly inhibited LTP (Veh + A\( \beta \), 105.4 ± 4.9%, \( n = 4 \); \( p < 0.05 \) compared with control). (ii) Pretreatment with the same dose of donepezil completely prevented the inhibition of LTP by A\( \beta \) (Don + A\( \beta \), 139.0 ± 5.0%, \( n = 5 \); \( p < 0.05 \) compared with A\( \beta \) alone, \( p > 0.05 \) compared with control). However, donepezil no longer prevented the inhibition of LTP by A\( \beta \) in animals pre-administered with the general nicotinic receptor antagonist mecamylamine (Mec + Don + A\( \beta \), 1 mg kg\(^{-1} \), i.p.) or the more selective \( \alpha_7 \)-nicotinic receptor antagonist MLA (MLA + Don + A\( \beta \), 11 nmol in 5 \( \mu \)l, i.c.v.) (100.4 ± 2.2 and 105.9 ± 2.3%, at −150 and −130 min, respectively, \( n = 5 \) per group, \( p < 0.05 \) compared with donepezil + A\( \beta \), \( p > 0.05 \) compared with vehicle + A\( \beta \)). (b) By contrast, treatment with donepezil after the administration of A\( \beta \) did not prevent the inhibition of LTP (A\( \beta \) + Don, 104.7 ± 4.9%, \( n = 5 \); \( p > 0.05 \) compared with 101.3 ± 3.8%, \( n = 5 \), in animals injected with A\( \beta \) alone, A\( \beta \) + vehicle, \( p < 0.05 \) compared with 131.4 ± 2.8%, \( n = 4 \), in control animals, Veh + Veh). Insets show EPSP traces at the time indicated. Horizontal bar, 10 ms; vertical bar, 2 mV.
4. Persistence of the synaptic plasticity disrupting actions of soluble Aβ

Because the deleterious effects of soluble Aβ oligomers on synaptic plasticity and plasticity-related mechanisms are studied in hippocampal slices and anaesthetized animals, most research has focused on their acute effects and how these effects can be prevented by pretreatment with potential therapeutic interventions. Little attention has been paid to investigating the duration of action of acutely applied soluble Aβ on LTP. Consequently, much less is known about the reversibility, either spontaneous or intervention-based, of these disruptive effects. Indeed, Aβ oligomers are not cleared very efficiently from the brain compared with Aβ monomers [49], so there is a strong likelihood that the Aβ oligomer concentration in the brain decays relatively slowly with time.

Because the time window to study the duration of action of Aβ in the acutely anaesthetized rat is limited to a period of several hours, we also studied its effects in chronically implanted animals. In the present experiments, initially we directly compared the ability of Aβ to inhibit LTP when administered 30 min or 3 h before HFS in the acute preparation. We found that the same dose that strongly inhibited LTP with 30 min pretreatment had a similar disruptive action when injected 3 h prior to HFS (figure 4a,b).

We planned to examine the effects of Aβ over a longer time course in chronically implanted animals. We chose to re-anaesthetize the animals when assessing the magnitude of LTP in order to have a similar level of CNS arousal to that present in the experiments on acutely anaesthetized animals [50]. To our surprise, the dose of Aβ that strongly inhibited LTP in the acute preparation failed to disrupt LTP in chronically implanted re-anaesthetized animals when injected 30 min before the HFS (figure 4c). We found evidence that the threshold dose of Aβ necessary to inhibit LTP was raised. When four times the acute effective dose was injected 30 min prior to the HFS in re-anaesthetized animals, LTP was strongly inhibited. At this higher dose a

Figure 4. The synaptic plasticity disrupting action of Aβ₁₋₄₂ persists for several hours in acutely anaesthetized rats, whereas chronically implanted re-anaesthetized rats develop tolerance to this action of Aβ. (a,b) Injection of Aβ₁₋₄₂ (asterisk (*), 80 pmol i.c.v.) in acutely anaesthetized rats completely inhibited NMDAR-dependent LTP measured 3 h post-HFS when administered either (a) 30 min prior to HFS (arrow, standard 200 Hz protocol) (95.9 ± 5.3%, n = 5; p < 0.05 compared with 129.5 ± 4.5%, n = 4, in vehicle-injected rats, unpaired t-test) or (b) 3 h prior to HFS (98.2 ± 5.8%, n = 6; p < 0.05 compared with 127.4 ± 5.7%, n = 5, in vehicle-injected rats). (c) Although HFS induced similar magnitude LTP in chronically implanted re-anaesthetized rats (127.0 ± 3.1%, n = 5), injection of the same dose of Aβ₁₋₄₂ now failed to significantly impair LTP (121.9 ± 4.7%, n = 5; p > 0.05 compared with vehicle, ANOVA). A dose four times higher (320 pmol) inhibited LTP in the chronic animals (98.4 ± 4.6%, n = 7; p < 0.05 compared with vehicle). (d) Whereas the injection of either vehicle or 80 pmol Aβ₁₋₄₂ did not significantly affect baseline transmission (n = 5–6 per group), the higher dose of 320 pmol Aβ₁₋₄₂ caused a small but significant decrease in EPSP amplitude that was similar in magnitude in both the acute and chronic preparations (87.5 ± 5.1 and 89.4 ± 3.0% pre-injection baseline at 3 h post-injection, n = 6 and 7, respectively; p < 0.05 compared with respective vehicle controls, p > 0.05 compared between acute and chronic, ANOVA). (i) Acutely anaesthetized rats, (ii) chronically implanted re-anaesthetized rats. Insets show EPSP traces at the time indicated. Horizontal bar, 10 ms; vertical bar, 2 mV; open circles, vehicle; filled circles, Aβ (80 pmol); filled triangles, Aβ (320 pmol).
slow-onset, small reduction in baseline synaptic transmission developed 2–3 h after the injection (figure 4d), which probably contributed partly to the apparent magnitude of LTP inhibition in the chronically implanted rats. The higher dose of Aβ had a similar baseline depressant effect in the acutely anaesthetized rats (figure 4d). These findings indicate that relatively selective tolerance develops in chronically implanted rats to the acute synaptic plasticity disrupting action, but not the baseline depressant action, of Aβ. Further investigation will be needed to elucidate the mechanisms of such selective tolerance and to determine whether similar resistance to other acute effects of Aβ develops in chronically implanted animals.

Rather than determine the persistence of LTP inhibition in animals chronically implanted with electrodes and theור, which lowered the potency of Aβ, we used a modified protocol. The duration of action of Aβ after single injection using a temporary cannula under recovery anaesthesia was assessed a week later under non-recovery anaesthesia. For these experiments, we turned to studying the pathophysiologically most relevant form of water-soluble Aβ species and assemblies, those present in AD brain. Soluble extracts of AD brain contain Aβ monomers and sodium dodecyl sulfate (SDS)-stable dimers at dramatically higher concentrations than in non-AD controls (figure 5a) [15,51]. Acute application of such Aβ-containing soluble extracts of AD brain rapidly and potently inhibits LTP both in vitro [15,52] and in vivo [53,54].

Similar to what we reported previously, Aβ-containing soluble extract of AD brain injected 15 min prior to HFS inhibited LTP in urethane-anaesthetized rats (figure 5c). Remarkably, we found that a single i.c.v. injection of the same AD brain extract under recovery anaesthesia still inhibited LTP induction when HFS was applied 7 days later under urethane anaesthesia (figure 5d). Importantly, a single i.c.v. injection of an equivalent volume of vehicle or the same soluble AD brain extract that had been immunodepleted of Aβ did not affect LTP when tested immediately or 7 days later. There was no evidence for a change in baseline synaptic transmission (figure 5b) or paired-pulse facilitation (1.83 ± 0.05 and 1.79 ± 0.09 facilitation ratio at 40 ms interstimulus interval in controls and AD brain-injected group, respectively, n = 5 per group, data not shown).

Because we did not examine the acute effect of Aβ injection on LTP in animals that had a vehicle injection under recovery anaesthesia a week previously, we do not know if the potency of AD brain Aβ was affected by the modified experimental protocol. Moreover, baseline properties of synaptic transmission in other hippocampal pathways need to be investigated because i.c.v. injection of similar AD brain extract in rats can cause ultrastructural changes, including reduced synaptic density, in the stratum lacunosum moleculare of the CA1 region and outer

Figure 5. Water-soluble Aβ in AD brain extract strongly and persistently inhibits LTP. (a) AD brain tris-buffered saline (TBS) extract (AD) was characterized by immunoprecipitation and Western blot and found to contain approximately 5.06 ng ml⁻¹ Aβ monomer (M) and approximately 2.7 ng ml⁻¹ SDS-stable dimer (D). The concentration of soluble Aβ in a TBS brain extract from a non-AD control (Con) was below the detection limit. (b) There was no significant difference in baseline excitability between the controls and AD brain extract-treated animals as measured by (i) EPSP amplitude or (ii) stimulation intensity. (c) The acute injection of AD brain extract (8 μl, i.c.v.) 15 min prior our standard 200 Hz HFS protocol (arrow) completely inhibited LTP measured 3 h post-HFS (105.4 ± 3.8%, n = 5, p < 0.05, unpaired t-test, compared with 149.6 ± 10.6% in Aβ-immunodepleted AD brain extract, n = 4, or vehicle-injected controls, n = 3, combined n = 7) in urethane-anaesthetized rats. (d) Similarly, a single injection (20 μl, i.c.v. under ketamine–xylazine anaesthesia) of Aβ-containing AD brain TBS extract 7 days before HFS completely inhibited LTP (103.8 ± 5.2%, n = 5, p < 0.05, compared with 140.9 ± 6.6% in Aβ-immunodepleted AD brain extract, n = 2, or vehicle-injected controls, n = 3, combined n = 5) in urethane-anaesthetized rats.Insets show EPSP traces at the time indicated. Horizontal bar, 10 ms; vertical bar, 1 mV; open circles and bars, vehicle or Aβ-immunodepleted AD brain extract; filled circles and bars, AD.
molecular layer of the dentate gyrus when measured 2 days later, after performing a passive avoidance learning task [55].

The persistence of the disruptive effect of Aβ 7 days after a single injection may be caused by residual Aβ assemblies that are poorly cleared from the brain. Alternatively, transient exposure to Aβ may trigger a cascade of events that persist for long periods. For example, intracerebral injections of either AD brain Aβ or soluble Aβ from amyloid precursor protein transgenic (APP) transgenic mouse brain have been reported to act as seeds for the aggregation of endogenous Aβ in APP transgenic, but not wild-type, mice, several months after inoculation [56,57]. It will be of interest to determine how much longer, beyond the week we report here, that the synaptic plasticity disrupting action of soluble Aβ from AD brain persists in wild-type rats.

5. Conclusion

Taken together, these findings indicate that non-NMDAR mechanisms are important in regulating the deleterious effects of Aβ oligomers on synaptic plasticity and that once initiated, reversing persistent disruption in vivo remains a major challenge for the future.

6. Material and methods

(a) Animals and surgery

All experiments were carried out in accordance with guidelines under licence from the Department of Health and Children, Ireland (86/609/EEC) using methods similar to those described previously [58]. Male Wistar rats (250–350 g) were anaesthetized with urethane (1.5 g/kg, i.p.) and core body temperature was maintained at 37.5 ± 0.5°C. A stainless steel guide cannula (22 gauge, 0.7-mm outer diameter, length 13 mm) was implanted above the right lateral ventricle before the electrodes were implanted ipsilaterally. Injections were made via a Hamilton syringe which was connected to the internal cannula (28 gauge, 0.36 mm outer diameter). The injector was removed 1 min post-injection and a stainless steel plug was inserted. The position of the cannula was verified postmortem by investigating the spread of ink dye after i.c.v. injection. Teflon-coated tungsten wire (external diameter 75 μm bipolar or 112 μm monopolar) electrodes were positioned in the stratum radiatum of area CA1. Screw electrodes located over the contralateral cortex were used as reference and earth. The stimulation and recording electrodes were optimally located using a combination of physiological and stereotactic indicators.

In the case of chronically implanted animals, the insertion of electrodes and cannula was carried out under recovery anaesthesia using a mixture of ketamine (80 mg kg−1) and xylazine (8 mg kg−1) (both i.p.) as described above for acute experiments. Rats were housed individually in their home cages with free access to food and water and a 12 L:12 D cycle for at least a week postsurgery. Electrophysiological recordings were carried out under urethane anaesthesia, as described above. To investigate the delayed effect of human brain extract, the injection was carried out under recovery anaesthesia and the cannula was then removed. The electrode implantation and recording was carried out under urethane anaesthesia 7 days later.

(b) Electrophysiological recording

Test stimuli were delivered to the Schaffer-collateral/commissural pathway every 30 s to evoke field excitatory postsynaptic potentials (EPSPs) that were 45–60% maximum amplitude. With one exception, LTP was induced using our standard 200 Hz protocol of a single series of 10 trains of 20 stimuli with an intertrain interval of 2 s. A repeated strong 40 Hz protocol (three sets of 10 trains of 20 pulses, intertrain interval of 2 s and interset interval of 5 min) was used to investigate NMDAR-independent LTP [17]. The stimulation intensity was increased to 75% maximum during both induction protocols. The magnitude of control LTP varied considerably over the period of carrying out these experiments. In order to minimize the possible confounding effect of such variation in control LTP, the experiments in each study were interleaved.

(c) Chemicals

Stock solution (70 or 100 μM) of synthetic Aβ 1–42 (Bachem) was prepared in 0.1% ammonium hydroxide and stored at –80°C. To produce soluble Aβ, for the experiments illustrated in figures 3 and 4, the solution was centrifuged (100 000 g × 3°C × 3 h) [59] and the upper 75% of the supernatant used. Dnonezepil hydrochloride (Aricept, purchased from St. James’s Hospital Pharmacy, Dublin) was dissolved in water and injected s.c. (0.5 ml kg−1). The vehicle + vehicle group in figure 3 received an i.c.v. injection of vehicle 90 min before the HFS and the second injection (s.c.) either 30 min before or after the i.c.v. injection. Mecamylamine hydrochloride (Sigma) was dissolved in saline and injected i.p. (3 ml kg−1). D-AP5 (Tocris), methyllycaconitine citrate (Sigma) and methoctramine (Sigma) were dissolved in water for i.c.v. injection.

(d) AD brain extracts

AD brain extracts were prepared as described previously [55]. Human brain tissue was kindly provided by Matthew P. Frosch, Massachusetts General Hospital and used in accordance with local Ethics Committee guidelines. Samples of temporal cortex were from an 83 year old female, who died with AD and Lewy body dementia, and an 82 year old male control, who died free of neurodegeneration. Briefly, tri-buffered saline (TBS) extracts of cortex were prepared by dounce homogenization and then clarified by high-speed centrifugation. To eliminate bioactive small molecules the supernatant was exchanged to ammonium acetate using a 5 ml Hi-trap de-salting column (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). One aliquot of the extract was immunodepleted of Aβ with the rabbit polyclonal anti-Aβ antibody, AW8. Aliquots of samples were stored at ~80°C or used to assess Aβ content with a sensitive immunoprecipitation (IP)/western blotting (WB) procedure. AW8 was also used for IP and a combination of the anti-Aβ40 and Aβ42 monoclonal antibodies for WB. Aβ concentration was estimated by reference to known quantities of synthetic Aβ 1–42.

(e) Data analysis

The magnitude of potentiation is expressed as the percentage of baseline during the initial 30-min period, expressed as mean ± s.e. of the mean, unless otherwise stated. For statistical analysis, EPSP amplitudes were grouped into 10-min epochs. Standard one-way ANOVA was used to compare the magnitude of LTP between multiple groups followed by post hoc Tukey’s tests. Unpaired Student’s t-tests were used for two-group comparisons. A value of p < 0.05 was considered statistically significant.

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