Soluble amyloid-β peptides potently disrupt hippocampal synaptic plasticity in the absence of cerebrovascular dysfunction in vivo

Neng-Wei Hu,1,2 Imelda M. Smith,3 Dominic M. Walsh3 and Michael J. Rowan1,2

1Trinity College Institute of Neuroscience, 2Department of Pharmacology and Therapeutics, Trinity College, Dublin 2 and 3Laboratory for Neurodegenerative Research, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland

Correspondence to: Michael J. Rowan, Department of Pharmacology and Therapeutics, Biotechnology Building, Trinity College, Dublin 2, Ireland
E-mail: mrowan@tcd.ie.

Long before the onset of clinical Alzheimer’s disease non-fibrillar, soluble assembly states of amyloid-β (Aβ) peptides are believed to cause cognitive problems by disrupting synaptic function in the absence of significant neurodegeneration. Since many of the risk factors for Alzheimer’s disease are vascular, impairment of cerebral blood flow by soluble Aβ has been proposed to be critical in triggering these early changes. However, it is not known if soluble Aβ can affect cerebrovascular function at the concentrations required to cause inhibition of synaptic plasticity mechanisms believed to underlie the early cognitive deficits of Alzheimer’s disease. Here we developed a new method to simultaneously assess the ability of soluble Aβ to impair plasticity at synapses and to affect resting and activity-dependent local blood flow in the rat hippocampus in vivo. Intracerebroventricular injection of soluble synthetic Aβ40 dimers rapidly inhibited plasticity of excitatory synaptic transmission at doses (10–42 pmol) comparable to natural Aβ, but failed to affect vascular function measured using laser-Doppler flowmetry (LDF). Like wild-type Aβ40, the more vasculotropic Aβ produced by people with familial hemorrhagic stroke of the Dutch type (Aβ40E22Q), impaired hippocampal plasticity without causing a significant change in local blood flow. Furthermore, neither resting nor activation-evoked hippocampal perfusion was affected by soluble Aβ42, even at a concentration that markedly (25%) reduced baseline synaptic transmission. These findings demonstrate that the putative synaptotoxic soluble Aβ species of early Alzheimer’s disease cause synaptic dysfunction in the absence of detectible changes in local blood flow. This strongly indicates that early cognitive deficits can be caused by soluble Aβ independently of deleterious effects on cerebrovascular dynamics.

Keywords: cerebral blood flow; functional hyperaemia; synaptic transmission; amyloid-β oligomers; Alzheimer’s disease

Abbreviations: Aβ = amyloid-β protein; AMPA = α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; D-AP5 = D-(–)-2-amino-5-phosphonopentanoic acid; EPSP = excitatory postsynaptic potential; HBF = hippocampal blood flow; HFS = high frequency stimulation; LDF = laser-Doppler flowmetry; LTP = long-term potentiation; NMDA = N-methyl-d-aspartate

Introduction

Alzheimer’s disease is characterized pathologically by the deposition of insoluble aggregates of fibrillar amyloid-β protein (Aβ) in neuritic plaques and blood vessels (termed cerebral amyloid angiopathy), and tau protein in neurofibrillary tangles (Ikonomovic et al., 2008). Although neuritic and vascular pathology are major contributors to the late stages of the disease process, there is growing evidence that synaptic dysfunction, in the absence of significant pathology, may cause cognitive impairment especially during early stages (Walsh et al., 2002; Walsh and Selkoe, 2004). Moreover, brain levels of soluble Aβ, in contrast to the
levels of the insoluble protein deposits, correlate well with the severity of dementia (Lue et al., 1999; McLean et al., 1999; Wang et al., 1999). Consequently, current Aβ hypotheses of the aetiology of Alzheimer’s disease propose that non-fibrillar soluble assemblies of Aβ, in particular Aβ oligomers, disrupt synaptic physiological processes underlying cognition (Klein et al., 2004; Walsh and Selkoe, 2004).

There is strong evidence that soluble oligomers of Aβ initially selectively impair synaptic plasticity mechanisms necessary for memory processing (Cooke and Bliss, 2006; Rowan et al., 2007). Both cell-derived and synthetic soluble Aβ strongly inhibit long-term potentiation (LTP), a persistent form of plasticity at glutamatergic synapses that can be readily induced by conditioning stimulation in the hippocampus, one of the most vulnerable areas affected in early Alzheimer’s disease (Lambert et al., 1998; Walsh et al., 2002; Klyubin et al., 2004, 2005; Origlia et al., 2008). Human CSF and conditioned media from cells that contain small oligomers such as dimers can inhibit LTP at extremely low Aβ concentrations (Townsend et al., 2006; Klyubin et al., 2008). The ∼100-fold lower potency of standard preparations of soluble synthetic Aβ to inhibit plasticity may be due to the lower relative abundance of stable Aβ oligomers to less active Aβ species, in particular Aβ monomers, which have been reported not to inhibit LTP (Walsh et al., 2002; Wang et al., 2004; Klyubin et al., 2005).

It is now clear that soluble Aβ also can cause cerebrovascular dysfunction by acting directly on blood vessels (Townsend et al., 2002; Iadecola, 2004; Zlokovic, 2005; Cole and Vassar, 2008; de la Torre, 2008). Since synaptic function is critically dependent on adequate supply of oxygen and essential nutrients, some authors have proposed that oligemia may precede cognitive dysfunction (Townsend et al., 2002; Iadecola, 2004; de la Torre, 2008). Consistent with this possibility, in conditions of sufficient ischaemia synaptic transmission and plasticity is profoundly disrupted in vulnerable brain areas (Hori and Carpenter, 1994; Calabresi et al., 2002; Row, 2007; Di Filippo et al., 2008). Furthermore, soluble Aβ has been reported to reduce cortical blood flow and functional hyperaemia without apparently reducing energy requirements, as measured by local cerebral glucose utilization (Niwa et al., 2000). These findings, and similar observations in β-amyloid precursor protein over-expressing transgenic mice, suggest that soluble Aβ potentially could cause a mismatch between nutrient supply and demand very early in Alzheimer’s disease (Niwa et al., 2000). Indeed such mechanisms have been proposed to explain why many of the risk factors for Alzheimer’s disease are vascular, in particular those associated with cerebral hypoperfusion and why early Alzheimer’s disease is associated with reduced resting cerebral blood flow or especially functional hyperaemia (Iadecola, 2004; Hansson et al., 2007; Cole and Vassar, 2008; de la Torre, 2008).

If soluble Aβ-elicited hypoperfusion is indeed associated with the disruption of synaptic plasticity in vivo, then doses of Aβ that are sufficient to inhibit LTP should also cause a reduction in resting blood flow or functional hyperaemia. We tested this hypothesis by simultaneously measuring the effect of Aβ on synaptic plasticity and local perfusion in the hippocampus in the same animals. We describe the potent inhibitory effects of different preparations of synthetic Aβ, including Aβ dimers, the smallest assembly state of Aβ aggregation and vasculotropic mutant Aβ on LTP, in the absence of any detectible change in hippocampal blood flow (HBF).

Material and Methods

Animals and surgery

Male Wistar rats (250–350 g) had stimulating and recording electrodes and a laser-Doppler flowmetry (LDF) probe implanted under urethane (1.5–1.6 g/kg, i.p.) anaesthesia. A small craniotomy (1 × 1.5 mm) was performed and the dura and arachnoid membranes were carefully removed. The craniotomy hole was sealed with dental cement after the implantation procedure. In some experiments, the left femoral vein and artery were cannulated for intravenous injections and the continuous monitoring of arterial blood pressure, respectively. The body temperature was maintained at 37–38°C with a feedback-controlled heating blanket. The animal care and experimental protocols were approved by the Department of Health and Children, Republic of Ireland.

Cannula implantation and intracerebroventricular injections

A stainless steel guide cannula (22 gauge, 0.7 mm external diameter) was implanted in the right lateral ventricle (1 mm lateral to midline, 0.5 mm posterior to bregma and 4 mm below the surface of the dura). An internal cannula (28 gauge, 0.36 mm external diameter) was used for i.c.v. injections. Solutions were injected in a 5 μl volume over a 3 min period. Verification of the placement of the cannula was performed post-mortem by checking the spread of ink dye after i.c.v. injection.

Electrode implantation

Electrodes were made and implanted as described previously (Cullen et al., 1997). Briefly, twisted bipolar electrodes were constructed from Teflon-coated tungsten wires (62.5 μm inner core diameter, 75 μm external diameter). Field excitatory postsynaptic potentials (EPSPs) were recorded in the stratum radiatum of the dorsal hippocampus in response to stimulation of the ipsilateral Schaffer collateral-commisural pathway. The recording site was located 3.4 mm posterior to bregma and 2.5 mm lateral to midline, and the stimulating site was located 4.2 mm posterior to bregma and 3.8 mm lateral to midline. The final depths of the electrodes were adjusted to optimize the electrically evoked EPSP and confirmed by post-mortem analysis.
Electrical stimulation and electrophysiological recording

The intensity of the test constant current square wave stimulation pulses (0.2 ms duration, 0.033 Hz) was adjusted to evoke an EPSP that was 50% of the maximum. Conditioning high frequency stimulation (HFS) to induce LTP consisted of 10 trains of 20 pulses at 200 Hz with an inter-train interval of 2 s and the stimulation intensity was raised to evoke an EPSP that was 75% of the maximum.

Hippocampal and cortical blood flow recording

HBF was monitored continuously using LDF (Periflux 5010, Perimed, Sweden) (Fig. 1A) (Fowler et al., 2003). The LDF probe (450 μm external diameter) was located 3.8 mm posterior to bregma and 3.0 mm lateral to midline. It was lowered through the cortex to a depth of ~2 mm, just above the surface of the right hippocampus. As LDF only provides a relative measure of flow measurements were expressed as perfusion units relative to zero measured after cardiac arrest at the end of the experiment.

We tested the relative sensitivity of blood flow recorded from the surface of the dorsal hippocampus and cortex by simultaneously measuring the vascular response to a reduction in systemic blood pressure caused by i.v. injection of the vasodilator acetylcholine in some animals. In order to carry out this test a second LDF probe was stereotaxically placed (3.8 mm posterior to bregma and 3.00 mm lateral to midline) above the intact dura after a second craniotomy (1.5 mm diameter) had been performed on the contralateral side. Injection of a dose of acetylcholine (15 μg in 0.3 ml saline into the femoral vein that evoked a 25–30% reduction in mean arterial blood pressure caused an 11–31% reduction in both hippocampal and cortical blood flow with near identical time course.

Drugs and chemicals

Stock solutions of wild-type full-length Aβ40, Aβ42 (both Bachem, UK) and Dutch Aβ40 E22Q (Biopolymer Laboratory, UCLA Medical School, USA) were prepared by dissolving known weights of peptides in 0.1% ammonium hydroxide in milliQ water (Millipore Corporation, Ireland) to produce a concentration of 100 μM, respectively. These solutions were then centrifuged under conditions (100 000 g for 3 h) that readily pellet fibrils and protofibrils (Walsh et al., 2002; Klyubin et al., 2004) and the upper 75% of the supernatant removed. The relative concentration of the corresponding stock solutions were determined using the micro BCA protein assay (Thermo-Fisher Scientific Life Science Research Products, Rockford, IL) and the amount of peptide in the supernatant determined accordingly.

Covalently cross-linked synthetic dimers of full-length Aβ40 were prepared using Aβ40S26C (Biopolymer Laboratory, UCLA Medical School). Briefly, Aβ dimers were generated by atmospheric oxidation of a 20 μM solution of Aβ40S26C in 20 mM ammonium bicarbonate, pH 8.0, for 4 days at room temperature. To facilitate disassembly of aggregates formed during the oxidation reaction, the peptide solution was lyophilized and subsequently incubated in 5 M GuHCl, Tris–HCl, pH 8.0, for 4 h. Disulphide cross-linked Aβ dimers were isolated from unreacted monomer and higher aggregates by size exclusion chromatography. A Superdex 75 10/30 HR column was eluted with 50 mM ammonium acetate, pH 8.5 at a flow rate of 0.8 ml/min.

Fig. 1 Activity-dependent hyperaemia in the rat hippocampus: simultaneous recording of excitatory glutamatergic synaptic transmission and blood flow in the CA1 area. (A) Schematic diagram of a transverse section of the rat brain showing the approximate locations of the laser-Doppler probe and the electrodes used to simultaneously record HBF and synaptic field potentials, respectively. The stimulating (STIM) and recording (REC) electrodes were located in the stratum radiatum of the dorsal hippocampus. The laser-Doppler probe was located just above the surface of the adjacent hippocampus. (B) Examples of simultaneously recorded typical electrophysiological and blood flow responses evoked by different stimulation protocols. Whereas single pulse stimulation (upper STIM) evoking the test field EPSP did not evoke a discernable increase in HBF (dots), application of a brief burst stimulus (×1, 10 pulses at 200 Hz, middle STIM) or the conditioning high frequency stimulus train (×10, HFS, 10 trains of 20 pulses at 200 Hz, lower STIM) caused summation of EPSPs and elicited clear, activity-dependent increases in HBF. Calibration bars: vertical, 20 perfusion units; horizontal, 30 s.

Fractions (0.5 ml) were collected and an aliquot of each electrophoresed on 16% Tris–tricine polyacrylamide gels and protein detected by silver staining. Fractions that contained predominantly Aβ dimer or unreacted Aβ40S26C monomer were
identified, and these were used as the dimer and monomer stocks, respectively. SDS–PAGE analysis revealed that the monomer fraction contained no detectable dimer, and the dimer fraction contained cross-linked dimer and a trace of unoxidized Aβ monomer (i.e. <10% of the total peptide detected).

These preparations together with Aβ40 solutions of known concentration, were electrophoresed in the presence of β-mercaptoethanol on the same gel and proteins stained with silver so that the peptide content of the monomer and dimer fractions could be estimated relative to known standards. The intensity of the Aβ bands was determined using Densitometric analysis using the Scion Image for Windows Beta 3 program (http://www.scioncorp.com) and protein concentrations determined by linear regression analysis (R² = 0.998).

Stock solutions were stored in small aliquots at −80°C and were diluted with milliQ water to the desired final concentration. To control for any effects attributable to the vehicle equal volumes of saline were injected. The comparison of Aβ40 fractions could be estimated relative to known standards. The intensity of the Aβ bands was determined using Densitometric analysis using the Scion Image for Windows Beta 3 program (http://www.scioncorp.com) and protein concentrations determined by linear regression analysis (R² = 0.998).

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The doses of Aβ40 (500 pmol in 5 μl), Aβ42E22Q (50 pmol, i.c.v.) and Aβ42 (80 pmol, i.c.v.) were chosen to selectively inhibit LTP without affecting baseline transmission (Klyubin et al., 2004; Wang et al., 2007). Similarly, the dose (42 pmol) of dimer tested in the LTP experiments was found not to affect baseline synaptic transmission in pilot experiments.

D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5) (Tocris, Bristol, UK) was dissolved in distilled water and a dose that completely inhibits LTP under our in vivo recording conditions was used (I. Klyubin et al., unpublished observations).

**Results**

**Activity-dependence of electrically evoked functional hyperaemia in the CA1 area**

Single pulse stimulation at the intensity used to evoke the test EPSP (50% of maximum) did not trigger an observable change in HBF (Fig. 1B). Increasing the degree of activation by the application of a brief burst of 10 pulses at 200 Hz at the test pulse intensity caused summation of EPSPs (~35 ms duration) and evoked a significant increase in HBF that had an onset latency of ~1 s and a duration of ~7 s. Multiple burst HFS (10 trains of 20 pulses at 200 Hz), similar to the conditioning stimulation protocol used to induce LTP, triggered prolonged summated EPSPs (~105 ms duration of each train over a period of ~18 s) and evoked a hyperaemia that had an onset latency of ~1 s and lasted for ~60 s.

**Soluble Aβ40 dimers potently inhibit LTP without affecting HFS-evoked hyperaemia**

The relative sensitivity of synaptic plasticity and functional hyperaemia to Aβ was initially assessed using soluble Aβ40, which is reported to be more vasoactive than Aβ42 (Niwa et al., 2000). We compared our standard preparation of Aβ, which is centrifuged to remove fibrils and protofibrils but otherwise is uncharacterized with regard to assembly forms of soluble Aβ, with size exclusion fractions of Aβ40S26C monomers or dimers. Stable Aβ dimers were prepared using a novel technique in which the serine at position 26 of Aβ40 was substituted with a cysteine (Aβ40S26C) and the cysteine oxidized to form an intermolecular disulphide bond (Fig. 2). The cysteine substitution involves the change of a single atom, i.e. –OH of serine changes to –SH of cysteine; thus, besides a difference in redox potential Aβ40 dimers were used for injection in vivo. 

![Fig. 2 Production and characterization of covalently cross-linked Aβ dimers. Aβ dimers were generated by atmospheric oxidation of a 20 μM solution of Aβ40Ser26Cys for 4 days. (A) SDS–PAGE revealed two bands (lane 3), the lower of which co-migrated with the unoxidized peptide (lane l) and a dimer band which migrated 9 kDa; molecular weight markers are in lane 2. (B) Oxidized peptide was lyophilized, reconstituted in 5 M GuHCl, Tris, pH 8.0 and chromatographed on a Superdex 75 column eluted with 50 mM ammonium acetate, pH 8.5; peak fractions corresponding to the elution of Aβ monomer and dimer were used for injection in vivo.](http://brain.oxfordjournals.org/).
and Aβ40S26C are otherwise highly similar. In order to study the properties of unreacted monomer and disulphide cross-linked dimers mixtures containing the two were separated using size exclusion chromatography and fractions containing only monomer or predominantly dimer were studied (Fig. 2B).

In control, vehicle-injected animals (5 μl, i.c.v.) application of HFS induced a robust and stable LTP measuring 127.6 ± 2.8% at 3 h post-HFS (mean ± SEM% pre-HFS baseline, n = 11; P < 0.05 compared with baseline) (Fig. 3A). In the same animals, the HFS simultaneously evoked a transient hyperaemia that measured 120 ± 1.8% (P < 0.05 compared with baseline) (Figs 3A and 7). The functional hyperaemia was reproducible in the same animals since application of a second HFS, 3 h after the first HFS, evoked a similar increase in HBF (n = 11, 125 ± 2.6%; P < 0.05 compared with baseline and P > 0.05 compared with the response to the first HFS).

Due to the relatively low potency of Aβ40 at impairing LTP (Cullen et al., 1997; Klyubin et al., 2004) we tested the stock Aβ40 solution, the maximum concentration of Aβ40 that we were confident contained only soluble species. Injection i.c.v. of our standard preparation of soluble Aβ40 (500 pmol in 5 μl), 30 min prior to the conditioning HFS significantly impaired the induction of LTP (110.4 ± 2.3%, n = 5; P < 0.05 compared with pre-HFS baseline and compared with vehicle-injected controls at 3 h post-HFS) (Fig. 3A, upper panel). In the same animals the injection of Aβ40 did not significantly affect the simultaneously recorded hyperaemia evoked by the HFS (119.3 ± 3.3%; P < 0.05 compared with baseline and P > 0.05 compared with vehicle-injected controls) or resting HBF (Figs 3A and 7).

In contrast to the partial disruptive effect of our standard preparation of soluble Aβ40, the dimer preparations potently and fully inhibited LTP, whereas Aβ monomer was inactive (Fig. 3B). Thus, HFS failed to induce LTP in animals injected with Aβ dimer (42 pmol) (97.3 ± 2.5%, n = 5; P > 0.05 compared with pre-HFS baseline and compared with vehicle-injected controls at 3 h post-HFS). In animals injected with Aβ monomer, at the slightly higher dose of 56 pmol, HFS induced robust LTP that was indistinguishable from that observed in vehicle-injected animals (124 ± 3.5%, n = 5; P < 0.05 compared with pre-HFS baseline and P > 0.05 compared with vehicle-injected controls at 3 h post-HFS). We investigated the potency of Aβ dimer further by testing the effects of the lower doses of 25 and 10 pmol, which also inhibited LTP (107.8 ± 8.0 and 108.0 ± 5.2%, respectively; P > 0.05 compared with pre-HFS baseline, n = 3 per group).

Neither Aβ dimer nor monomer affected the simultaneously recorded hyperaemia evoked by HFS (117.3 ± 2.5 and 122.6 ± 4.6%, respectively; P < 0.05 compared with baseline and P > 0.05 compared with vehicle-injected controls) or resting HBF (n = 5 per group, Figs 3B and 7).
Mutant Aβ_{40} with the Dutch sequence (E22Q) inhibits LTP without affecting HBF

Patients with hereditary cerebral haemorrhage with amyloidosis, Dutch type, have a point mutation in β-amyloid precursor protein that leads to the production of mutant Aβ_{E22Q}, which is known to cause vascular and synaptic disruption (Klyubin et al., 2004; Maat-Schieman et al., 2005; Levy et al., 2006). Therefore, we compared the ability of soluble Aβ_{40E22Q} (50 pmol, i.c.v.) to affect synaptic plasticity and HBF. Injection of Aβ_{40E22Q}, 30 min prior to the HFS completely inhibited LTP measured 3 h later (104 ± 1.8%, n = 6; P > 0.05 compared with baseline and P < 0.05 compared with vehicle-injected controls). Similar to the other forms of Aβ_{40}, Aβ_{40E22Q} did not significantly affect the hyperaemia evoked by the HFS (119.5 ± 3.1%; P < 0.05 compared with baseline and P > 0.05 compared with vehicle-injected controls) or resting HBF (n = 6, Figs 4 and 7).

Low dose soluble Aβ_{40} inhibits LTP without affecting HFS-evoked hyperaemia

Although Aβ_{40} has been reported to be more vasoactive than Aβ_{42}, the latter has been shown to share some of the vascular disruptive actions of Aβ_{40} (Crawford et al., 1998; Niwa et al., 2001) and is considered a major synaptotoxic species in Alzheimer’s disease. Administration of soluble Aβ_{40} (80 pmol, i.c.v.) 30 min prior to the HFS strongly inhibited LTP at 3 h (100.9 ± 2.5%, n = 7; P > 0.05 compared with baseline and P < 0.05 compared with controls). In the same animals the functional hyperaemia evoked by the HFS was not significantly affected (119.8 ± 2.2%; P < 0.05 compared with baseline) by this relatively low dose of Aβ_{40} (Figs 5A and 7). Similarly, there was no significant change in resting HBF.

High dose soluble Aβ_{42} reduces baseline synaptic transmission without affecting baseline HBF or HFS-evoked hyperaemia

The higher dose of Aβ_{42} (320 pmol, i.c.v.) caused a depression of baseline synaptic transmission that gradually developed during the recording period, reaching 74 ± 2.3% at 3 h after the injection (n = 5; P < 0.05 compared with pre-injection baseline). There was no significant change in baseline HBF in these animals (e.g. 101.8 ± 4% at 3 h; P > 0.05). Furthermore, the functional hyperaemia evoked by HFS was not significantly affected at 3 h after the injection of this dose of Aβ_{42} (121.3 ± 5.1%; P < 0.05 compared with baseline, P > 0.05 compared with vehicle-injected controls, Figs 5B and 7).

HBF and activation-induced hyperaemia are partly N-methyl-D-aspartate receptor-dependent

Pre-treatment with the NMDA (N-methyl-D-aspartate) receptor antagonist d-AP5 (100 nmol in 5 μl, i.c.v.) 10 min prior to the application of HFS completely inhibited the induction of LTP (98 ± 2.1 and 104 ± 2.7% at 10 and 90 min post-HFS, respectively; P > 0.05 compared with pre-injection baseline, n = 5). Although there was no significant effect on baseline synaptic responses there was a small transient reduction in resting HBF, reaching 94.7 ± 2.1% at 10 min (P < 0.05 compared with pre-injection baseline and P > 0.05 compared with vehicle-injected animals). HFS triggered a small but significant hyperaemia after d-AP5 treatment (109.4 ± 4.2%; P < 0.05 compared with baseline). However, the increase in HBF was markedly attenuated compared with the controls (P < 0.05). This reduction in functional hyperaemia by d-AP5 was reversible since application of a second HFS, at a time when LTP induction had fully recovered from the inhibitory effect of d-AP5 (90 min after the first HFS), now evoked a robust increase in HBF (118.7 ± 3.0%; P < 0.05 compared with baseline) that was not significantly different from the vehicle-injected controls (P > 0.05, Figs 6 and 7).
Discussion
The vascular and neuronal effects of soluble Aβ were directly compared in the same animals in order to determine if disruption of synaptic plasticity was associated with altered vascular function. We report here that intracerebral infusion of Aβ40 dimers or the vasculotropic Aβ40E22Q potently inhibited synaptic plasticity without affecting either resting local blood flow or the functional hyperaemia evoked by the plasticity-inducing conditioning stimulation. Doses of soluble Aβ42 that inhibited plasticity or reduced baseline synaptic transmission also failed to affect simultaneously recorded local functional hyperaemia or resting blood flow. These findings provide strong evidence that soluble Aβ can acutely disrupt hippocampal synaptic plasticity without affecting local blood supply.

By carefully placing a fine needle laser Doppler probe on the surface of the dorsal hippocampus adjacent to the stimulating and recording electrodes it was possible to record local HBF in response to Schaffer collateral/commissural pathway stimulation and to record the CA1
pyramidal cell synaptic responses at the same time in vivo. The sensitivity of the local blood flow to activity was demonstrated by the finding that, whereas single pulse synaptic stimulation failed to evoke a measurable change in blood flow, high frequency burst stimulation triggered a reliable and marked transient hyperaemia that became larger and longer with increasing tetanization. The increase in flow, which had an onset of ∼1 s after the stimulation, is likely to be a homeostatic response to maintain synaptic function in response to the increased metabolic demand caused by the burst activity, a type of functional hyperaemia.

In the present experiments different Aβ preparations completely inhibited LTP, but did not significantly affect either basal blood flow or evoked functional hyperaemia in the dorsal hippocampus in vivo. Furthermore, in the case of Aβ42 a dose sufficient to markedly (∼25%) reduce baseline synaptic transmission did not affect hippocampal perfusion. These findings do not support the proposal that soluble Aβ may cause synaptic disruption by triggering oligemia. Some years ago soluble Aβ species, including dimers, were detected in the cerebral vasculature of Alzheimer’s disease patients (Frackowiak et al., 1994). Although initially these species were considered just transitional assembly states prior to fibrillization, evidence that soluble Aβ can disrupt vascular function led to the hypothesis that they may impair the synaptic mechanisms underlying cognitive processes by reducing the local supply of essential nutrients and oxygen (de la Torre, 2008; Iadecola, 2004). Why, if soluble Aβ can have such marked cerebrovascular effects, did we fail to detect any vascular response to Aβ in our experiments? One of the most obvious differences between the present and previous studies is the method of application of Aβ. Previously direct in vitro application of soluble Aβ was found to inhibit vasodilation and enhance vasoconstriction of a variety of blood vessels and to directly constrict small cerebral arteries (Townsend et al., 2002; Iadecola, 2004). Similarly, direct in vivo intraluminal or topical application of soluble Aβ reduced resting blood flow and greatly inhibited functional hyperaemia (Townsend et al., 2002; Deane et al., 2003; Iadecola, 2004). In the present study, Aβ was injected into the lateral cerebral ventricle where the Aβ is predominantly transferred initially to the brain parenchyma and hence to the blood vessels across the blood brain barrier (Ghersi-Egea et al., 1996). Although it is not possible to calculate the exact final concentrations of Aβ after in vivo application, concentrations of Aβ in the parenchyma and hence nearby capillaries or small vessels would be very low even after i.c.v. administration of the highest doses (500 pmol of Aβ40) used in the present study. Thus the vascular Aβ concentration was presumably insufficient to reliably disrupt vascular function as reported when it was directly applied to blood vessels. Clearly, our paradigm of Aβ application is more akin to a situation where Aβ is principally parenchymal in origin, which is believed to be the most important source of Aβ in Alzheimer’s disease and pure cerebral amyloid angiopathy (Kandimalla et al., 2005; Herzig et al., 2006).

The lack of significant effect of Aβ on functional hyperaemia in the present study is unlikely to be due to the nature of the hyperaemia that was evoked by the conditioning stimulation protocol used to induce LTP. We found that at a concentration of d-AP5 that completely inhibited LTP induction there was a small (∼5%) but significant reduction in resting blood flow but a much greater inhibition (∼50%) of the functional hyperaemia. The greater NMDA receptor-dependence of the functional hyperaemia.
hyperaemia over resting blood flow is explained by the increased activation of NMDA receptors consequent to the depolarization caused by the summation of EPSPs evoked by the conditioning stimulation. Activation of NMDA receptors is believed to elicit an increase in HBF by triggering release of vasodilators, especially NO from dendrites (Fergus and Lee, 1997; Lovick et al., 1999). The D-AP5 resistant component of the functional hyperaemia evoked in the present experiments is likely to be at least partly triggered by the increased activation of non-NMDA receptor-mediated synaptic transmission and increased neuronal firing caused by the conditioning stimulation (Fergus and Lee, 1997; Lovick et al., 1999). Since application of Aβ(42) can rapidly reduce NMDA receptor surface expression and NMDA evoked currents in certain cortical neurons (Snyder et al., 2005), Aβ might be expected to reduce NMDA receptor-dependent hyperaemia. The lack of significant effect of Aβ in the present studies on hippocampal hyperaemia, although partly NMDA receptor-dependent, is consistent with the relatively weak effect of Aβ on synaptic NMDA receptor-mediated transmission at concentrations sufficient to strongly inhibit LTP in the CA1 area of the hippocampus (Raymond et al., 2003; Nomura et al., 2005). The lack of a reduction in the HFS-triggered hyperaemia following the injection of the high dose of Aβ(42) is somewhat surprising, assuming that the reduction in baseline synaptic transmission was mediated by Aβ-induced internalization of synaptic AMPA (z-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors (Almeida et al., 2005; Hsieh et al., 2006), since AMPA receptor-mediated depolarization during the HFS would be expected to increase metabolic demand. However, AMPA receptor activation is unlikely to play a major role in the control of local blood supply in the hippocampus (Fergus and Lee, 1997; Lovick et al., 1999). Clearly, the conditioning stimulation that we used to induce synaptic plasticity was increasing metabolic demand and vasodilator release through a variety of signalling mechanisms that are commonly observed in different brain regions (Iadecola, 2004) but the local hyperemic response to this transient activation was not affected by doses of soluble Aβ that strongly inhibited synaptic plasticity or partially reduced baseline synaptic transmission.

The present findings are consistent with the majority of studies in transgenic mouse β-amyloid precursor protein-linked Alzheimer’s disease models, which did not report evidence of disruption of brain perfusion in the presence of raised soluble Aβ until significant amounts of fibrillar Aβ had deposited in blood vessels (Christie et al., 2001; Mueggler et al., 2002; Beckmann et al., 2003; Shin et al., 2007; Meyer et al., 2008), but see also (Van Dorpe et al., 2000; Iadecola, 2004). Similar conclusions were made in people with familial cerebral amyloid angiopathy, including the Dutch type and related transgenic animal models (Maat-Schieman et al., 2005). Intriguingly, whereas in the case of familial cerebral amyloid angiopathy of the Dutch type the presence of clinical dementia has been strongly associated with the degree of amyloid deposition, we found that our standard soluble preparation of mutant Aβ with the Dutch sequence disrupted synaptic plasticity, but less potently than fibril containing preparations (Klyubin et al., 2004). Similarly, transgenic mice expressing the E22Q mutation showed behavioural deficits in advance of amyloid deposition in either the vasculature or parenchyma (Kumar-Singh et al., 2000). Together these results suggest that preclinical cognitive problems may be present before significant angiopathy, but that frank cerebral amyloid angiopathy may be required for the fulminant expression of the disease (Maat-Schieman et al., 2005; Levy et al., 2006; Xu et al., 2007).

It has been suggested that the previously reported high sensitivity of pial vessel function to disruption by soluble Aβ may result from the recording of blood flow on the exposed cortex which could enhance free radical production since the vascular actions of Aβ are critically dependent on oxidative stress mechanisms (Iadecola, 2004; Shin et al., 2007). Thus, one could envision that under conditions of increased oxidative stress the vascular effects of Aβ may become more apparent. Indeed, blood vessels themselves can synthesize Aβ and the key β-amyloid precursor protein- cleaving enzyme BACE is upregulated in cerebral arteries following hypoxia (Coma et al., 2008; Cole and Vassar, 2008). It will be interesting to discover if oxidative stress differentially affects the vascular and synaptic actions of Aβ since the plasticity disrupting action of Aβ is also highly dependent on oxidative stress mechanisms (Rowan et al., 2007). Similarly, it will be important to determine if Aβ applied from the vascular lumen affects synaptic function at concentrations that alter cerebrovascular function (Su et al., 1999). Interestingly, there is evidence that the uptake of Aβ from the luminal side is largely mediated by receptors for advanced glycosylation end-products (RAGE), which has also been implicated in the inhibition of LTP by soluble Aβ (Deane et al., 2003; Origlia et al., 2008).

Importantly, when we compared the relative potency of different Aβ40 preparations we discovered that whereas our standard preparation only partially inhibited synaptic plasticity at the highest dose tested, a dose of at least 50-fold lower Aβ dimers completely inhibited LTP. This dramatic increase in potency of the Aβ dimer was not a simple consequence of the S26C substitution since comparable amounts of the corresponding S26C monomer did not block LTP. Moreover, the difference in potency of the Aβ dimer and wild-type Aβ40 preparations containing unspecified assembly forms suggests that spontaneously associating non-covalent low n oligomers (Bitan et al., 2001, 2003) have structural elements analogous to the stable dimer and differences in the potency between the cross-linked dimer and non-covalent low n oligomers are reflective of the relative stability and abundance of the active species. Of particular note, the high potency of synthetic Aβ dimers
mimics the high potency of Aβ dimer-containing human CSF (Klyubin et al., 2008), brain extracts (Townsend et al., 2006) and cultured cell medium (Walsh et al., 2002). The discovery that synthetic dimers have similar activity on synaptic plasticity to natural dimer-containing Aβ is of considerable interest, since the latter are composed of variable lengths of Aβ in the presence of other, potentially modulatory, co-factors. Thus, the present experiments strongly support the proposal that Aβ dimers are the earliest synaptic disrupting species in Alzheimer’s disease.

Although it is currently unclear what underlies the relatively high susceptibility of synaptic plasticity to Aβ dimers it will be of interest to test their affinity for the many putative receptors for Aβ, some of which have been shown to be necessary for the inhibition of LTP by soluble Aβ, including RAGE and integrins (Wang et al., 2007; Origlia et al., 2008). Of great potential clinical significance, if current strategies of targeting Aβ, for example by using antibodies or small molecules, are to be successful in the treatment of early Alzheimer’s disease the present data strongly indicate that ability to neutralize or clear Aβ dimers should be prioritized. Furthermore, given the strong relationship between cognitive status and post-mortem soluble Aβ load (Lue et al., 1999; McLean et al., 1999; Wang et al., 1999), and the present findings strongly implicating Aβ dimers, future studies aimed at improving the diagnostic utility of in vivo Aβ imaging should attempt to develop high sensitivity to Aβ oligomers, including dimers.

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