

**AMYLOID BETA PROTEIN
RESTORES HIPPOCAMPAL
LONG TERM POTENTIATION: A
CENTRAL ROLE FOR
CHOLESTEROL?**

**ALEXEI R. KOUDINOV &
NATALIA V. KOUDINOVA**

There is no understanding of the role of amyloid beta protein (Ab) in brain function and Alzheimer's disease. In the present study we attempted to dissect out the role for Ab in the synaptic plasticity in brain slices from adult male rat hippocampus. The prolonged maintenance of slices *ex vivo* (20+ hours) in our experimental set up preserved basic synaptic physiology but abrogated tetanus induced long term potentiation (LTP). Peptide Ab1-40 rescued LTP while cholesterol synthesis inhibition abolished the restorative action of the peptide. Our observation implies that Ab protein is a functional player in cholesterol neurochemical pathways and in synaptic structure-functional plasticity. The finding also supports our proposed hypothesis that the change in Ab biochemistry in Alzheimer's disease and related disorders is a functional (but not pathologic) phenomenon aiming to compensate impaired cholesterol dynamics and associated neurotransmission and synaptic plasticity. Such cholesterol mediated failure of synaptic function and neural degeneration (*Science*, 22 March 2002, **295**, p.2213) may cause the major sporadic form of Alzheimer's disease (*Neurology*, 2002, **58**, p.1135; posters 21.10/21.11, poster boards BB10/BB11).

INTRODUCTION

These days an increasing number of scientific papers implicates cholesterol in Alzheimer's disease (AD)(1-3). It is generally accepted that cholesterol is involved in AD pathogenesis by affecting brain amyloid β protein ($A\beta$) manufacturing and deposition (2). In our recent contribution we further proposed that cholesterol homeostasis biological misregulation itself has a key role for synaptic plasticity impairment, neuronal degeneration and is the primary cause for several AD hallmarks not limited to brain amyloid (3). We also proposed that the change in $A\beta$ neurochemistry in the disease represents physiological mechanism aiming to compensate impaired neural cholesterol dynamics and associated neurotransmission and synaptic plasticity failure (1-3).

Our recent study demonstrated that rats fed a cholesterol diet possess impaired hippocampal long-term potentiation (LTP), spatial behavior (not published observation), Alzheimer's-like brain amyloid and increased neural synthesis of cholesterol and phospholipids (3). Acute modulation of cholesterol dynamics (particularly, an increase of cholesterol efflux) in the hippocampal slices injured both neurotransmission and synaptic plasticity (1,4), caused disruption of neurofilament and increase of PFH-like tau phosphorylation (reproduced in several studies, see (1,3) for additional references), but preserved the pattern of A β immunofluorescence (1). Except of the above reports (1,3), there were no other study that would examine interrelation of brain cholesterol turnover and A β metabolism with respect to synaptic plasticity, deficit which represents the major Alzheimer's functional abnormality. In the present study we evaluated the effect of A β on hippocampal LTP and attempted to test its dependency on hippocampal cholesterol synthesis.

EXPERIMENTAL

All experimental procedures were previously reported in details (1,3-6). Briefly, brains from adult albino Wistar rats (3-4 months, 300-380g) were rapidly removed and placed into cold (2⁰C) artificial CSF (ACSF). Transverse hippocampal slices (400 μ) were prepared with a McIlwain tissue slicer within 4 min of an animal decapitation. After standard recreating incubation at room temperature for 1.5 h in ACSF, slices were subjected to pharmacological treatment (for 18-24 hrs) with the human peptide Aβ₁₋₄₀ (0 and 0.33 μg/ml [83 nM] in ACSF, see Ref. 1) and 0.3 μM mevinolin (4,7,8), followed by washing for 3 min (5x) in ACSF and extracellular field recording of evoked postsynaptic potentials (fEPSP) in stratum radiatum of CA1. Electrophysiological recording was performed as described (1,3,5) except that tetanic stimulation was delivered at the stimulus intensity that yielded double (compared to baseline responses) EPSP amplitude. The input-stimulus/output-response (I/O) relationship and LTP were

expressed as a fEPSP amplitude and slope change versus stimulus intensity and time, respectively. In selected experiments hippocampal slices were subjected to metabolic labeling with [¹⁴C]acetate to evaluate the synthesis of cholesterol and phospholipids (1). The data are presented as mean±SEM. Nonparametric Mann-Whitney signed rank test was used for determining significant differences between experimental values. A probability of 0.05 (one-tailed) or less was accepted as statistically significant.

RESULTS AND DISCUSSION

Prolonged incubation of slices for 20+ hours in our experimental condition did not affect basic synaptic physiology (**Fig. 1**), but adsorbed the ability of slices to induce and maintain LTP in CA1 area of the hippocampus; an initial postpotentiation level reached ~150% and fell down to 120% in 3 min after the tetanic stimulation (Fig. 1), compared

to significantly ($P < 0.05$, one tailed) higher standard values obtained for slices incubated in identical condition for 4-8 hours (not shown). The incubation of slices with the peptide A β 1-40, however, resulted in recovery of LTP. After the incubation with A β (83 nM) initial post-potential level reached 200% and remained steady at 175% above the baseline during the remaining recording time (Fig. 1), thus expressing no statistical difference with the slices subjected to 4-8 hrs of *in-vitro* maintenance without A β ($P > 0.05$). The treatment of slices with A β and mevinolin abolished the restoration of LTP by A β , and reversed the curve back to the condition of the prolonged incubation with no A β (Fig.1,2).

In our study we focused on the role of A β 1-40 in synaptic plasticity in the condition characterized previously with regard to cholesterol and phospholipid status (1,3). This condition, however, is different from those used in other reports on the role of A β in synaptic function (see Supplement 1). Hippocampal slices are characterized (1) by the

basal efflux of lipoprotein cholesterol ($4.0 \pm 1.23\%$ of the hippocampal cholesterol in 6 h). This efflux rate in 18 hours could yield the efflux of $\sim 12\%$ hippocampal cholesterol. Such loss of cholesterol in the medium is compatible with preserving basic synaptic physiology (1) but may well explain the impairment of LTP in slices (1,4) subjected to prolonged incubation in the absence of the external cholesterol donor (9). On the other hand, peptide A β 1-40 increases cholesterol synthesis ($\sim 145\%$ above the control value in 21 h) and enhances the uptake of cholesterol ($132.5 \pm 11.25\%$ above the control value in 21 h) by the hippocampal slices (1). Thus, the restoration of the hippocampal LTP by the peptide A β 1-40 may be caused by replenishment of hippocampal cholesterol to the level likely exceeding the loss of lipoprotein cholesterol during the prolonged incubation of the slices (1). Additional support for restoration of LTP by A β -mediated improvement of the hippocampal cholesterol supply comes from the inhibition of A β effect by mevinolin. Mevinolin is a member of a group of drugs, called statins, which inhibit 3-

hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme that catalyzes rate-limiting reaction in cholesterol biosynthesis. The concentration of mevillin used in this study causes 50 % inhibition of HMG-CoA reductase, does not affect nonsterol isoprenoid pathway products, and was successfully utilized to inhibit neuronal cholesterol content to near 50 % (4,7,8).

Our observation is in accord with the previous data that showed the dependency of hippocampal LTP on the inhibition of cholesterol biosynthetic pathway (1,4). The above discussion is well compatible with several articles on the role for cholesterol proper in the synaptic plasticity and synaptogenesis (1,10). Moreover, reported by others acute facilitation of tetanus-induced LTP (11) and the development of slow onset potentiation in the absence of tetanic stimulation (12) by low dose of A β 1-40 may be well due to an increase of A β -mediated neuronal cholesterol synthesis and lipoprotein-receptor-

mediated (1,13) uptake of cholesterol, the processes that are critical for synaptic plasticity, learning and memory (14,15).

The data presented here imply functional importance for the relation of the neurochemistry of A β and cholesterol in synaptic plasticity. It is known that A β modulates neuronal cholesterol esterification, influx, and efflux, and thus likely regulates neural cholesterol intracellular compartmentation and extracellular trafficking (see Refs.1,3 for detailed bibliography). A β also modulates neuronal physical property of cell membrane fluidity suggested to be important for cholesterol-dependent receptor impairment (discussed in Ref. 1). Most relevant to the current study seems A β -mediated increase of cholesterol synthesis and cholesterol uptake by *ex vivo* hippocampal slices (1), also reported in PC12 and rat primary neuronal cell cultures and in fetal brain (1,4,6,13).

Function of A β in cholesterol metabolism and recent data on the importance of cholesterol compartmentation for A β generation (16,17) indicate feedback functional relation between cholesterol and A β homeostasis. It is additionally supported by a dependency of amyloid precursor protein processing and A β production on the site 2 processing of sterol regulatory binding proteins (SREBP, the major regulatory protein in cholesterol metabolism) and associated inability of cells to upregulate the expression of several enzymes and proteins involved in cholesterol synthesis and turnover (18). The specific intraneuronal routing of A β (6)(Fig.2) and other C-terminal products of amyloid precursor protein processing to the nucleus (19) open the possibility that A β exerts the action on neuronal cholesterol dynamics by intranuclear modulation of SREBP or serving itself a nuclear transcription factor regulating genes encoding enzymes, receptors and (apolipo)proteins involved in cholesterol and possibly other lipids homeostasis.

Our observation confirms that A β protein is a physiological player in structure-functional plasticity of synapses. This is supported by several recent reports by others, particularly, by an increase of synaptic amyloid β precursor protein with learning capacity in rats (20); by neuronal activity dependent secretion of A β (21); and by A β -mediated upregulation of a synaptic vesicle protein transcript (22). Another recent articles showed that there is a bidirectional modulation between A β , its precursor protein and both metabotropic and ionotropic receptor molecules (23-25). A β also potentiates Ca²⁺ influx through voltage-sensitive Ca²⁺ channels (26) and was reported to form calcium-permeable channels in lipid vesicles (27). Taken together, the above articles support our proposed hypothesis that the change in A β neurochemistry in AD is a functional phenomenon aiming to compensate impaired neurotransmission and synaptic plasticity failure.

Our data also suggest that A β improves synaptic plasticity by modulating neural cholesterol dynamics (1-3). The role of A β (as a normal human protein) in mediating essential neurochemical pathways, however, is unlikely limited to cholesterol homeostasis. The other pathways can not be excluded and should be studied further in greater details. One such candidate is oxidative stress cascade (26), also shown to be critical for synaptic function and plasticity (28). The slow onset LTP similarly pharmacologically induced by vitamin E (29) and A β (12,30), but impaired in the transgenic mice overexpressing enzyme SOD-1 (28) may be well attributed to the lipid antioxidant properties modulation by vitamin E or A β (28,31) and dependency of slow LTP component (5) on a unique molecular mechanism (5,29-31).

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SUPPLEMENT

THERE IS NO CONSENSUS ON A β EFFECT ON SYNAPTIC FUNCTION

The role of A β in the mechanisms of synaptic function, plasticity, learning and memory remains to be intriguing and yet unresolved issue. The three early studies reported A β -mediated increase of LTP in rat dentate gyrus in *in vitro* experiments. Thus, it was shown (11) that whereas acute treatment of young rat (70-120 days) hippocampal slices with the low concentration (100-200 nM) of bath applied A β 1-40 did not change basal synaptic transmission, there was an increase in tetanus induced LTP. Moreover, intracellular (100 nM, via the recording pipette) or bath (200 nM) application of A β 1-40 triggered the slow onset potentiation of the NMDA receptor-mediated synaptic currents (12) in the hippocampal slices from young rats (70-120 g weight), and did not affect the basal AMPA receptor-mediated transmission, resting membrane potential or input resistance of the granule cells. Similar results were presented by Schulz, who showed no effect of A β 1-42 on AMPA currents, and demonstrated the increase of NMDA currents by the peptide (30). This report proposed that A β peptides (A β 1-42, A β 1-28 and A β 1-40) increase the probability of LTP under the paradigm that induced little LTP in control slices (30), as supported by our present study.

Another recent report (1S), presented data on A β 1-42 and A β 25-35 inhibition of hippocampal LTP at the concentration of 200nM to 1 μ M and no effect at 20 nM. This paper, however, employed different from earlier reports (11,12,30) protocol (particularly, Sprague-Dawley [and not Wistar] rats; 30⁰C recording temperature; stimulus duration of 0.1 msec delivered through sharpened monopolar tungsten electrodes; the decline of bath applied peptide just prior to the tetanic stimulation), and missed detailed consideration of A β 1-40, also suggested (despite of the lack of data) to inhibit the hippocampal LTP.

Several other articles reported on A β infusion into the rat brain followed by electrophysiological (2S-4S) or behavioral analysis (5S-7S). The paper of Cullen *et al.* (2S) showed no effect of low concentration of A β 1-40 (0.4 or 3.5 nmol in 5 μ l [equal to the injection of 5 μ l of 0.8mg/ml solution for 3.5 nmol A β 1-40] I.V.) on the ability to induce LTP in hippocampal slices *in vitro*, and the delayed (presented 24 and 48 hrs after the injection and not observed 75 min after injection) reduction in the NMDA receptor-mediated responses recorded *in vivo*. Another study (3S) investigated the effect of intra-cerebroventricular injection of A β fragments (A β 15-25, A β 25-35 and reverse sequenced A β 35-25) on synaptic transmission and LTP in the CA1 region of the hippocampus *in vivo*. This study (3S) showed an impairment of LTP in a time- (for A β 25-35) and concentration-dependent manner (for A β 25-35 and A β 35-25) but left open the question what would be the effect of A β 1-40 or A β 1-42. Another earlier article (4S) recorded waveforms in *in vitro* hippocampal slices at

25⁰C (and not at standard 32⁰C) after the injection of A β 1-40, and expressed LTP as a population spike (but not EPSP) change versus time. The authors suggested that A β 1-40 injection at a dose of 300 pmol/day (the volume of injection, however, remained unclear) for 10-11 days impaired the hippocampal LTP (4S). Recent behavioral study reported increase of the synaptic β -amyloid precursor protein with learning capacity in rats (20). Another earlier report showed no evidence of A β 1-40 accumulation or neurotoxicity after the injection of the peptide into the rat hippocampus (5S). This experimental condition was characterized by both the absence and the presence of A β effect on learning and memory in a different behavioral maze experiments (5S-7S).

Several reports further addressed the puzzling issue of the role of A β structural properties for neural function. These reports showed that oligomeric (8S) and plaque (5,9S-11S) amyloid is capable to impair synaptic and behavioral plasticity, possibly due to breaking the neuronal microcircuitry (5). Another recent study suggested age-related impairment of synaptic transmission (but not LTP) in transgenic mice that overexpress human amyloid precursor protein possessing “Swedish” mutation (12S). Such observation however unlikely contradicts the consensus of cited above four studies (5, 9S-11S) due to the experimental differences (particularly, the lack of estimation of A β load at the site of the recording, and employing for recording the stimulus intensity of 20 percent of maximum).

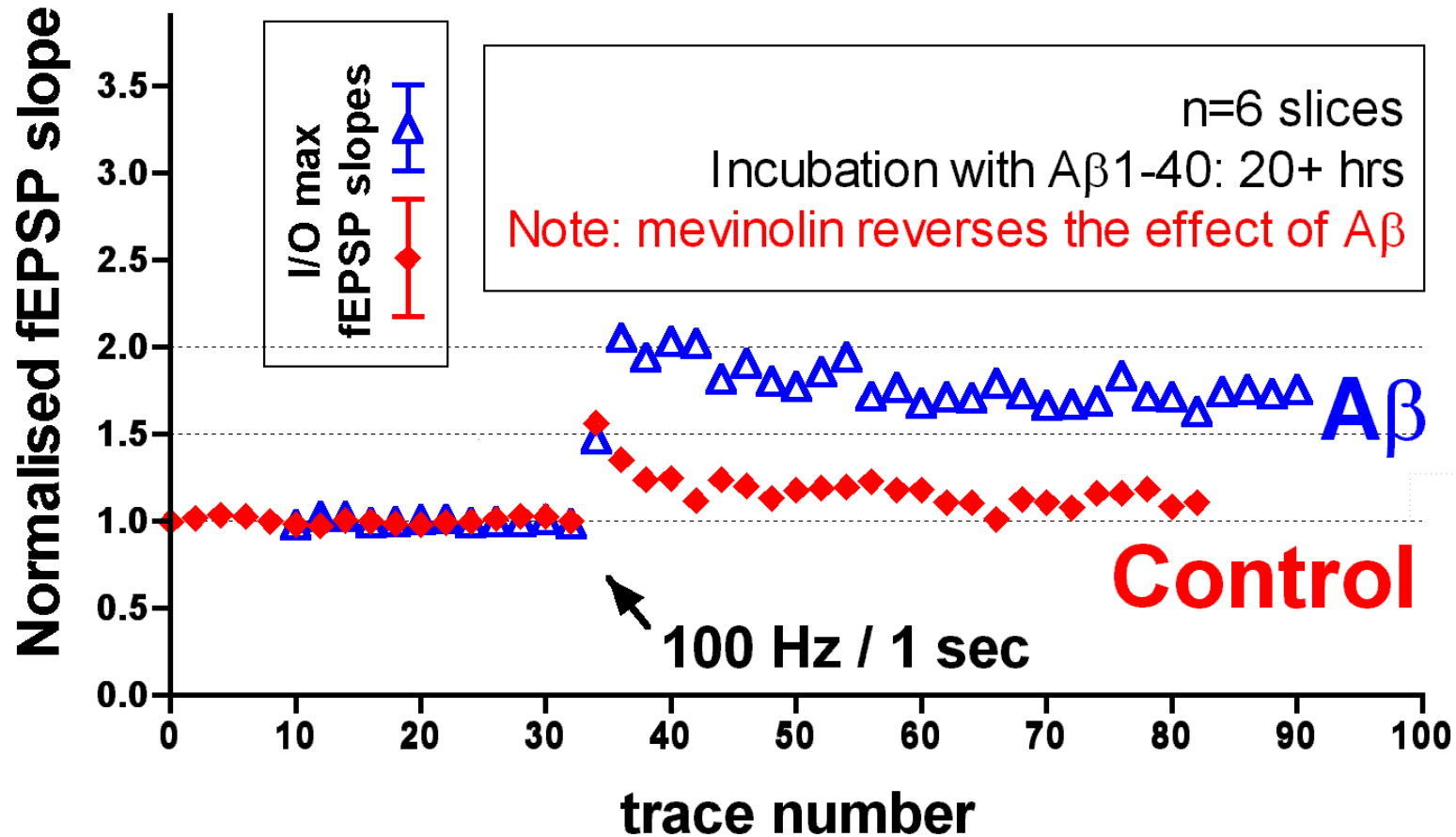
At present it is difficult to unite all cited above *in vitro* and *in vivo* electrophysiological and behavioral studies (5,11,12,29,12S) and conclude on the relevance of their experimental conditions to brain physiology and Alzheimer's disease (5,13S). For this reason in our study we focused on a different (from above studies) experimental condition that we characterized well with regard to cholesterol and phospholipid metabolism status (1,3).

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Effect of Alzheimer's A β 1-40 on synaptic plasticity in CA1 area of adult rat hippocampus



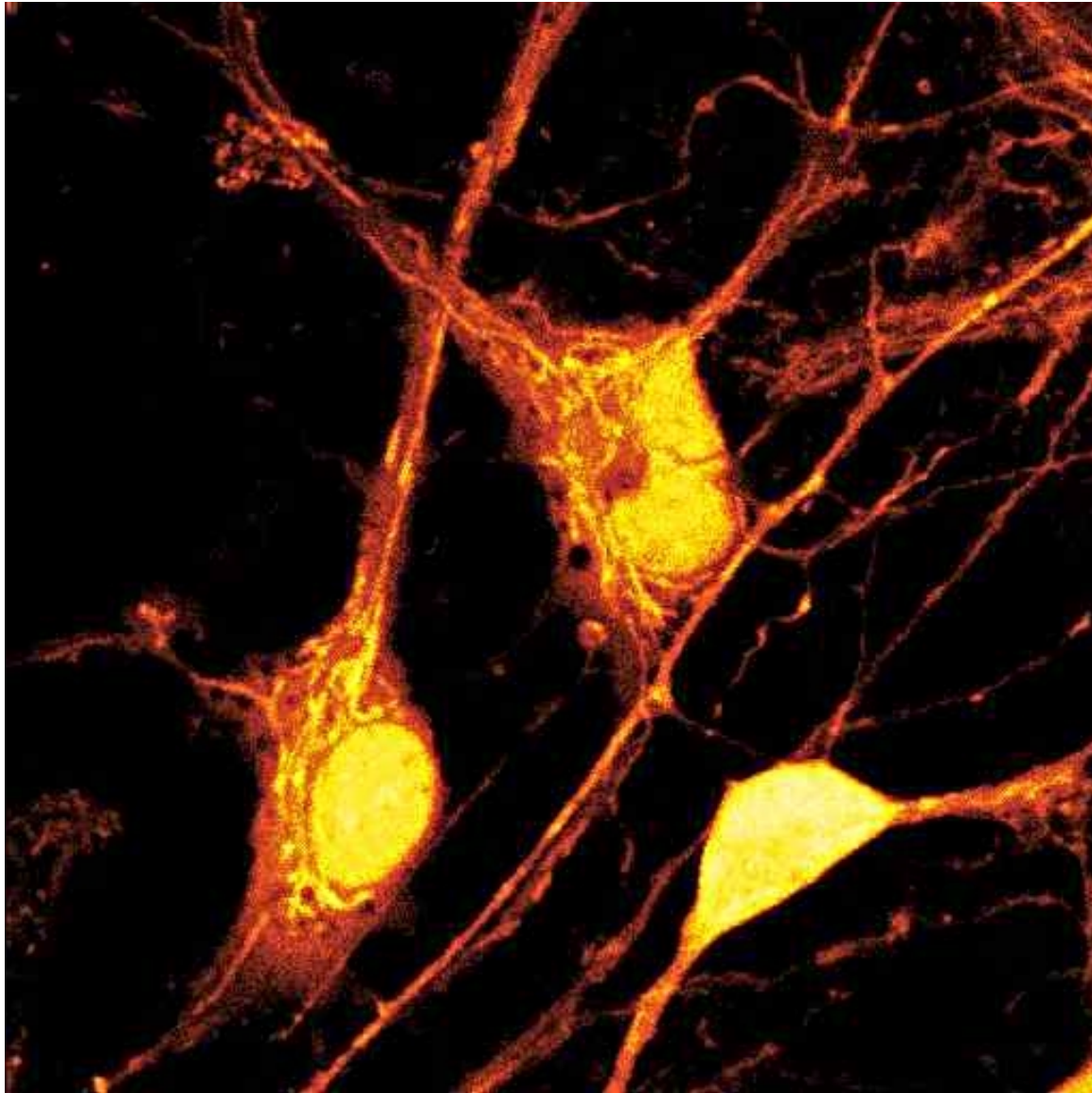


Figure 2

Fluorescence microscopy of A β (added to neuronal cell culture) inside neuronal cells: the mechanism of A β action on lipid synthesis remains obscure. see Koudinova *et al* (2000)(Ref. 6)

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