Neurotransmitters and Energy Metabolites in Amyloid-bearing APP$^{\text{SWE}}$ x PSEN1dE9 Mouse Brain

Joachim Hartmann, Cornelia Kiewert, and Jochen Klein

Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Science Center, Amarillo, Texas (J.H., C.K., J.K.); Department of Pharmacology, Goethe University of Frankfurt, Frankfurt, Germany (J.K.)
Running title page

Running title: Influence of amyloid on brain neurochemistry

Correspondence

Jochen Klein, Ph.D.
Department of Pharmacology, Goethe University of Frankfurt
Max-von-Laue-Str. 9, 60438 Frankfurt, Germany
E-mail: klein@em.uni-frankfurt.de
Phone: +49-69-798 29366. Fax: +49-69-798 29277

Number of text pages: 31
Number of tables: 1
Number of figures: 6
Number of references: 45
Number of words in the abstract: 238
Number of words in the Introduction: 684
Number of words in the Discussion: 1,493

Nonstandard abbreviations used in the paper: ACh, acetylcholine; AChE, acetylcholinesterase; APP, amyloid precursor protein; BChE, butyrylcholinesterase; HACU, high-affinity choline uptake; PS-1, presenilin-1.

Recommended section assignment: Neuropharmacology
Abstract

Alzheimer´s disease is characterized by amyloid peptide formation and deposition, neurofibrillary tangles, synaptic loss and central cholinergic dysfunction, dysfunction of energy metabolism, and dementia; however, the interactions between these hallmarks remain poorly defined. We studied a well-characterized mouse model of amyloid deposition, the doubly transgenic APPSWE x PSEN1dE9 mouse. At 10-14 months of age, these mice had high levels of amyloid peptides (6.6 µg/g wet wt.) and widespread amyloid plaques. Extracellular levels of acetylcholine were determined by microdialysis in the hippocampus and were comparable to non-transgenic mice from the same colony. In the open field, both mouse strains responded with a threefold increase of hippocampal acetylcholine release. Exploratory behavior of the transgenic mice appeared normal. Infusion of scopolamine evoked 5-6fold increases of acetylcholine levels in both mouse strains. High-affinity choline uptake (HACU) and cholinesterase activities were identical in both mouse lines. Extracellular levels of glucose and glycerol were similar in control and transgenic mice while lactate levels were slightly (p=0.06), and glutamate levels significantly (p=0.02) lower in transgenic mice. Exploration caused increases of glucose and lactate while infusion of scopolamine (1 µM) increased glucose but not lactate. Glutamate levels were increased by scopolamine while glycerol remained constant under all conditions. We conclude that amyloid peptide production and plaque deposition causes minor changes in cholinergic function and energy metabolites in transgenic mice in vivo. Amyloid peptide formation and/or deposition may not be sufficient for long-term cholinergic or metabolic dysfunction.
Introduction

Alzheimer’s disease (AD) is the most frequent type of dementia in humans and is characterized by cognitive dysfunction and early memory loss (Blennow et al., 2006; Burns and Iliffe, 2009). AD brains display amyloid plaques and neurofibrillary tangles as well as neuronal degeneration and generalized atrophy. Cholinergic fibres originating in the basal forebrain and innervating hippocampus and cortex seem to be degenerating relatively early in the disease although the clinical evidence is somewhat controversial (Mesulam, 2006; Schliebs and Arendt, 2006). In later stages of the disease, cholinergic dysfunction correlates well with dementia, and current therapies of AD use inhibitors of acetylcholinesterase (AChE) to enhance cholinergic transmission in the brain. While treatment of AD with AChE inhibitors as a monotherapy has only limited effects, successful treatment of dementia will probably not be possible without correcting the cholinergic deficit. Thus, the understanding of the cholinergic dysfunction and its development remains highly relevant for drug treatment of AD.

Clinically, AD can be distinguished in early-onset disease (familial AD) and late-onset, sporadic disease (Blennow et al., 2006; Burns and Iliffe, 2009). The rare familial AD cases have mutations in genes coding for amyloid precursor protein (APP) and the presenilins (PS); both mutations have been shown to cause amyloid peptide formation and amyloid plaque depositions in human brain. These characteristic features have given rise to the “amyloid hypothesis” which postulates that the formation of amyloid peptides is responsible for the etiology of AD (Hardy and Selkoe, 2002). Present animal models of AD are therefore largely based on mutations in APP and PS genes (see below). It should be noted, however, that the relevance of amyloid peptides and plaque formation for the
etiology of the common, “sporadic” form of AD is unclear. Major risk factors for sporadic AD include old age and the presence of apolipoprotein E4 alleles (Blennow et al., 2006). In addition, metabolic changes, damage to mitochondrial components as well as changes of glucose utilization in the brain were observed in sporadic AD (Blass et al., 2002; Eckert et al., 2003; Mosconi et al., 2008).

Based on the “amyloid hypothesis”, transgenic mice have been generated that express (usually overexpress) various APP and PS1 genes containing mutations that were identified in human familial AD (McGowan et al., 2006). While overexpression of human APP mutations in mice produces high levels of amyloid peptides but not overt neuronal loss or behavioral dysfunctions, transgenic mice with double mutations in APP and PS1 genes were associated with various degrees of dysfunction in electrophysiological and behavioral models (McGowan et al., 2006). Central cholinergic function in these mice has been addressed in a variety of experimental studies (reviewed by Kar et al., 2004; Schliebs and Arendt, 2006) but gave contradictory results. For instance, several markers of cholinergic function (choline acetyltransferase, vesicular ACh transporter, high-affinity choline uptake) were found to be unchanged in Tg2576 mice during aging (Gau et al., 2002). In contrast, high-affinity choline uptake (HACU) was found to be reduced in aged (21 month old) Tg2576 mice while the density of the vesicular ACh transporter was increased (Apelt et al., 2002). In doubly transgenic mice overexpressing human APP and PS mutations, the number of forebrain cholinergic cells was normal but cholinergic synapse density was decreased (Bell and Cuello, 2006). In previous work from our group, we observed a small increase of septal cholinergic neurons in a double transgenic APP/PS1 mouse model with moderate amyloid plaque
deposition (Hartmann et al., 2004). Basal ACh levels in the hippocampus were slightly but significantly reduced while stimulated release was unchanged. In contrast, in TgCRND8 mice which have very high levels of amyloid, basal forebrain neurons showed signs of atrophy and inflammation at age 7 months, and both basal and stimulated ACh release was reduced (Bellucci et al., 2006).

In the present study, we used microdialysis to study a well-known mouse model, the APPswe x PSEN1dE9 mouse, which is characterized by aggressive amyloid deposition (Borchelt et al., 1997; Jankowsky et al., 2004). In addition to acetylcholine and cholinergic markers, we monitored extracellular levels of glucose and lactate, two major metabolites reflecting neuronal energy metabolism, and of the neurotransmitter glutamate.

Methods

Animals

Male B6C3-Tg(APPswe, PSEN1dE9)85Dbo/J mice and the appropriate controls were obtained from the Jackson laboratory (JAX004462, Bar Harbor, Maine). The transgenic mice were generated by simultaneously co-injecting the transgenic constructs into the founder mouse pronucleus (Jankowsky et al., 2004); expression of both genes is controlled by the mouse prion promoter. The mutant mice express amyloid precursor protein (APP) harboring the Swedish mutation (K594M/N595L) and a presenilin-1 gene in which exon 9 is deleted (PSEN1dE9). Breeding of the transgenic mice occurred as hemizygotes, and the control mice in this study were wild-type mice from the same
colony (Jackson Labs, Bar Harbor, Maine). All mice were housed in a facility with controlled temperature and humidity and a day/night cycle of 12/12 hours. They had free access to food and water. All experiments were approved by the Animal Care and Use Committee at TTUHSC (protocol 04030-08).

Mouse behavior was observed in the “open field”, a dark grey, rectangular plastic box (45 x 30 x 15 cm). We scored line crossings, rearing, leaning, freezing and grooming during 3 minutes of observation as measures of spontaneous behavior.

Reagents

Most chemicals were obtained from Sigma (St. Louis, MO) in the highest purity available. The ELISA kit for determination of amyloid peptide was from Biosource and was ordered through Invitrogen (Carlsbad, CA), additional chemicals for ELISA (molecular biology grade) and the protease inhibitor cocktail were from Calbiochem (La Jolla, CA). $[^3]H$-choline (specific activity 60-90 Ci/mmol) was from American Radiolabeled Chemicals (St. Louis, MO), scintillation cocktail for the HACU assay (Scintisafe 30%) was from Fisher Scientific (Pittsburgh, PA).

Histochemistry

Amyloid plaques were stained in right brain hemispheres of control and transgenic animals as described (Bussiere et al., 2004) and visualized on a Nikon fluorescence microscope.

Quantification of amyloid peptide
Amyloid Aβ₁₋₄₂ was quantified using a colorimetric ELISA kit from Invitrogen (Biosource). Briefly, cortex and hippocampus from one hemisphere were separated, weighed and homogenized in 10 volumes of 5 M Guanidine HCl/ 50 mM Tris, pH 8.0, supplemented with protease inhibitor cocktail from Calbiochem. The homogenates were frozen at -20°C. On the day of the assay, homogenates were thawed, diluted 100-fold and centrifuged (16,000 x g, 20 min, 4°C). Aβ in the supernatant was quantified according to kit instructions. Each sample was measured in triplicate.

Cholinergic parameters

Total choline uptake, HACU and LACU were determined in synaptosomal (P₂) fractions obtained from cortex and hippocampus as described in a previous publication (Hartmann et al., 2004). Cholinesterase activities were assessed as previously described (Li et al., 2000).

Microdialysis experiments

Mice were anesthetized with isoflurane (induction dose 4%, maintenance dose 1-1.5% v/v) in a 25%/75% mixture of oxygen and nitrous oxide and placed in a stereotaxic frame. Self-made, I-shaped, concentric dialysis probes with an exchange length of 1 mm were implanted in the right dorsal hippocampus using the following coordinates (from bregma): AP −2.2 mm; L −1.8 mm; DV −2.3 mm. Mice were allowed to recover over night, and experiments were carried out on two consecutive days after probe implantation in freely moving animals.
On the experimental days, the microdialysis probes were perfused with artificial cerebrospinal fluid (aCSF; 147 mM NaCl, 4 mM KCl, 1.2 mM CaCl$_2$ and 1.2 mM MgCl$_2$), supplemented with 1 µM neostigmine bromide. The perfusion rate was 2 µl/min, and efflux from the microdialysis probe was collected in intervals of 15 minutes. On the first day of microdialysis, samples were collected for 90 minutes to establish baseline values of ACh. Subsequently, animals were placed into a novel environment, consisting of a dark grey, rectangular plastic box (45 x 30 x 15 cm), termed the “open field”. After 90 minutes in the open field, mice were returned to their home cages and dialysis continued for 2 hours. On the second day, baseline values for ACh were collected again, then the perfusion fluid was switched to aCSF containing 1 µM scopolamine bromide. After 90 minutes, the perfusion fluid was switched back to aCSF, and mice were further monitored for a period of at least 2 hours.

On day 3 after probe implantation, mice were sacrificed, probe locations were verified and contralateral hemispheres were extracted for subsequent staining or assessment of amyloid burden by ELISA.

**Chemical analysis of microdialysates**

ACh and Ch in dialysates were determined by microbore HPLC-ECD using a metal-free system from Bioanalytical Systems (West Lafayette, IN). The procedure was performed as previously described (Kopf et al., 2001; Hartmann et al., 2004). At an injection volume of 5 µl, the detection limit of this system was 5-10 fmol/injection.
Glucose, lactate, glutamate and glycerol concentrations in the microdialysis samples were determined by a CMA 600 microanalyzer (CMA, Stockholm, Sweden) using a kinetic photometric assay as described by the manufacturer.

**Statistical analysis**

Data are given as mean ± S.E.M. of \( N \) experiments and were compared by Student t-test (Table 1; Fig. 2). Time courses of microdialysis data (Figs. 3-6) were compared by two-way ANOVA. Statistical significance was derived from the column factor comparing the two curves (software: GraphPad Prism 4.0).

**Results**

**Characterisation of mice**

Amyloid formation in APPswe-PS1dE9 mice was extensively characterized in previous work by the group who created these mice. These doubly transgenic mice have high amyloid peptide levels and amyloid plaques were already observed at 4-6 months of age (Jankowsky et al., 2004; Savonenko et al., 2005; Garcia-Alloza et al., 2006). In the present study, we used these mice at age 10-14 months. As expected, these mice had plenty of amyloid plaques which could be visualized with thioflavin-S (Fig. 1). Formation of amyloid peptide A\(\beta_{1-42}\) was measured by ELISA and yielded a value of 6.6 ± 0.4 µg/g wet wt. (Table 1). Control mice had neither amyloid plaques (Fig. 1) nor measurable amounts of amyloid peptides.
Cholinergic parameters

Enzymatic activities of cholinesterases in control mice were: AChE, $2.80 \pm 0.28$ U/g wet weight and BChE, $0.14 \pm 0.02$ U/g wet weight (N=5). In transgenic mice, the values were very similar, and differences between the two groups were not statistically significant (Table 1). Choline uptake measurements also did not reveal differences between the two groups of mice. High-affinity choline uptake, an ex vivo-marker for acetylcholine turnover, was identical in both mouse strains (Table 1). Total choline uptake and low-affinity choline uptake were also unchanged (not illustrated).

Microdialysis: acetylcholine and choline

In control mice, basal efflux was $100.3 \pm 12$ fmol/5 µL for acetylcholine (ACh) and $2.62 \pm 0.31$ pmol/5 µL for choline (N=8). After correction for in vitro recoveries (9.6% for ACh and 14.6% for Ch), extracellular concentrations for ACh were estimated at $209 \text{ nM}$, and for choline $3.59 \text{ µM}$ (Table 1). In transgenic APPswe/PS1dE9 mice, basal efflux was $92.4 \pm 12$ fmol/5 µL for ACh and $2.69 \pm 0.23$ pmol/5 µL for choline (N=8) yielding extracellular concentrations for ACh of $193 \text{ nM}$, and $3.69 \text{ µM}$ for choline in transgenic mice, which were not statistically different from control mice (Table 1).

Two paradigms were used to stimulate release of ACh, namely exposure to a novel environment and infusion of scopolamine (see below). When mice were placed into a novel environment (the “open field”), control as well as transgenic mice showed exploratory behaviour. There were no statistically significant differences in motor activity or in the frequency of rearing, leaning or grooming in the open field (p>0.2 in all cases; Fig. 2). Freezing, a sign of great stress or anxiety, was not observed. During
exploration, ACh release in the hippocampus was stimulated by up to fourfold in control mice (Fig. 3A). Maximum increases were slightly higher for control mice (343 ± 59 % of basal release) than for transgenic mice (278 ± 47 %) but the differences between these two time points or between the time courses did not reach significance (two-way ANOVA: $F_{1,252}=2.09; p=0.12$). While ACh levels rose, choline levels dropped in a mirror-like fashion to ACh and reached a minimum of 75-80% of basal levels after 30 min in both strains (not illustrated).

Upon infusion of scopolamine, hippocampal ACh levels were increased more than five-fold on average (Fig. 4B). Again, maximum increases were slightly higher for control mice (665 ± 144 %) than for transgenic mice (594 ± 123 %) but differences between the two curves were due to chance (two-way ANOVA: $F_{1,216}=0.05; p=0.83$). During scopolamine infusion, choline levels were slightly reduced to 80-85% of basal levels in both mouse strains (not illustrated).

**Glutamate and glycerol**

In microdialysates from untreated mice, basal levels of glutamate were 0.44 ± 0.08 µM in control mice and 0.20 ± 0.04 µM in transgenic APP<sub>swe</sub>/PS1dE9 mice. After correction for recovery, extracellular glutamate levels were estimated at 1.99 µM and 0.90 µM in controls and transgenics, respectively. This difference was significant ($p=0.02$; Table 1).

Upon exposure to the open field, brain extracellular glutamate concentrations rose slowly but steadily to ca. 150% of basal values (Fig. 4A). In comparison, infusion of scopolamine caused a doubling of glutamate levels within 60 min (Fig. 4B). Changes of glutamate levels were identical in the two mouse strains ($p>0.3$).
Basal levels of glycerol were 1.23 ± 0.40 µM in control mice and 1.04 ± 0.58 µM in transgenic mice. After correction for recovery, extracellular glycerol levels were estimated at 9.4 µM and 8.9 µM in controls and transgenics, respectively (p=0.74; Table 1). Glycerol levels did not appreciably change during either behavioural activation or scopolamine infusion (data not shown).

**Energy metabolites: glucose and lactate**

In microdialysates from untreated mice, basal levels of glucose were 56.8 ± 11.1 µM in control mice (N=5) and 47.1 ± 3.5 µM in transgenic APP<sub>swe</sub>/PS1dE9 mice (N=8). After correction for recovery, extracellular glucose levels were estimated at 0.9 mM and 0.76 mM in controls and transgenics, respectively (p=0.34; Table 1). Basal levels of lactate were 39.4 ± 6.2 µM in control mice and 27.1 ± 3.5 µM in transgenic mice, yielding true extracellular lactate levels of 0.43 mM and 0.30 mM in controls and transgenics, respectively. This difference was borderline significant (p=0.06; Table 1).

When mice were placed into the open field, glucose levels rose in a delayed fashion during exploration and remained elevated even after the mice had been put back into their home cages (Fig. 5A). Extracellular concentrations of lactate increased rapidly by more than 2-fold during exploration but returned to normal within two hours (Fig. 5B). These metabolic responses were similar in both control and transgenic mice.

Infusion of scopolamine (1 µM) caused a slow increase of glucose levels which remained high after three hours (Fig. 6A). Lactate levels, however, were not affected by scopolamine (Fig. 6B). No differences were noted between the two strains of mice (p>0.5).
Discussion

Mouse models of AD present with variable degrees of amyloid peptide formation and amyloid deposition (McGowan et al., 2006). For the present study, we chose a double transgenic mouse strain in which the Swedish APP mutation is expressed together with the presenilin-1 gene deleted in exon 9 (Borchelt et al., 1997; Jankowsky et al., 2004). The PS1 mutation specifically increases the formation of Aß42 peptide which is highly amyloidogenic (Jankowsky et al., 2004) and causes the development of amyloid plaques early in life. Our finding of amyloid plaques in 10-14 month old mice corresponds to an earlier report (Garcia-Alloza et al., 2006), and the high Aß1-42 peptide levels observed in this previous study (about 1.0 nmol/g wet tissue, corresponding to 4.5 µg/g) were confirmed in our measurements (6.6 µg/g wet weight). In another study, a slightly lower value (2.2 µg/g) was reported for hippocampal tissue of these animals (Liu et al., 2004). It should be noted, however, that most of this Aß1-42 is “insoluble” peptide that was solubilised from tissue by guanidine (see Methods).

Microdialysis is ideally suited to evaluate presynaptic cholinergic function in vivo because sampling of acetylcholine (ACh) in the extracellular fluid of the brain reflects the activity of central cholinergic fibres. In the present study, basic parameters of cholinergic function were largely preserved in APPswe x PS1dE9 mice, in spite of high Aß1-42 levels and amyloid deposits. Thus, the level of extracellular ACh in the hippocampus – representing the activity of septohippocampal cholinergic fibres – was almost identical in controls and transgenics (209 nM vs. 193 nM, a reduction of <10%, p>0.2).
activity, a measure of ACh turnover and cholinergic firing frequency, was unchanged in amyloid-bearing mice. AChE as well as BChE activities were the same in both mouse strains. These findings are in line with an earlier study in which cholinergic markers were unchanged in these mice at 6 months of age and mildly decreased at age 18 months (Savonenko et al., 2005).

The focus of our present work was to investigate changes of neurotransmitters and energy metabolites under conditions of behavioral and pharmacological stimulation. For this purpose, we first exposed the mice to an “open field”. Exploration of a novel environment is known to enhance hippocampal ACh release (Kopf et al., 2001; Hartmann et al., 2004), and this response was also observed in this study (Fig. 3A). Our second paradigm of stimulation was an infusion of scopolamine, a muscarinic antagonist which increases ACh release by blocking presynaptic inhibitory autoreceptors of the muscarinic (M₂/M₄) subtype. Upon scopolamine infusion, ACh release was strongly enhanced to 500-600% of basal levels (Fig. 3B) while choline levels decreased (not illustrated). Amyloid-bearing mice had similar cholinergic responses as control mice in both paradigms (Fig. 3A and B).

While most studies in AD neurochemistry focused on cholinergic dysfunction, changes of glutamatergic function have also been repeatedly described early in AD. Glutamate concentrations are lowered in AD brains (Lowe et al., 1990), and loss of glutamatergic neurons was suggested by reductions in vesicular glutamate transporters (Kirvell et al., 2006). In contrast, excitotoxicity was postulated as a contributor to neurodegenerative diseases and increased extracellular glutamate levels were postulated in AD brain (Hynd et al., 2004). In our study, extracellular levels of glutamate were 2 µM
in control mice, a value that is similar to previous measurements in mice using microdialysis or enzyme electrodes (1-5 µM; Hascup et al., 2008). Surprisingly, basal glutamate concentrations were significantly lower in transgenic mice (Table 1), a finding that supports glutamatergic dysfunction in amyloid-bearing mice (Kirvell et al., 2006), but is at variance with the idea of ongoing cell death due to excitotoxicity. Moreover, both glycerol and choline are products of membrane breakdown (Marklund et al., 1997; Klein, 2000), but neither was increased in the doubly transgenic mice (Table 1); thus, no obvious degeneration of brain cells occurred in these mice during the experimental phase.

Extracellular levels of glutamate, as sampled by microdialysis, are not easy to interpret because they are partially from non-vesicular sources and are affected by changes of glutamate release and uptake into glial cells (van der Zeyden et al., 2008). In the present study, glutamate levels did not strongly respond to behavioural activation (Fig. 4A); however, they were increased in both strains by scopolamine (Fig. 4B). This effect may be due to an increase of acetylcholine release evoked by scopolamine (Fig. 3B) or by the blockade of postsynaptic muscarinic receptors. Both mechanisms are possible because cholinergic activation increases glutamate levels in the striatum (via muscarinic receptors; Rawls and McGinty, 1998) and in the prefrontal cortex (via nicotinic receptors; Gioanni et al., 1999) while in the hippocampus, glutamate release is inhibited by ACh acting on scopolamine-sensitive M₂-type muscarinic receptors (Marchi and Raiteri, 1989). Both mouse strains had similar responses for glutamate levels when expressed as relative changes of basal values (Fig. 4) although the lower glutamate levels were preserved in transgenic mice when absolute changes of glutamate were graphed (not
illustrated); thus, our results are in agreement with reduced glutamate release in aged amyloid-bearing mice (Minkeviciene et al., 2008).

In addition to neurotransmitters, we also measured extracellular levels of the energy metabolites, glucose and lactate. Glucose is the standard fuel for brain energy production; in addition, it is a precursor of acetyl-CoA and therefore, of ACh synthesis, and ACh levels depend on glucose oxidation in periods of increased cholinergic activity (Ragozzino et al., 1996; Löffelholz and Klein, 2006). AD patients show glucose hypometabolism early in the disease, and reductions of the cerebral metabolic rate of glucose correlate with disease severity (Blass et al., 2002; Mosconi et al., 2008). Lactate is a product of ischemic metabolism in muscle, but in brain lactate is formed by astrocytes and supports neuronal function in periods of low glucose availability and high neuronal demand (Pellerin and Magistretti, 2004; Schurr, 2006). In mice, extracellular levels of glucose were 0.7-1.0 mM in hippocampus, a value that corresponds closely to previous measurements in rats using the more sophisticated zero-net-flux method (McNay and Gold, 1996). Lactate levels were 0.3-0.4 mM and were slightly lower in transgenic mice (p=0.06), a finding of potential significance that is presently unexplained.

Behavioural stimulation in the open field caused glucose levels to rise in a delayed fashion (Fig. 4A). This observation may reflect an increase of blood sugar or of blood flow in the hippocampus because blood glucose, blood flow and glucose uptake in the brain are tightly coupled (Messier and Gagnon, 1996; Löffelholz and Klein, 2006). Compared to the small changes of glucose, extracellular lactate levels were immediately increased more than two-fold upon placing the animals into the open field (Fig. 5B). As lactate is now recognized as fuel for neurons (Schurr, 2006), this increase of lactate levels
is probably a sign of increased energy demand in the hippocampus during exploration and spatial orientation. Importantly, the extent of glucose and lactate mobilization was identical in transgenic and control mice (Fig. 5) indicating that amyloid peptides and plaques do not hamper the ability of mouse brain to respond adequately to a physiological stimulus.

Pharmacological stimulation was induced by the infusion of scopolamine which caused a strongly increased release of ACh (Fig. 3B). It must be noted, however, that scopolamine also blocks excitatory postsynaptic M₁ receptors and therefore inhibits cholinergic activation of hippocampal pyramidal cells. The increase of glucose concentrations that was observed in the hippocampus during scopolamine infusion (Fig. 6A) may be due to a reduced energy demand in hippocampus during blockade of excitatory cholinergic input. Similar increases of glucose were seen during anesthesia (Fellows et al., 1992). This interpretation is in agreement with the fact that hippocampal lactate levels remained unaltered during scopolamine infusion (Fig. 6B).

Summarizing, a double transgenic mouse strain with high levels of amyloid peptides in the brain and widespread amyloid plaque formation displays only subtle changes in hippocampal activity. Cholinergic parameters were largely unchanged although transgenic mice had a slightly lower basal ACh concentration and a somewhat smaller response to the open field paradigm. This study, therefore, indicates that presynaptic cholinergic function is largely unchanged in amyloid-bearing mice. It should be noted that our results do not exclude potential interactions of amyloid peptides with postsynaptic cholinergic signalling; for instance, Aβ peptides interfere with both nicotinic (Dineley et al., 2001; Abbott et al., 2008) and muscarinic signalling (Goto et al., 2008;
Machova et al., 2008), but these interactions were not addressed in the present study. Importantly, basal glutamate levels were decreased in APPswe/PS1dE9 mice although the responses to stimulation were preserved. We also document significant and distinct changes of glucose and lactate levels upon brain stimulation, but again transgenic mice had similar or identical responses as controls. We conclude that – while amyloid peptide formation may be a required step for brain dysfunction in AD – it does not seem to be sufficient to induce impairments of brain metabolism and central cholinergic function. Additional factors are evidently required to model the full picture of neuronal dysfunction that is characteristic of human AD.


Age-related decrease in stimulated glutamate release and vesicular glutamate transporters in APP/PS1 transgenic and wild-type mice. *J Neurochem* **105**: 584-594.


Van der Zeyden H, Oldenziel WH, Rea K, Cremers TI and Westerink BH (2008)

Microdialysis of GABA and glutamate: analysis, interpretation and comparison
Footnotes

This work was supported by the Alzheimer´s Association [IIRG-04-1178].
Legends for Figures

Figure 1
Thioflavin-S staining of amyloid plaques
Staining with thioflavin-S for amyloid plaques in hemibrain sections from a control mouse (left panel) and a transgenic APPswe-PS1dE9 mouse (right panel) at age 13 months.

Figure 2
Behavioral parameters of mice in the open field
Mouse behavior was scored within 3 minutes of placing the mice into the open field. “Cross”, line crossings; “Rear”, rearing; “Lean”, leaning; “Groom”, grooming. Data are expressed as number of observations within 3 minutes and are shown as mean ± S.D. (N=5 in each group).

Figure 3
Stimulated release of acetylcholine in mouse hippocampi measured by microdialysis
(A) Changes of ACh efflux upon behavioral activation (exposure to a novel environment) from 0-90 min, followed by return to the home cage. (B) Changes of ACh efflux upon infusion of scopolamine (1 µM) from 0-90 min, followed by infusion of artificial CSF. Ctr, control mice (N=8 in A; N=7 in B). Tg, transgenic APP(swe)/PS1dE9 mice (N=8 in A; N=7 in B). Data are means ± S.D. of basal release which was determined as average efflux from six samples prior to treatment. Statistical analysis (two-way ANOVA,
GraphPad Prism®): (A) ACh (Ctr) vs. ACh (Tg), $F_{1,252}=2.09; p=0.12$ (N=8 each). (B) ACh (Ctr) vs. ACh (Tg), $F_{1,216}=0.05; p=0.82$ (N=7 each).

**Figure 4**

Extracellular concentrations of glutamate in mouse hippocampi measured by microdialysis

(A) Changes of glutamate efflux upon behavioral activation (exposure to a novel environment) from 0-90 min, followed by return to the home cage. (B) Changes of glutamate efflux upon infusion of scopolamine (1 µM) from 0-90 min, followed by infusion of artificial CSF. Ctr, control mice. Tg, transgenic APP(swe)/PS1dE9 mice (N=6 each). Data are means ± S.D. of basal release which was determined as average efflux from six samples prior to treatment. Statistical analysis (two-way ANOVA, GraphPad Prism®): (A) Glutamate (Ctr) vs. glutamate (Tg), $F_{1,170}=1.02; p=0.34$ (N=6 each). (B) Glutamate (Ctr) vs. glutamate (Tg), $F_{1,170}=0.77; p=0.40$ (N=6 each).

**Figure 5**

Microdialysis of energy metabolites during behavioral activation

Extracellular concentrations of (A) glucose and (B) lactate: Changes induced by behavioral activation (exposure to a novel environment) from 0-90 min, followed by return to the home cage. Ctr, control mice (N=7). Tg, transgenic APP(swe)/PS1dE9 mice (N=7). Data are means ± S.D. of basal release which was determined as average efflux from six samples prior to treatment. Statistical analysis (two-way ANOVA for repeated
measures, GraphPad Prism®): (A) Glucose (Ctr) vs. Glucose (Tg), F_{1,198}=2.30; p=0.13. (B) Lactate (Ctr) vs. Lactate (Tg), F_{1,198}=0.06; p=0.81.

**Figure 6**

**Microdialysis of energy metabolites during stimulated ACh release**

Extracellular concentrations of (A) glucose and (B) lactate: Changes induced by infusion of scopolamine (1 µM) from 0-90 min, followed by artificial CSF. Ctr, control mice (N=7). Tg, transgenic APP(swe)/PS1dE9 mice (N=7). Data are means ± S.D. of basal release which was determined as average efflux from six samples prior to treatment. Statistical analysis (two-way ANOVA for repeated measures, GraphPad Prism®): (A) Glucose (Ctr) vs. Glucose (Tg), F_{1,198}=0.02; p=0.89. (B) Lactate (Ctr) vs. Lactate (Tg), F_{1,198}=0.03; p=0.87.
### TABLE 1

**Comparison of control and transgenic APP(swe)/PS1dE9 mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control mice</th>
<th>Transgenic mice</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ₁₋₄₂</td>
<td>n.d.</td>
<td>6.6 µg/g wet wt.</td>
<td></td>
</tr>
<tr>
<td>AChE activity</td>
<td>2.80 U/mg wet wt.</td>
<td>2.81 U/mg wet wt.</td>
<td>0.98</td>
</tr>
<tr>
<td>BChE activity</td>
<td>0.14 U/mg wet wt.</td>
<td>0.14 U/mg wet wt.</td>
<td>1.00</td>
</tr>
<tr>
<td>HACU</td>
<td>36.5 dpm/µg protein</td>
<td>35.1 dpm/µg protein</td>
<td>0.81</td>
</tr>
<tr>
<td>ACh (basal)</td>
<td>209 nM</td>
<td>193 nM</td>
<td>0.64</td>
</tr>
<tr>
<td>Choline (basal)</td>
<td>3.59 µM</td>
<td>3.69 µM</td>
<td>0.86</td>
</tr>
<tr>
<td>Glutamate (basal)</td>
<td>1.99 µM</td>
<td>0.90 µM</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose (basal)</td>
<td>902 µM</td>
<td>760 µM</td>
<td>0.34</td>
</tr>
<tr>
<td>Lactate (basal)</td>
<td>428 µM</td>
<td>295 µM</td>
<td>0.06</td>
</tr>
<tr>
<td>Glycerol (basal)</td>
<td>9.41 µM</td>
<td>8.87 µM</td>
<td>0.74</td>
</tr>
</tbody>
</table>

n.d., not detectable
Figure 1

Control mouse

Transgenic mouse
Fig. 2

The figure shows a bar graph comparing the number of observations between controls and transgenics. The x-axis represents different behaviors: Cross, Rear, Lean, Groom. The y-axis represents the number of observations. Transgenics show higher values for Cross and Lean compared to controls.
FIG. 5

A

Glucose (% basal)

Time [min]

Ctr
Tg

B

Lactate (% basal)

Time [min]

Ctr
Tg