Experimental Gerontology xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Effects of galantamine on β-amyloid release and beta-site cleaving enzyme 1 expression in differentiated human neuroblastoma SH-SY5Y cells

Qianqian Li [a], Donglai Wu [b], Liming Zhang [a], Yan Zhang [a,*]

[a] Department of Neurology, the First Affiliated Hospital of Harbin Medical University, PR China
[b] Harbin Veterinary Research Institute, PR China

ARTICLE INFO

Article history:
Received 21 February 2010
Received in revised form 23 June 2010
Accepted 24 June 2010
Available online xxxx

Keywords:
Galantamine
Amyloid precursor protein
BACE1
β amyloid

ABSTRACT

Galantamine (Gal) is an acetylcholinesterase inhibitor and used to treat the symptoms of Alzheimer's disease (AD). Recent studies show that Gal may affect amyloid precursor protein (APP) metabolism and increase release of secretory APPα (sAPPα). However the effect of Gal on amyloid-β peptide (Aβ) release and β-site cleaving enzyme 1 (BACE1) expression is still unknown. Consequently, we investigated the effect of Gal on the level of Aβ and BACE1. In a differentiated human neuroblastoma cell line (SH-SY5Y), Gal (0.3 μM) was found to significantly decrease Aβ release and BACE1 expression following treatment for 6, 12, and 24 h. Increasing Gal to 0.9 μM or 10 μM had no further effect. The effect of Gal (0.3 μM for 18 h) was maximal on BACE1 expression but not on Aβ secretion. At higher concentration (0.9 μM and 10 μM), Gal had no effect on the level of full-length APP but could still stimulate further decrease in Aβ secretion and release of sAPPα. These observations suggested that 0.3 μM Gal exerts its effect on Aβ production by inhibiting BACE1 expression, while 0.9 μM or 10 μM Gal mainly reduces Aβ production by stimulating the non-amyloidogenic pathway to decrease the amount of APP substrate available for β-secretase cleavage. In addition, α7 nicotinic acetylcholine receptor (α7nAChR) and multiple second messengers (including PKC, MEK, and p38MAPK) were found to be involved in the regulation of Gal-inhibited Aβ release and BACE1 expression.

1. Introduction

One of the hallmarks of Alzheimer's disease (AD) is the presence in the brain of extracellular senile plaques composed mainly of extracellular deposits of amyloid-β peptide (Aβ) (Selkoe, 1994). Aβ is generated from the amyloid precursor protein (APP) through an initial cleavage with β-secretase at the amino-terminal side of the Aβ sequence to generate a soluble peptide, secretory APPβ (sAPPβ), and an intracellular carboxyl-terminal fragment C99. Thereafter, γ-secretase cleaves C99 to release Aβ (Haass et al., 1992; Shoji et al., 1992). In the non-amyloidogenic pathway, α-secretase cleaves APP within the Aβ peptide sequence to generate a secreted form of APP fragment (sAPPα), preventing the formation of Aβ peptide (Esch et al., 1990; Sisodia et al., 1990). α- and β-secretases are believed to compete for APP, and thereby determine the amount of Aβ (Buxbaum et al., 1998). In one study, overexpression of α-secretases shifted APP processing towards the non-amyloidogenic pathway and resulted in decreased Aβ generation (Postina et al., 2004). The most likely candidate α-secretases are a membrane-bound disintegrin and metalloprotease (ADAM)10 and ADAM17 (Lammich et al., 1999; Nunan and Small, 2000). β-site APP cleaving enzyme 1 (BACE 1; an aspartic protease) was identified as a candidate β-secretase (Sinha et al., 1999; Vassar et al., 1999).

Galantamine (Gal), a plant-derived alkaloid, is a third-generation acetylcholinesterase inhibitor (AChEI) (Maelicke et al., 2001). Gal treatment has been shown to alleviate cognitive deficits in patients with AD (Raskind et al., 2000; Tariot et al., 2000). In contrast to other AChEIs, such as tacrine, donepezil, and rivastigmine, Gal has a weak cholinesterase inhibitor effect. However, it interacts allosterically with nicotinic acetylcholine receptors (nAChRs) to potentiate the action of agonists of these receptors and enhance the sensitivity of the receptors to acetylcholine (Maelicke et al., 2001; Texido et al., 2005). Recent findings have demonstrated that Gal is also involved in APP processing (Lenzken et al., 2007).

The purpose of the present study was to determine the effect of Gal on Aβ generation and BACE1 expression in the differentiated human neuroblastoma cell line (SH-SY5Y), which expresses choline acetyltransferase and both muscarinic and nicotinic receptors (Adem et al., 1987; Gould et al., 1992; Halvorsen et al., 1995). Our findings may suggest an additional mechanism of the pharmacological action of Gal on APP metabolism.

2. Materials and methods

2.1. Reagents

The cell signaling inhibitors PD98059, SB203580, and GF109203X (Santa Cruz Biotechnology, CA, USA), together with LY294002 and SP600125 (Sigma-Aldrich, St. Louis, MO, USA) were each dissolved in

* Corresponding author. Mailing address: No 23rd, You Zheng Street, Nan Gang District, Harbin, Hei Longjiang Province, PR China. Tel.: +86 0451 85555025.
E-mail address: hydfsy@163.com (Y. Zhang).

0531-5565/$ – see front matter © 2010 Published by Elsevier Inc.
doi:10.1016/j.exger.2010.06.008

dimethylsulfoxide and stored at −20 °C. Gal, atropine (a muscarinic acetylcholine receptor antagonist), and methyllycaconitine (MLA, an α7-nicotinic acetylcholine receptor antagonist) all from Sigma Chemical Co. were each dissolved in distilled water and stored at −20 °C. Trans-retinoic acid (Sigma Chemical Co.) was dissolved in dimethylsulfoxide and stored at −20 °C. All stocks were diluted in medium to their respective working concentrations immediately before use. Aβ1-40 colorimetric sandwich ELISA kit was obtained from Invitrogen (Carlsbad, CA, USA) and ELISA kit for Aβ1-42 was from Covance (Denver, PA). We also purchased the following antibodies: polyclonal APP antibody, which recognizes residues 44–60 of APP (Sigma), anti-BACE1 polyclonal antibody (Abcam, Cambridge, UK), anti-β-actin antibody (Santa Cruz Biotechnology), and anti-sAPPα (2B3) mouse IgG MAb (Immuno-Biological Laboratories Co., Ltd., Tokyo Japan). All culture media, supplements, and fetal bovine serum (FBS) were from Gibco (Carlsbad, CA, USA). Electrophoresis reagents were from Bio-Rad (Hercules, CA, USA). All other reagents were of the highest grade available and were purchased from Sigma Chemical Co. unless otherwise indicated.

2.2. Cell cultures

The human neuroblastoma SH-SY5Y cells (Institute of Basic Medical Sciences Chinese Academy of Medical Sciences) were cultured in medium with equal amount of Eagle's minimum essential medium and Nutrient Mixture Ham's F-12, supplemented with 10% FBS, glutamine (2 mM), penicillin/streptomycin, non-essential amino acids, at 37 °C in 5% CO_2 and 95% air. For differentiation, cells were plated at a density of 5×10^3 cells/cm^2 in 60-mm diameter culture dishes and exposed to 10 μM all trans-retinoic acid for 6 days as previously described (Lenzken et al., 2007). Differentiation was considered to be complete when the growth of cone-terminated neurites was up to three times longer than one cell body diameter.

2.3. Treatment of cells with Gal and cell signaling inhibitors

Cells grown to 80% confluence were washed with serum-free medium, maintained in serum-free medium for 2 h, and then treated with Gal (0, 0.3, 0.9, or 10 μmol/ml for 6, 12, 18, or 24 h). In inhibition studies, cells were exposed to 2.5 μM GF109203X, 30 μM PD98059, 30 μM SB203580, 10 μM LY294002, and 20 μM SP600125, which are inhibitors of protein kinase C (PKC), mitogen-activated protein kinase kinase (MEK), p38 mitogen-activated protein kinase (MAPK), phosphoinositol-3-kinase (PI3K), and c-Jun N-terminal protein kinase (JNK), respectively. Atropine (10 nM) and MLA (10 nM) were also used. Cells were exposed to inhibitors for 30 min before Gal treatment.

After incubation with the drugs for the indicated periods, conditioned media were collected and mixed with a complete protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA). The media were centrifuged (13,000×g for 5 min) to remove detached cells and debris, and supernatants were concentrated with centrifugal filter devices (Amicon Ultra-4; Millipore Corp., Bedford, MA) and then stored at −70 °C. Cell monolayers were washed twice with ice-cold PBS, lysed on the tissue culture dish by addition of ice-cold RIPA buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecylsulfate, and protease inhibitors cocktail), and centrifuged (14,000×g for 30 min at 4 °C). The supernatants were transferred to new microtubes and stored at −70 °C. Protein levels were determined using the BCA Protein Assay kit (Beyotime Institute of Biotechnology, Shanghai, China) and the amount of protein on each blot was equalized by loading a volume of sample of conditioned medium standardized to total cell lysate protein concentration.

2.4. Western blot assay

SDS-polyacrylamide gel electrophoresis (PAGE; 7.5% gel) was carried out on each sample (30 μg of protein). Separated proteins were subsequently transferred to 0.2-μm nitrocellulose membranes at 16 V for 30 min. The membranes were blocked with 5% fat-free milk in Tris-buffered saline containing 0.05% Tween 20 (TBST; 2 h at RT) and washed in TBST. The blots were probed with polyclonal antibodies to APP N-terminal (1:200 dilution) and BACE1 (1:200), and monoclonal antibodies to sAPPα (1:50) and β-actin (1:2000) at 4 °C overnight, washed for 30 min in TBST, incubated with horseradish peroxidase conjugated goat anti-mouse IgG and goat anti-rabbit IgG (both at 1:5000) for 2 h at RT, and visualized using a DAB kit. Densitometric quantification of blots was carried out using Alphapart11Ease version 5.0, and a ratio relative to control was calculated.

2.5. Quantification of secreted Aβ levels

Aβ released from control and drug-treated cells into the cell culture media was measured in lyophilized samples using ELISA kits for Aβ1-40 and Aβ1-42 according to the manufacturer's protocol. The release of Aβ1-40 and Aβ1-42 was evaluated using standard curves generated in duplicate. The quantity of Aβ in each sample was measured in duplicate and expressed as mean ± standard error of quadruplicate experiments. Aβ1-40 and Aβ1-42 levels are expressed in pg/ml.

2.6. Statistical analysis

Data are presented as the mean ± the standard error of the mean (S.E.M). Each procedure was performed in duplicate in four independent experiments. Statistical analyses were carried out using one-way ANOVA, followed by a least significant difference post-hoc test. A difference at $p<0.05$ was considered to be significant. All statistical analyses were performed using SPSS software version 13.0.

3. Results

3.1. Effect of Gal on Aβ1-40 and Aβ1-42 levels

Aβ1-40 and Aβ1-42 peptide levels were measured in medium conditioned by differentiated SH-SY5Y cells. Gal (0.3 μM) for 6, 12, and 24 h significantly decreased Aβ1-40 and Aβ1-42 generation. The magnitude of the effect was unaffected by further increases in Gal concentration (0.9 or 10 μM). However, the decrease in Aβ1-40 level (in pg/ml) after 18 h of Gal treatment was concentration-dependent: from 119.05 ± 2.01 to 95.25 ± 1.16 for 0.3 μM, 89.14 ± 2.96 for 0.9 μM, and 81.06 ± 2.28 for 10 μM Gal (Fig. 1A, $p<0.001$). Also Aβ1-42 level decreased similarly: from 38.35 ± 0.48 to 32.10 ± 0.87 for 0.3 μM, 28.07 ± 1.05 for 0.9 μM, and 24.18 ± 0.24 for 10 μM (Fig. 1B, $p<0.001$).

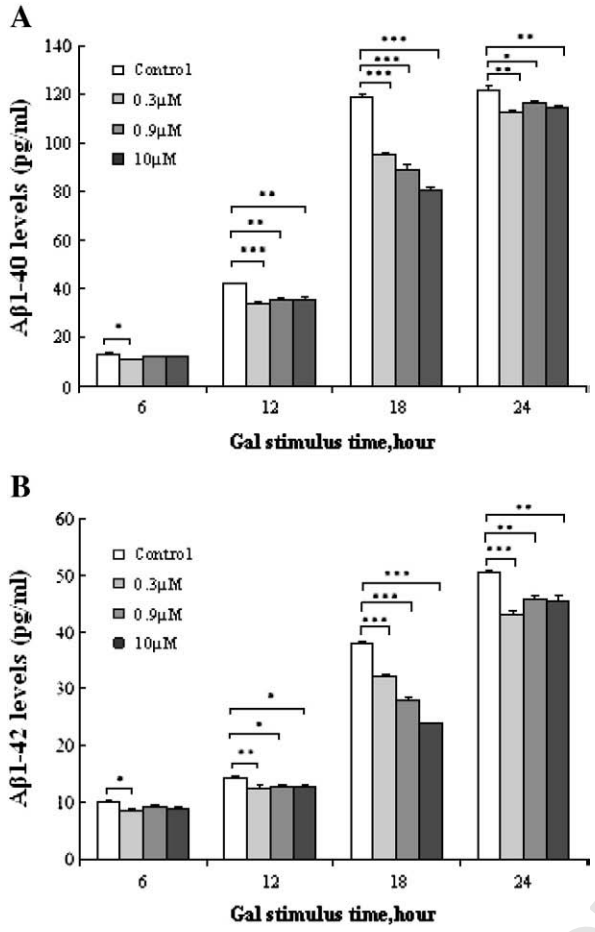
3.2. Effect of Gal on BACE1 and cellular APP expression

BACE1, which plays an essential role in Aβ generation, was monitored. Gal (0.3 μM) for 6, 12, 18, and 24 h significantly decreased the expression of BACE1. However, further increase in the concentration of Gal increased BACE1 expression. Gal was maximally effective at a concentration of 0.3 μM after 18 h of treatment, which resulted in a 44% decrease in BACE1 level compared with the control level (Fig. 2, $p<0.001$). Western blot analysis of the effect of Gal (after treatment for 6, 12, 18, and 24 h) on cellular APP levels found no effect on APP steady-state levels (Fig. 3).

3.3. Effect of Gal stimulation on sAPPα release

Western blotting was used to determine the amount of sAPPα released in serum-free medium by differentiated SH-SY5Y cells exposed to Gal (0–10 μM) for 18 h. The effect of Gal on the release of sAPPα proved to be concentration-dependent, with the maximal effect (2.4-fold increase above control level) observed at a concentration of 10 μM (Fig. 4, $p<0.001$).

Please cite this article as: Li, Q., et al., Effects of galantamine on β-amyloid release and beta-site cleaving enzyme 1 expression in differentiated human neuroblastoma SH-SY..., Exp. Gerontol. (2010), doi:10.1016/j.exger.2010.06.008



Q6 **Fig. 1.** Galantamine decreased Aβ release from differentiated SH-SY5Y cells. Cells were incubated with Gal (0, 0.3, 0.9, or 10 μM) for 6, 12, 18, and 24 h. Aβ1-40 and Aβ1-42 levels in the medium were assayed using an Aβ1-40 and Aβ1-42 sandwich ELISA. The results are expressed in pg/ml. Gal dose-dependently decreased (A) Aβ1-40 release and (B) Aβ1-42 release after 18 h of treatment. Results are shown as the mean ± SEM for each condition and tested for statistical significance using repeated-measures one-way ANOVA with post-hoc LSD test (n = 4 for each condition). *p<0.05 ,**p<0.01, ***p<0.001, compared with control.

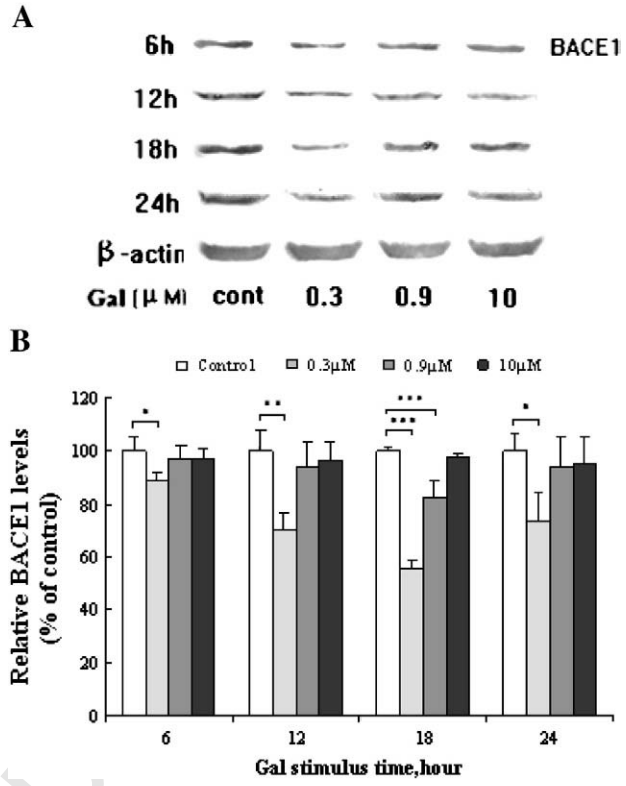


Fig. 2. Galantamine decreased BACE1 level in spent medium from differentiated SH-SY5Y cells. Cells were incubated with Gal (0, 0.3, 0.9, or 10 μM) for 6, 12, 18, and 24 h. BACE1 level was detected by polyclonal antibody using Western blot. (A) Representative Western blot showing BACE1 bands (~68 kDa). (B) Densitometric analysis of BACE1 protein level normalized to β-actin level. Results are shown as the mean ± SEM for each condition and tested for statistical significance using repeated-measures one-way ANOVA with post-hoc LSD test (n = 4 for each condition). *p<0.05 ,**p<0.01, ***p<0.001, compared with control.

3.4. Pharmacological characterization of the cholinergic effect of Gal

To determine whether Gal decreases Aβ or BACE1 level through receptor mediated mechanisms, differentiated SH-SY5Y cells were exposed to 0.3 μM Gal in the presence or absence of 10 nM atropine or 10 nM MLA. Treatment with these inhibitors alone had no effect on BACE1 expression (Fig. 5, Table 1) or Aβ1-40 and Aβ1-42 secretion (Table 1). Treatment (18 h, 0.3 μM) with Gal reduced the level (in pg/ml) of Aβ1-40 from 119.56 ± 2.22 to 97.00 ± 3.25 ($p<0.001$) and of Aβ1-42 from 38.49 ± 1.46 to 32.38 ± 0.72 ($p<0.001$). Addition of MLA reversed this effect, restoring the Aβ1-40 level to 116.74 ± 3.98 pg/ml ($p<0.05$) and the Aβ1-42 level to 37.24 ± 1.34 pg/ml ($p<0.001$). Pre-treatment with 10 nM atropine had no effect on the Gal-induced reduction in Aβ1-40 or Aβ1-42 level, which was 95.93 ± 3.15 pg/ml and 32.10 ± 0.64 pg/ml, respectively. (Table 1).

Similarly, 10 nM MLA inhibited the Gal-induced decrease in BACE1 expression by 82.7% ($p<0.001$), while 10 nM atropine had no effect on this decrease (Fig. 5, Table 1).

3.5. Signal transduction molecules involved in Gal-induced decrease in BACE1 expression and Aβ release

To identify the signal transduction pathways involved in this effect on BACE1 expression and Aβ secretion, several protein kinase inhibitors were added prior to Gal (0.3 μM) treatment. Treatment with these inhibitors alone had no effect on BACE1 expression (Fig. 5, Table 1) or Aβ 1-40 and Aβ1-42 secretion (Table 1), compared to control levels. Pre-treatment with PKC inhibitor GF109203X (2.5 μM), MEK inhibitor PD98059 (30 μM), p38MAPK inhibitor SB203580 (30 μM), however, prevented Gal-induced decrease in Aβ1-40 and Aβ1-42 secretion, raising Aβ1-40 level (in pg/ml) to 102.30 ± 3.37($p<0.05$), 113.92 ± 1.03 ($p<0.001$), and 113.54 ± 1.14($p<0.001$), respectively and Aβ1-42 level to 34.74 ± 0.87 ($p<0.05$), 36.68 ± 0.48 ($p<0.001$), and 36.40 ± 1.27 ($p<0.001$), respectively. Pre-treatment with PI3-K specific inhibitor LY294002 (10 μM) and the JNK pathway inhibitor SP 600125 (20 μM), on the other hand, failed to prevent either the Gal-induced decrease in level of Aβ1-40 (which was 96.23 ± 2.74 pg/ml in the presence of LY + Gal and 84.44 ± 1.35 pg/ml in the presence of SP + Gal) or Aβ1-42 (which was 33.90 ± 1.58 pg/ml in the presence of LY + Gal and 27.24 ± 0.24 pg/ml in the presence of SP + Gal) (Table 1).

These inhibitors had similar effects on Gal-induced changes in BACE1 expression. The effect of Gal on BACE1 was reduced approximately 54.1%, 59.7%, and 34.2%, respectively, by pre-treatment with GF109203X (2.5 μM), PD98059 (30 μM), and SB203580 (30 μM), compared to Gal-treated cultures without inhibitors ($p<0.001$). However, LY29400202 and SP600125 failed to modulate Gal-induced changes (Fig. 5, Table 1).

4. Discussion

AChEIs are mainly used for the treatment of AD (Giacobini, 2000). The clinical efficacy has been related to the ability of the drug to inhibit acetylcholinesterase activity, thus preventing the hydrolysis of

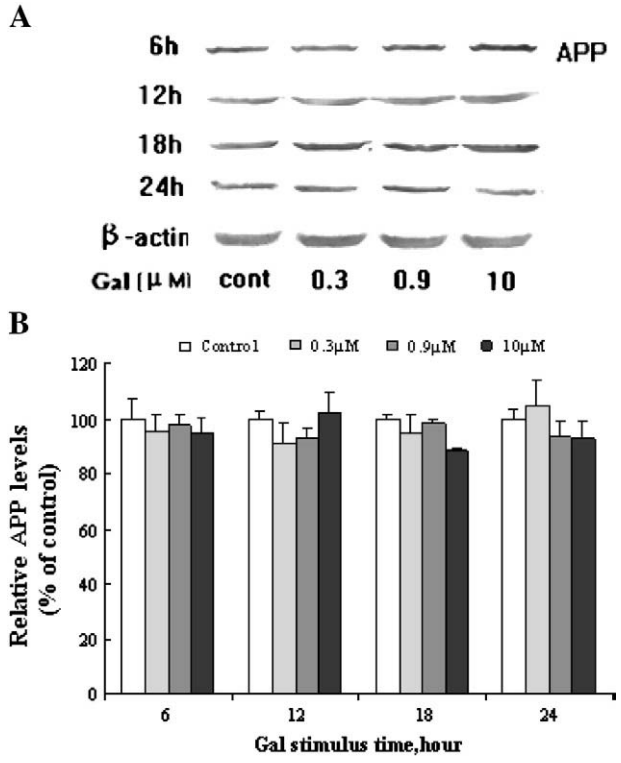


Fig. 3. Galantamine had no effect on the steady-state levels of APP in differentiated SH-SY5Y cells. Cells were incubated with Gal (0, 0.3, 0.9, or 10 μM) for 6, 12, 18, and 24 h. Full-length APP in the cell lysates were detected by APP polyclonal antibody. (A) Representative Western blot showing APP bands (~125 kDa). (B) Densitometric analysis of APP protein levels normalized to β-actin level. Results are shown as the mean ± SEM for each condition and tested for statistical significance using repeated-measures one-way ANOVA with post-hoc LSD test (n = 4 for each condition).

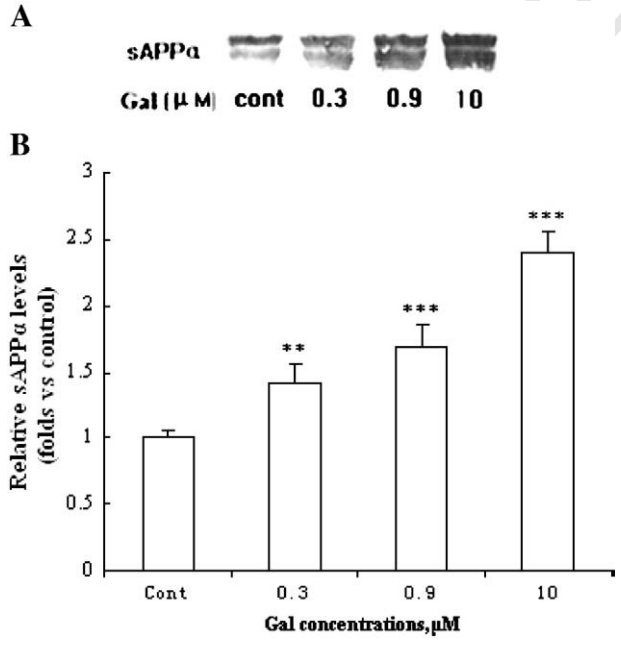


Fig. 4. Galantamine stimulated sAPPα release from differentiated SH-SY5Y cells. Cells were incubated with Gal (0, 0.3, 0.9 or 10 μM) for 18 h. sAPPα was detected in spent medium with anti-sAPPα monoclonal antibody using Western blot. (A) Representative Western blot showing sAPPα bands (110~120 kDa). (B) Densitometric analysis of sAPPα protein levels. Results are shown as the mean ± SEM for each condition and tested for statistical significance using repeated-measures one-way ANOVA with post-hoc LSD test (n = 4 for each condition). **p<0.01, ***p<0.001, compared with control.

released acetylcholine, increasing the efficiency of cholinergic transmission, and reducing memory and cognitive impairment. In addition to improving disease symptoms, AChEIs may impact biochemical pathways involved in APP processing. Lenzken et al. (2007) demonstrated that Gal (10 μM) for 2 h promoted a strong increase (on average three times baseline value) in sAPPα release. Peng et al. (2007) showed that the acetylcholinesterase inhibitor, huperzine A (Hup A), also increased sAPPα secretion and that this increase involved ADAM10 and tumor necrosis factor (TNF)-alpha convertase (TACE)/ADAM17. However, the effect of Gal on Aβ level and BACE1 expression is still unknown.

Our study demonstrated a Gal-induced decrease in Aβ levels in differentiated SH-SY5Y cells, but the precise mechanism is still unclear. Three mechanisms are possible: (i) decreased synthesis of APP after Gal treatment; (ii) Gal stimulation of the non-amyloidogenic pathway resulting in decrease of the APP substrate available for β-secretase cleavage; (iii) Gal-induced decrease in β-secretase activity. Consequently, we investigated the effect of Gal on the expression of APP, BACE1, and sAPPα.

In agreement with a previous study (Lenzken et al., 2007), we found that Gal had no effect on the steady-state levels of APP, indicating that Gal-inhibited secretion of Aβ was due to the reduced cleavage of APP via the β-secretase pathway rather than decreased synthesis of APP. We also observed a concentration-dependent effect of Gal (in the range, 0–10 μM) on both BACE1 expression and Aβ secretion after 18 h of exposure. The effect of Gal on BACE1 expression was maximal at 0.3 μM, but Aβ secretion was decreased further at higher Gal concentration, which suggested that at these higher concentrations (0.9 and 10 μM) Gal was acting on Aβ level through a mechanism other than decreasing β-secretase activity. Therefore, higher concentrations (0–10 μM) of Gal were used in subsequent experiments on sAPPα level.

Interestingly, treatment with Gal (0.9 and 10 μM) for 18 h was found to stimulate sAPPα release, which suggests that Gal in higher concentration can shift APP processing towards the non-amyloidogenic pathway and thereby possibly decrease Aβ generation. Overall, it appears that the effect of Gal on Aβ production is complex and concentration-dependent. We speculate that Gal at lower concentration affects Aβ production by inhibiting BACE1, while at higher concentration it mainly reduces Aβ production via the non-amyloidogenic pathway and thereby reduces the availability of APP substrate for β-secretase cleavage.

It is worth mentioning that the effective concentration of galantamine for significantly decreasing levels of BACE1 and Aβ in our experiment (0.3 μM) differs from its IC50 (1.85–16 μM) for blocking AChE (Arias et al., 2004; Lenzken et al., 2007; Thomsen et al, 1991). It Q1 therefore seems that the galantamine activities reported here are not directly related to AChE inhibition and may be mediated in a receptor-dependent manner. Several studies have demonstrated the involvement of the cholinergic receptor in APP processing. However the main focus has been on the role of the cholinergic receptor in the non-amyloidogenic pathway of APP processing (Lenzken et al., 2007; Peng et al., 2007; Zimmermann et al., 2004). Only a few studies have investigated the involvement of nAChRs in the regulation of Aβ release or BACE1 expression. Srivareerat et al. (2009) indicated that 6 weeks of nicotine treatment reduced the levels of Abeta (1–40) and BACE1 peptides in the hippocampal area CA1 and prevented Abeta-induced impairment of learning and short-term memory in AD rats. Hellstrom-Lindahl et al. (2004) also found that long- and short-term nicotine treatment significantly reduces the amount of insoluble Aβ1-40 and Aβ1-42 in brains from APPsw mice. This reduction might be, in part, mediated via the α-7 nicotinic receptor. However, nicotine has a negative effect on both α- and β-secretase activities. Since data from Lenzken et al. (2007) show that Gal modulates non-amyloidogenic Q2 processing of APP in a fully receptor-dependent manner (mainly through α7nAChR), we herein investigated whether the action of Gal on amyloidogenic processing of APP involved the participation of

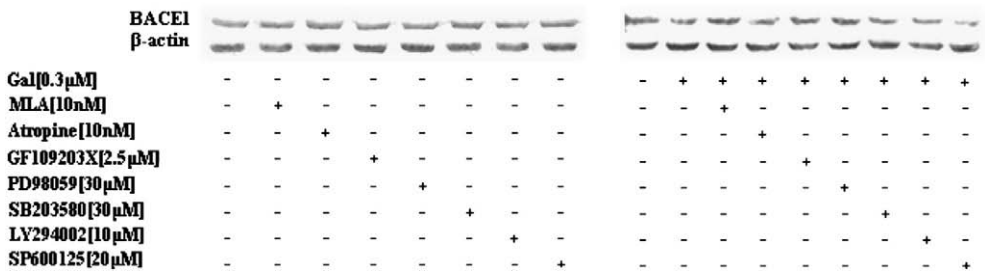


Fig. 5. Involvement of cholinergic receptors and specific signal transduction molecules in galantamine-reduced BACE1 expression. Cells were treated or not with 10 nM methyllycaconitine citrate hydrate (MLA), 10 nM atropine , 2.5 μM GF109203X, 30 μM PD98059, 30 μM SB203580, 10 μM LY 294002 or 20 μM SP600125 in the presence or absence of 0.3 μM Gal for 18 h . Inhibitors were added 30 min prior to Gal treatment. BACE1 levels were detected by polyclonal antibody using Western blot .The figure shows representative blots for BACE1, out of four independent experiments.

α7nAChR. We found that 10 nM MLA largely prevented both Gal-inhibited Aβ release and BACE1 expression, suggesting that Gal-modulation of BACE1 expression and Aβ release involved α7nAChR. These evidences further support our proposal that Gal-induced modulation of the amyloidogenic processing of APP involves α7nAChR rather than cholinergic enhancement due to AChE inhibition.

Several publications argued that atropine (10 μM) may also affect nicotinic receptors and thereby lead to overestimates of the importance of muscarinic receptors (Efthimiopoulos et al., 1996; Racchi et al., 2001; Mazzucchelli et al., 2003). Therefore, we used 10 nM atropine, a concentration putatively blocking only muscarinic receptors. We observed no blocking effect of atropine on either Gal-modulated BACE1 expression or Aβ release. Considering the evidence provided by Lenzken et al. (2007) that 10 nM atropine extensively blocks (60%) the effect of carbachol (100 μM), a non-specific cholinergic receptor agonist, and not that of Gal, we hypothesized that muscarinic receptors were not involved in the action of Gal described here.

To investigate the role of various second messengers in the Gal-modulated Aβ release and BACE1 expression, cells were treated with inhibitors shown to be involved in APP processing (i.e., PKC, MAPK, PI3K inhibitors) before treatment with Gal (Mazzucchelli et al., 2003; Solano et al., 2000; Yogev-Falach et al., 2002). PKC inhibitors and various MAP kinase inhibitors partially reversed the effect of Gal on Aβ release, suggesting the involvement of the PKC pathway and p38 MAPK and MEK in Gal-modulated Aβ release. However, the failure of LY294002 or SP600125 pre-treatment to block the effect of Gal seemed to exclude a role for the PI3K and JNK pathways. The effect of inhibitor pre-treatment on BACE1 expression was similar, which supported our proposal that Gal (0.3 μM) reduces Aβ production through BACE1 reduction. However, details of the activation of these signaling molecules and their relationship to α7nAChR deserves further investigation.

In conclusion, our data indicate that Gal inhibited both Aβ generation and BACE1 expression and that this effect involves α7nAChR and several signal transduction molecules such as PKC, p38MAPK, and MEK.

Table 1
Involvement of cholinergic receptors and specific signal transduction molecules in the galantamine-inhibited BACE1 expression and Aβ release from differentiated SH-SY5Y cells.

Treatment	BACE1 levels (% of control)	Aβ1-40 levels (pg/ml)	Aβ1-42 levels (pg/ml)
Treatment 1[a]			
Control	100.00 ± 2.09	110.80 ± 2.84	40.43 ± 2.37
MLA	98.35 ± 0.45	109.61 ± 3.22	39.88 ± 2.73
Atropine	101.75 ± 2.37	109.82 ± 2.93	40.15 ± 2.51
GF109203X	96.80 ± 3.03	109.52 ± 3.08	40.01 ± 2.51
PD98059	97.07 ± 4.87	109.78 ± 3.52	39.18 ± 1.73
SB203580	99.80 ± 3.78	110.46 ± 2.55	40.01 ± 1.27
LY294002	95.10 ± 2.70	110.33 ± 3.20	40.57 ± 1.58
SP600125	95.66 ± 3.19	106.19 ± 3.90	38.90 ± 1.58
Treatment 2[b]			
Control	100.00 ± 5.13	119.56 ± 2.22	38.49 ± 1.46
Gal	48.54 ± 2.12***	97.00 ± 3.25***	32.38 ± 0.72***
MLA + Gal	88.70 ± 0.56**$^{\Delta\Delta\Delta}$	116.74 ± 3.98$^{\Delta\Delta\Delta}$	37.24 ± 1.34$^{\Delta\Delta\Delta}$
Atropine + Gal	48.79 ± 2.06***	95.93 ± 3.15***	32.10 ± 0.64***
GF109203X + Gal	74.82 ± 2.96***$^{\Delta\Delta\Delta}$	102.30 ± 3.37***$^{\Delta}$	34.74 ± 0.87***$^{\Delta}$
PD98059 + Gal	77.50 ± 3.41***$^{\Delta\Delta\Delta}$	113.92 ± 1.03*$^{\Delta\Delta\Delta}$	36.68 ± 0.48$^{\Delta\Delta\Delta}$
SB203580 + Gal	65.14 ± 6.91***$^{\Delta\Delta\Delta}$	113.54 ± 1.14*$^{\Delta\Delta\Delta}$	36.40 ± 1.27*$^{\Delta\Delta\Delta}$
LY294002 + Gal	48.27 ± 0.70***	96.23 ± 2.74***	33.90 ± 1.58***
SP600125 + Gal	46.94 ± 3.63***	84.44 ± 1.35***$^{\Delta\Delta\Delta}$	27.24 ± 0.24***$^{\Delta\Delta\Delta}$

MLA, methyllycaconitine citrate hydrate; Gal, galantamine.

a. Cells were treated separately in serum-free medium with these inhibitors or not for 18 h. b. Cells were treated for 18 h with Gal (0.3 μM) or not in the presence or absence of these inhibitors, which were added 30 min prior to Gal treatment .Each value shows the mean ± SEM of four cultures.*p<0.05, **p<0.01,***p<0.001, compared with control; $^{\Delta}$p<0.05,$^{\Delta\Delta\Delta}$ p<0.001, compared with Gal alone treatment group.

Acknowledgements

This research was supported by the Natural Science Foundation of Heilongjiang Province of China (NO D200629) and the Scientific Research Fund of Heilongjiang Provincial Education Department(NO 11531158).

References

Adem, A., Mattsson, M.E., Nordberg, A., Pahlman, S., 1987. Muscarinic receptors in human SH-SY5Y neuroblastoma cell line: regulation by phorbol ester and retinoic acid-induced differentiation. Brain Res. 430 (2), 235–242.

Arias, E., Ales, E., Gabilan, N.H., Cano-Abad, M.F., Villarroya, M., Garcia, A.G., Lopez, M.G., 2004. GLA prevents apoptosis induced by beta-amyloid and thapsigargin: involvement of nicotinic acetylcholine receptors. Neuropharmacology 46 (1), 103–114.

Buxbaum, J.D., Liu, K.N., Luo, Y., Slack, J.L., Stocking, K.L., Peschon, J.J., Johnson, R.S., Castner, B.J., Cerretti, D.P., Black, R.A., 1998. Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor. J. Biol. Chem. 273 (43), 27765–27767.

Efthimiopoulos, S., Vassilacopoulou, D., Ripellino, J.A., Tezapsidis, N., Robakis, N.K., 1996. Cholinergic agonists stimulate secretion of soluble full-length amyloid precursor protein in neuroendocrine cells. Proc. Natl. Acad. Sci. USA 93 (15), 8046–8050.

Esch, F.S., Keim, P.S., Beattie, E.C., Blacher, R.W., Culwell, A.R., Oltersdorf, T., McClure, D., Ward, P.J., 1990. Cleavage of amyloid beta peptide during constitutive processing of its precursor. Science 248 (4959), 1122–1124.

Giacobini, E., 2000. Present and future of Alzheimer therapy. J. Neural Transm. Suppl. 59, 231–242.

Gould, J., Reeve, H.L., Vaughan, P.F., Peers, C., 1992. Nicotinic acetylcholine receptors in human neuroblastoma (SH-SY5Y) cells. Neurosci. Lett. 145 (2), 201–204.

Haass, C., Schlossmacher, M.G., Hung, A.Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B.L., Lieberburg, I., Koo, E.H., Schenk, D., Teplow, D.B., et al., 1992. Amyloid beta-peptide is produced by cultured cells during normal metabolism. Nature 359 (6393), 322–325.

Halvorsen, S.W., Jiang, N., Malek, R., 1995. Regulation of nicotinic acetylcholine receptors on human neuroblastoma cells during differentiation. Biochem. Pharmacol. 50 (10), 1665–1671.

Hellstrom-Lindahl, E., Court, J., Keverne, J., Svedberg, M., Lee, M., Marutle, A., Thomas, A., Perry, E., Bednar, I., Nordberg, A., 2004. Nicotine reduces A beta in the brain and cerebral vessels of APPsw mice. Eur. J. Neurosci. 19 (10), 2703–2710.

Lammich, S., Kojro, E., Postina, R., Gilbert, S., Pfeiffer, R., Jasionowski, M., Haass, C., Fahrenholz, F., 1999. Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. Proc. Natl. Acad. Sci. USA 96 (7), 3922–3927.

Lenzken, S.C., Lanni, C., Govoni, S., Lucchelli, A., Schettini, G., Racchi, M., 2007. Nicotinic component of galantamine in the regulation of amyloid precursor protein processing. Chem. Biol. Interact. 165 (2), 138–145.

Maelicke, A., Samochocki, M., Jostock, R., Fehrenbacher, A., Ludwig, J., Albuquerque, E.X., Zerlin, M., 2001. Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer's disease. Biol. Psychiatry 49 (3), 279–288.

Mazzucchelli, M., Porrello, E., Villetti, G., Pietra, C., Govoni, S., Racchi, M., 2003. Characterization of the effect of ganstigmine (CHF2819) on amyloid precursor protein metabolism in SH-SY5Y neuroblastoma cells. J. Neural Transm. 110 (8), 935–947.

Nunan, J., Small, D.H., 2000. Regulation of APP cleavage by alpha-, beta- and gamma-secretases. FEBS Lett. 483 (1), 6–10.

Peng, Y., Lee, D.Y., Jiang, L., Ma, Z., Schachter, S.C., Lemere, C.A., 2007. Huperzine A regulates amyloid precursor protein processing via protein kinase C and mitogen-activated protein kinase pathways in neuroblastoma SK-N-SH cells over-expressing wild type human amyloid precursor protein 695. Neuroscience 150 (2), 386–395.

Postina, R., Schroeder, A., Dewachter, I., Bohl, J., Schmitt, U., Kojro, E., Prinzen, C., Endres, K., Hiemke, C., Blessing, M., Flamez, P., Dequenne, A., Godaux, E., van Leuven, F., Fahrenholz, F., 2004. A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. J. Clin. Invest. 113 (10), 1456–1464.

Racchi, M., Sironi, M., Caprera, A., Konig, G., Govoni, S., 2001. Short- and long-term effect of acetylcholinesterase inhibition on the expression and metabolism of the amyloid precursor protein. Mol. Psychiatry 6 (5), 520–528.

Raskind, M.A., Peskind, E.R., Wessel, T., Yuan, W., 2000. Galantamine in AD: A 6-month randomized, placebo-controlled trial with a 6-month extension. The Galantamine USA-1 Study Group. Neurology 54 (12), 2261–2268.

Selkoe, D.J., 1994. Normal and abnormal biology of the beta-amyloid precursor protein. Annu. Rev. Neurosci. 17, 489–517.

Shoji, M., Golde, T.E., Ghiso, J., Cheung, T.T., Estus, S., Shaffer, L.M., Cai, X.D., McKay, D.M., Tintner, R., Frangione, B., et al., 1992. Production of the Alzheimer amyloid beta protein by normal proteolytic processing. Science 258 (5079), 126–129.

Sinha, S., Anderson, J.P., Barbour, R., Basi, G.S., Caccavello, R., Davis, D., Doan, M., Dovey, H.F., Frigon, N., Hong, J., Jacobson-Croak, K., Jewett, N., Keim, P., Knops, J., Lieberburg, I., Power, M., Tan, H., Tatsuno, G., Tung, J., Schenk, D., Seubert, P., Suomensaari, S.M., Wang, S., Walker, D., Zhao, J., McConlogue, L., John, V., 1999. Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature 402 (6761), 537–540.

Sisodia, S.S., Koo, E.H., Beyreuther, K., Unterbeck, A., Price, D.L., 1990. Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing. Science 248 (4954), 492–495.

Solano, D.C., Sironi, M., Bonfini, C., Solerte, S.B., Govoni, S., Racchi, M., 2000. Insulin regulates soluble amyloid precursor protein release via phosphatidyl inositol 3 kinase-dependent pathway. FASEB J. 14 (7), 1015–1022.

Srivareerat, M., Tran, T.T., Salim, S., Aleisa, A.M., Alkadhi, K.A., 2009. Chronic nicotine restores normal Abeta levels and prevents short-term memory and E-LTP impairment in Abeta rat model of Alzheimer's disease. Neurobiol. Aging. Q5

Tariot, P.N., Solomon, P.R., Morris, J.C., Kershaw, P., Lilienfeld, S., Ding, C., 2000. A 5-month, randomized, placebo-controlled trial of galantamine in AD. The Galantamine USA-10 Study Group. Neurology 54 (12), 2269–2276.

Texido, L., Ros, E., Martin-Satue, M., Lopez, S., Aleu, J., Marsal, J., Solsona, C., 2005. Effect of galantamine on the human alpha7 neuronal nicotinic acetylcholine receptor, the Torpedo nicotinic acetylcholine receptor and spontaneous cholinergic synaptic activity. Br. J. Pharmacol. 145 (5), 672–678.

Thomsen, T., Kaden, B., Fischer, J.P.B., Bickel, U., Barz, H., CervosNavarro, J., Kewitz, H., 1991. Inhibition of acetylcholinesterase activity in human brain tissue and erythrocytes by galantamine, physostigmine and tacrine. Eur. J. Chem. Clin. Biochem. 29, 487492.

Vassar, R., Bennett, B.D., Babu-Khan, S., Kahn, S., Mendiaz, E.A., Denis, P., Teplow, D.B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edenson, S., Lile, J., Jarosinski, M.A., Biere, A.L., Curran, E., Burgess, T., Louis, J.C., Collins, F., Treanor, J., Rogers, G., Citron, M., 1999. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286 (5440), 735–741.

Yogev-Falach, M., Amit, T., Bar-Am, O., Weinstock, M., Youdim, M.B., 2002. Involvement of MAP kinase in the regulation of amyloid precursor protein processing by novel cholinesterase inhibitors derived from rasagiline. FASEB J. 16 (12), 1674–1676.

Zimmermann, M., Gardoni, F., Marcello, E., Colciaghi, F., Borroni, B., Padovani, A., Cattabeni, F., Di Luca, M., 2004. Acetylcholinesterase inhibitors increase ADAM10 activity by promoting its trafficking in neuroblastoma cell lines. J. Neurochem. 90 (6), 1489–1499.

Please cite this article as: Li, Q., et al., Effects of galantamine on β-amyloid release and beta-site cleaving enzyme 1 expression in differentiated human neuroblastoma SH-SY..., Exp. Gerontol. (2010), doi:10.1016/j.exger.2010.06.008