Neurochem Res (2011) 36:904–912
DOI 10.1007/s11064-011-0420-7

ORIGINAL PAPER

Nicotine Decreases Beta-Amyloid Through Regulating BACE1 Transcription in SH-EP1-α4β2 nAChR-APP695 Cells

Hui-Zhen Nie · Zuo-Qing Li · Qi-Xin Yan · Ze-Jian Wang · Wen-Juan Zhao · Ling-Chen Guo · Ming Yin

Accepted: 3 February 2011 / Published online: 19 February 2011

Abstract Alzheimer's disease (AD) is a neurodegenerative disorder that affects the elderly population. Deposition of beta-amyloid (Aβ) in the brain is a hallmark of AD pathology. In our previous study, we have constructed a cell line expressing human APP695 (hAPP695) in SH-EP1 cells stably transfected with human nicotinic receptor (nAChR) α4 subunit and β2 subunit gene. In present study, we found that activation of α4β2 nAChR by nicotine and epibatidine decreased secreted Aβ level in the cell line and hippocampal neurons, but had no effects on full-length APP695 and sAPP-α. Nicotine also decreases BACE1 and PSEN1 expression, as well as ERK1 and NFκB P65 subunit expression in the cell line. Furthermore, BACE1 promoter activity is, but PSEN1 not, decreased by nicotine in the cell line. All the results suggest that activation of α4β2 nAChR decreases Aβ through regulating BACE1 transcription by ERK1-NFκB pathway. Additionally, analysis of BACE1 promoter activity by dual-luciferase reporter assay may be useful for drug screening as a high throughput method.

Keywords Alzheimer's disease · Beta-amyloid · BACE1 · nAChR

H.-Z. Nie · Z.-J. Wang · W.-J. Zhao · L.-C. Guo · M. Yin (✉)
Laboratory of Neuropharmacology, School of Pharmacy, Shanghai Jiao Tong University, 200240 Shanghai, China
e-mail: myin@sjtu.edu.cn

Z.-Q. Li
Department of Pathophysiology, Key Laboratory of Cell Differentiation and Apoptosis of Chinese Ministry of Education, SJTU-SM, 200025 Shanghai, China

Q.-X. Yan
Shenzhen Neptunus Bioengineering Holdings Co., LTD, 518057 Shenzhen, China

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that affects the elderly population and is characterized clinically by progressive loss of memory and decline of multiple cognitive abilities [1]. Beta-amyloid (Aβ) accumulation is considered to play a central role in AD pathogenesis by causing synaptic damage, neuritic alteration, glial activation and neuronal death [2].

Aβ is a hydrophobic peptide usually of 40 or 42 residues in length and prone to aggregation [3]. It is produced by sequential proteolysis of the amyloid precursor protein (APP) by β- and γ-secretases, and cleavage of APP by α-secretase generates sAPP-α, which is thought to have neuroprotective effects by modulation of neuronal excitability, synaptic plasticity, neurite outgrowth, synaptogenesis, and cell survival [4].

β-site APP cleaving enzyme 1 (BACE1) is the primary transmembrane aspartyl protease responsible for β-secretase activity in the brain and carries out the first cleavage step leading to Aβ production [5, 6]. This cleavage of APP is an essential step in the generation of the potentially neurotoxic and amyloidogenic $A\beta_{42}$ peptides in AD. BACE1 and its activity are increased in the brains of sporadic and familial AD cases, and in various transgenic models of AD [7–9]. A more recent study has shown that BACE1 elevation occurred predominantly within neurons surrounding plaques in both AD and animal models of AD [10]. Presenilin proteins are the catalytic center of the γ-secretase complex [2]. The C-terminus of Aβ is generated by γ-secretase. Presenilin 1 (or PSEN1), and presenilin 2 (PSEN2) have been linked to early-onset familial AD (FAD) [11].

Reducing Aβ production may be important measure for prevention and treatment of AD. Great efforts have been

made to discover drugs against AD, for example cholinesterase inhibitor. To date, however, AD treatments only provide modest symptomatic relief. Further work aiming at reducing Aβ by different ways is needed.

Nicotinic acetylcholine receptors (nAChR) have been a target for drug discovery efforts, primarily for CNS indications, for the past two decades [12]. There is emerging evidence that nicotine improves cognitive function in human beings and nonhuman species and this has sparked interest in the development of novel nicotinic treatments for cognitive dysfunction [13]. Neuronal nAChR are ligand-gated ion channels consisting of α and β subunits. To date 12 neuronal nAChR subunits (α2–α10 and β2–β4) have been described and they are differentially expressed throughout the nervous system [14]. $\alpha4\beta2$ nAChR subtype is the most widely spread subtype in brain. The examination of selective $\alpha4\beta2$ nAChR agonists suggests that this receptor mediates improvement in attention, learning and working memory [15]. It is also known that loss of frontal cortical nAChR is correlated to declining memory function in AD, and frontal cortical $\alpha4\beta2$ nAChR is involved in working memory. Both patients with AD and mild cognitive impairment showed significant reductions in $\alpha4\beta2$ nAChR in brain regions typically affected by AD pathology.

Biochemical researches have shown that receptor activation induced by neurotransmitters affects protein expression including the ones involved in APP procession [16]. In our previous study [17], we constructed a cell line by transfecting human APP695 gene into SH-EP1 cells which have been transfected with human nAChR α4 subunit and β2 subunit genes. Now, we attempt to explore the effects and mechanism by which $\alpha4\beta2$ nAChR activation affects Aβ production in the cell line. Alteration in secretase promoter activity induced by $\alpha4\beta2$ nAChR activation was also detected.

Experimental Procedures

Materials

Nicotine and dihydro-β-erythroidine (DHβE) were purchased from Sigma-Aldrich (St. Louis, MO). Lipofectamine reagent was purchased from Invitrogen (California, USA). M-MLV reverse transcriptase and SYBR Green mixture were purchased from Takara (Shiga, Japan). pGL3-BASIC, pGL3-SV40 and pRL-CMV vectors were purchased from Promega (Madison, USA). Bgl II and Hind III DNAase were purchased from Fermentas (MD, USA). The antibodies used for immunoblotting included the following: APP (22C11, 1:2,000, Millipore, MA, USA) and sAPP-α (6E10, 1:2,000, Millipore, MA, USA), anti-PSEN1 C-term antibody and anti-BACE1 N-term antibody (1:1,000, Abgent, CA, USA), ERK1 and NFκB P65 (1:2,000, Cell Signaling Technologies, MA, USA), GAPDH (6C5, 1:1,000, Beyotime institute of Biotechnology, China). Human A$\beta_{40/42}$ ELISA kits were purchased from Biosource (Victoria, Australia) and rat A$\beta_{40/42}$ ELISA kits were purchased from Wako (Osaka, Japan).

Cell Culture

The constructed SH-EP1-$\alpha4\beta2$nAChR-APP695 cell line was cultured in DMEM (Invitrogen, CA, USA), supplemented with 5% fetal bovine serum, 10% horse serum (Invitrogen, CA, USA), zeocin (InvivoGen, CA, USA) to a final concentration of 200 μg/ml, hygromycin B (CalBiochem, CA, USA) to a final concentration of 130 μg/ml, and with neomycin(CalBiochem, CA, USA) to a final concentration of 500 μg/ml to actively select for transfectants. Cells were maintained at 37°C in a 5% CO_2 incubator.

Primary hippocampal neurons were prepared as described previously [18]. Briefly, the pregnant Sprague–Dawley rats on embryonic day 18 (E18) were deeply anesthetized with isofluorane and the fetal hippocampi were dissected, pooled together, triturated into a single cell suspension, and finally plated on a 60 mm^2 culture dish coated with polylysine in primary culture medium Dulbecco's modified essential medium (DMEM) containing 10% heat-inactivated defined fetal bovine serum (FBS), 10% heat-inactivated horse serum, 1% penicillin-streptomycin, and 20 mM D-glucose (all from Gibco). 12 h later, the medium was replaced by Neurobasal/B27 medium (Invitrogen). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 and medium was replaced every 3 days. Neurons were treated after 10 days for 24 h.

RNA Extraction/Purification

Total cytoplasmic RNA was isolated from cells growing at approximately 80% conlfuence in a 35-mm culture dish with 2 ml of Trizol™ reagent. RNA was extracted according to the manufacturer's protocol. Briefly, cells were lysed in Trizol™ reagent and then the protein was precipitated by adding chloroform. RNA was isolated by precipitation with isopropanol. RNA pellet was washed in 70% ethanol dissolved in diethylpyrocarbonate, air dried and dissolved in water. The quantity and quality of RNA were determined by optical density measurements at 260 and 280 nm. The integrity of RNA was also confirmed by visual inspection of 28 and 18 s rRNA on agarose gels. For real-time PCR experiments, the RNA was treated with DNase I (Fermentas, MD, USA) to avoid genomic DNA contamination. Typically, 20 μg of RNA was incubated with 2 units of DNase I in a 25 μl reaction at room

temperature for 30 min, and then the mixture was incubated by addition of 2.5 μl of 25 mM EDTA at 65°C for 10 min.

cDNA Generation for Real-Time PCR

Two micrograms of RNA was used for each reaction, together with 1 μl of 10 pmol/μl Oligo dT primers, 4 μl 5× reaction buffer, and 2 μl of 10 mM dNTP mixture. The mixture was incubated at 65°C for 5 min, then centrifuged and 1 μl RNase inhibitor (10 U/μl) (Fermentas, MD, USA) and 1 μl M-MLV reverse transcriptase added. The reaction was incubated at 37°C for 1 h 30 min, followed by 5 min at 95°C.

Real-Time PCR

Gene-specific primers, designed using Primer Premier 5.0 software, were 5′-agcacaacgacagacggag -3′ (forward) and 5′-agtcacagggacaaagagcat -3′ (reverse)for PSEN1, 5′-gg cgggagtggtattatga -3′ (forward) and 5′-gctgccttgatggatt tga -3′(reverse) for BACE1, and 5′-aaatcccatcaccatcttcc-3′ (forward) and 5′-atgacccttttggctccc -3′ (reverse) for glyceraldehydes 3-phosphate dehydrogenase (GAPDH). The real-time PCR was performed with the SYBR Green mixture following the manufacturer's directions using a Rotor-Gene RG-300A (Corbett Life Science, NSW, Australia). Expression of each gene was normalized to the GAPDH housekeeping gene. PCR amplification reaction conditions were 40 cycles of 94°C for 50 s, 56°C for 30 s, and 68°C for 40 s. Experiments were performed in triplicate in the same reaction.

ELISA

After being treated with nicotine, epibatidine and selective inhibitor, the conditioned medium was collected. Secreted Aβ was measured by a sandwich ELISA kit (Biosource, USA) according to the manufacturer's instructions Immunoblot.

Protein concentrations were determined with the Enhanced BCA Protein Assay kit (Beyotime institute of Biotechnology, Hangzhou, China). Samples (20 μg) were mixed with sample buffer, resolved by SDS–PAGE, transferred to nitrocellulose membrane, and then probed with the appropriate primary and secondary antibodies. Antibodies were visualized using chemiluminescence (ECL), and densitometric analysis was performed using the Tanon software (Tanon Innotech, Shanghai, China) from film exposures in the linear range for each antibody and normalized to control (untreated cell) samples. Normalized control values were determined from each film by averaging control values. By loading a fixed, equal amount of protein, several experiments may be compared with each other. Loading equivalency was verified by probing for an abundant ubiquitous protein such as GAPDH.

Analysis of Cell Viability by MTT Colorimetry

Cells were cultured in 96-well plates. After treatment with nicotine for 24 h, 20 μl MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-dip-henyltetrazolium bromide) soluted in culture medium at 5 mg/ml was added to each well followed by incubation for 4 h at 37°C. One hundred micro litter DMSO was then added to each well. After shaking for 10 min, the absorbance at 490 nm was determined.

Promoter Reporter Plasmids Construction

Human genome was used as a template to clone the BACE1 promoter region. Primers were designed for easy cloning to pGL3-Basic vector (Promega, Madison, WI). We followed the numbering system used by a previous report (GenBank accession number AY542689) [19]. The primer sequences were as follows: for BACE1-757 bp vector construction, -706 BglII (5′-cgcagatctagccatttctc ctcagtctg-3′) and +51Hind III (5′- ctcaagctttcaggccacca taatccag -3′), for BACE1-1876 bp vector construction, -1876 BglII (5′- catagatcactgggtacagtggtccatg-3′) and -1 Hind III (5′-ctcaagctttcaggccaccataatccag-3′), and for BACE 1–2 kb vector construction, -1949 BglII (5′- ccgagat cttttaagtcatcaaatccatccag-3′) and +51Hind III (5′-ctcaagctt tcaggccaccaatatccagct-3′) were used.

Transfection and Luciferase Assay

About 1.5×10^4 cells were seeded in 96-well culture plates and transfected with 50 ng BACE1 promoter constructs using Lipofectamine reagent (0.6 μl per well). pRL-CMV plasmid (5 ng per well) was co-transfected to normalize transfection efficiencies. To analyze the promoter activity, cells were harvested and lysed with passive lysis buffer (30 μl per well, Promega) 24 h after transfection, and luciferase assays were conducted according to the dual luciferase assay system protocol using a luminometer (Berthold technologies). Luciferase activity was normalized versus renilla luciferase activity from pRL-CMV plasmid.

Data Analysis

Statistical analysis was performed by using a *t*-test for comparison of independent means. Data are shown as means ± SD. Differences were considered statistically significant for $P < 0.05$. *$P < 0.05$, **$P < 0.01$, compare with control.

Results

Nicotine Has no Effect on Full-length APP695 and sAPP-α, but Decreases Aβ Level in SH-EP1-α4β2 nAChR-APP695 Cells

APP695 and sAPP-α expressions are not altered in SH-EP1-α4β2 nAChR-APP695 cells treated with nAChR agonist nicotine, and selective α4β2 nAChR antagonist DHβE did not change APP695 expression, either. The results suggest α4β2 nAChR activation has no effect on full-length APP695 and sAPP-α level (Fig. 1b, c). $A\beta_{42}$ and $A\beta_{40}$ levels in cells treated with nicotine and selective α4β2 nAChR agonist epibatidine are significantly lower than control (Fig. 2a, b). Blockade of α4β2 nAChR by DHβE reversed the Aβ reduction (Fig. 2a). To exclude interference of cell viability decrease, cell viability was detected by MTT colorimetry in cells treated with nicotine (1 and 10 μM). The result shows that cell viability is not affected by 1 μM of nicotine, but decreased by 10 μM of nicotine. These results suggest that Aβ reduction results from α4β2 nAChR activation, but not cell viability decrease (Fig. 2c).

BACE1 and PSEN1 Expressions are Decreased by Nicotine in SH-EP1-α4β2 nAChR-APP695 Cells

mRNA levels of BACE1 and PSEN1 in cells treated with nicotine for 24 h were substantially lower than control (Fig. 3a, b). BACE1 and PSEN1 protein expressions are also attenuated by nicotine (Fig. 3c, d). The results suggest that α4β2 nAChR activation down-regulates β- and γ-secretase pathway of APP processing, resulting in decrease of Aβ level.

Nicotine Reduces ERK1 and NFκB P65 Subunit Expression in SH-EP1-α4β2 nAChR-APP695 Cells

Significant reduction is found in ERK1 mRNA and protein expression in SH-EP1-α4β2 nAChR-APP695 cells when α4β2 nAChR is activated by nicotine (Fig. 4a, c). Nicotine is also shown to decrease NFκB P65 subunit mRNA and protein expression (Fig. 4b, d). These results indicate that α4β2 nAChR activation reduces ERK1 and NFκB P65 subunit in the cell line, and ERK1-NFκB pathway is involved in α4β2 nAChR activation.

BACE1 Promoter Activity is Reduced by Nicotine in SH-EP1-α4β2 nAChR-APP695 Cells

BACE1 gene promoter sequence (757 bp, 1876 bp, and 2 kb) was cloned into pGL3-Basic vector (Figs. 5, 6a, b, c). Three cloned constructs were transfected to test BACE1 promoter activity in SH-EP1-α4β2 nAChR-APP695 cells. After treatment with nicotine (1 μM) for 24 h, BACE1-1876 bp and BACE1-2000 bp promoter activity in the cells is down-regulated. The down-regulation is reversed by selective α4β2 nAChR inhibitor DHβE (1 μM). No promoter activity was detected in pGL3-BACE1-757 vector (Fig. 6). Nicotine has no effect on PSEN1 promoter activity (data not shown).

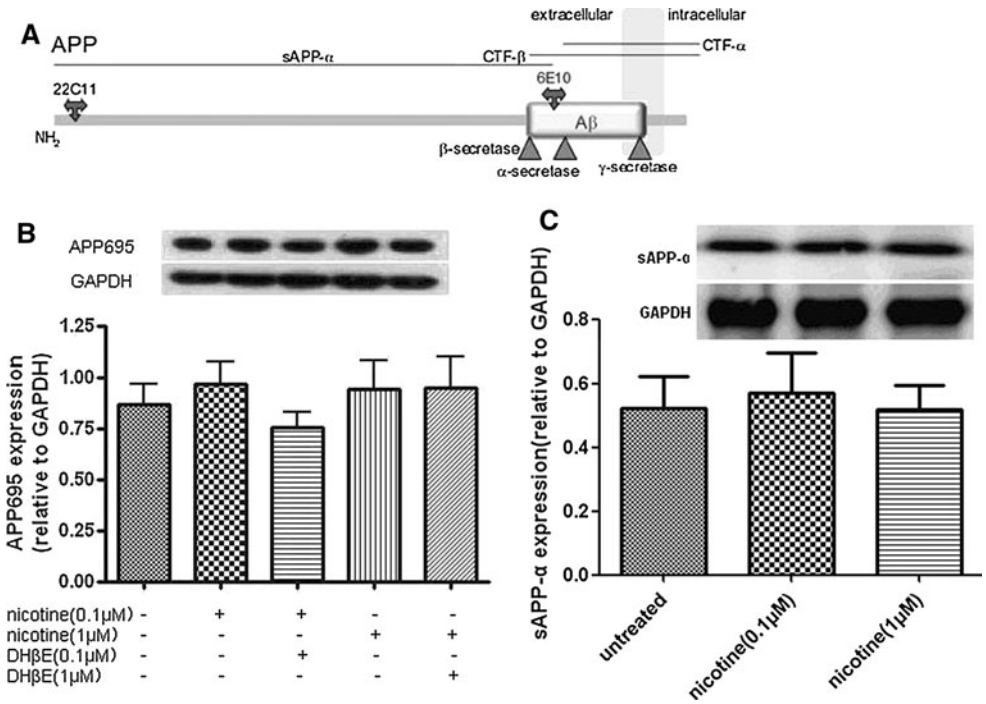

Fig. 1 Effects of nicotine on APP695 and sAPP-α in SH-EP1-α4β2 nAChR-APP695 cells. **a** Schematic depicting the APP within the cell membrane. Antibody epitopes and secretase cleavage sites are designated [20]. **b** No alteration was observed in APP695 expression after nicotine treatment (0.1 and 1 μM) for 24 h. **c** Nicotine has no effect on sAPP-α expression

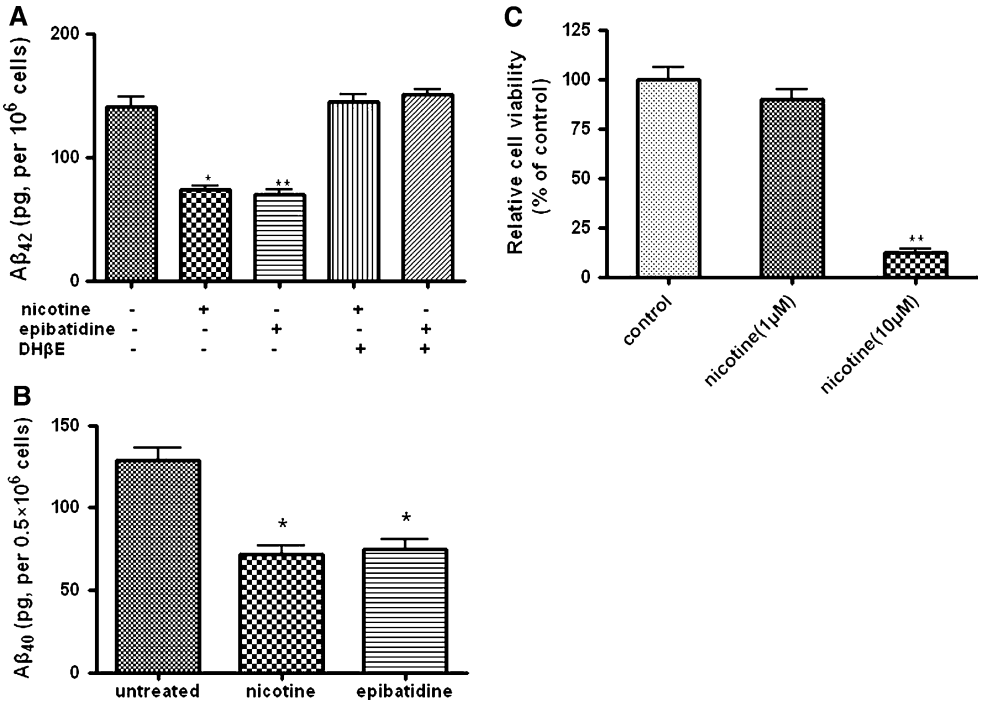

Fig. 2 Effects of nicotine and epibatidine on secreted Aβ_{40} and Aβ_{42} measured by ELISA. **a** Nicotine (1 μM) and selective $\alpha4\beta2$ nAChR agonist epibatidine (0.1 μM) decrease Aβ_{42} after 24 h treatment, and the decrease on Aβ_{42} is reversed by co-treatment with selective $\alpha4\beta2$ nAChR antagonist DHβE (1 μM). **b** Nicotine (1 μM) and epibatidine (0.1 μM) reduces secreted Aβ_{40}. **c** Cell viability (percentage of control) is not affected by 1 μM of nicotine, and is affected by 10 μM of nicotine

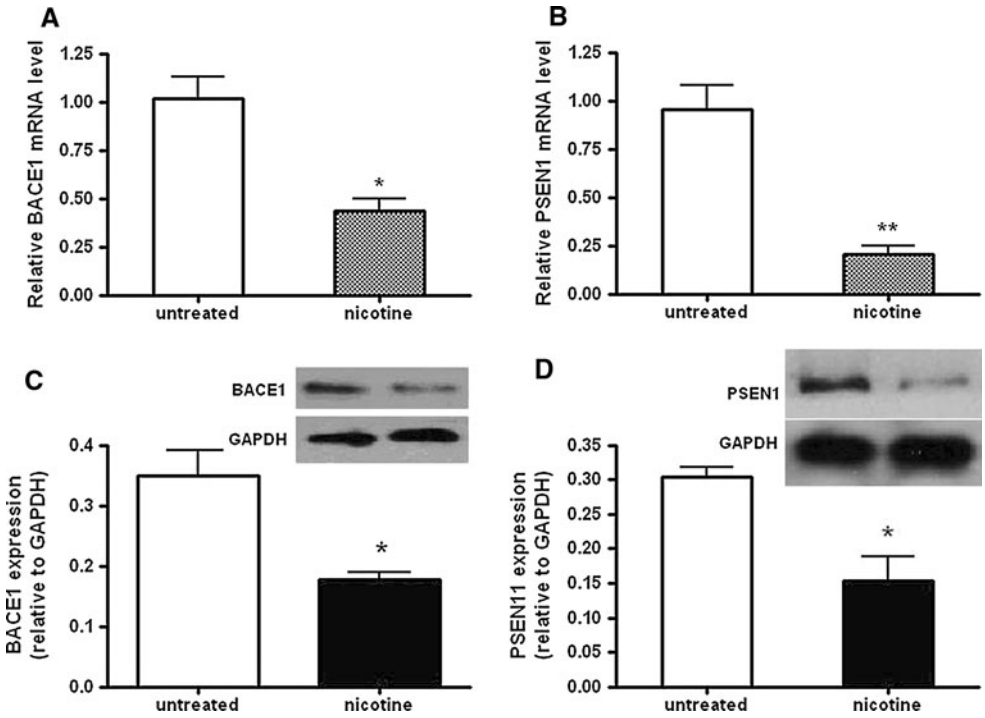

Fig. 3 Effects of nicotine on BACE1 and PSEN1 expression in SH-EP1-$\alpha4\beta2$ nAChR-APP695 cells. **a**, **b** mRNA levels of BACE1and PSEN1 are decreased after treatment with nicotine (1 μM) for 8 h in SH-EP1-$\alpha4\beta2$ nAChR-APP695 cells. **c**, **d** BACE1 and PSEN1 protein expression is attenuated after treatment with nicotine (1 μM) for 24 h in SH-EP1-$\alpha4\beta2$ nAChR-APP695 cell

Activation of $\alpha4\beta2$ nAChR Decreases Aβ Through Regulating BACE1 Transcription in Hippocampal Neurons

The results show that epibatidine has no effect on APP695 and sAPP-α expression (Fig. 7a, b), but decreases secreted Aβ_{40} and Aβ_{42} (Fig. 7d). BACE1 expression is reduced by treatment with epibatidine for 24 h in neurons (Fig. 7c). The decrease induced by epibatidine in Aβ and BACE1 is abolished by selective $\alpha4\beta2$ nAChR antagonist DHβE. BACE1 promoter activity is attenuated when treated with epibatidine for 8 h (Fig. 7e).

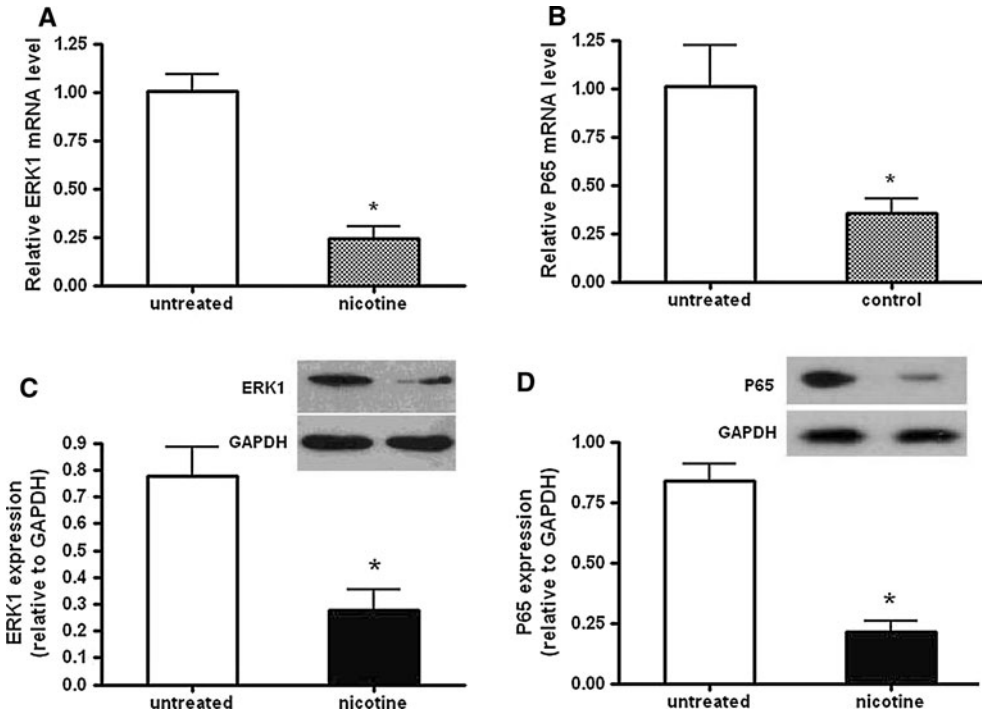

Fig. 4 Effects of nicotine (1 μM) on ERK1 and NFκB P65 subunit expression in SH-EP1-α4β2 nAChR-APP695 cells. **a**, **b** Nicotine decreases ERK1 and NFκB P65 subunit mRNA levels significantly. **c**, **d** ERK1 and NFκB P65 subunit protein expression is reduced after treatment with nicotine for 24 h in SH-EP1-α4β2 nAChR-APP695 cells

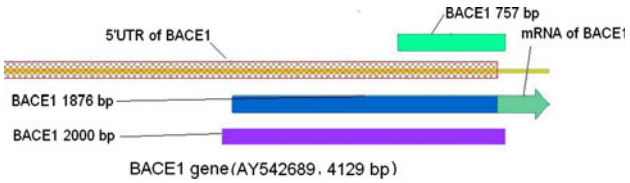

Fig. 5 Schematic figure of the promoter region of putative human BACE1 gene. *UTR* untranslated region. *TSS* transcription start site. BACE1-757 bp: −706 to +51, BACE1-1876 bp: −1876 to −1, BACE1-2 K: −1949 to +51

Discussion

Reports from neurobiology and biochemistry study have shown that activations of neuron surface receptors by neurotransmitters such as acetylcholine or 5-HT influences Aβ production [21, 22]. α4β2 nAChR is one of the major subtypes in mammalian brains and involves in the process of cognitive function including learning and memory [23]. Specific nAChR subtypes may represent unique targets for the treatment of neuropsychiatric and neurodegenerative disorders that feature cognitive impairment as a key symptom [24]. The results from 29 Chinese patients with AD showed that both α4 and β2 nAChR subunits at mRNA level were decreased and the changes were positively correlated with cognitive test scores [11].

To observe effects and mechanism of α4β2 nAChR subtype activation on Aβ production, we constructed a cell line expressing hAPP695 in SH-EP1 cells stably transfected with nAChR α4 subunit and β2 subunit genes [17]. Our present data show that α4β2 nAChR activation decreases Aβ level in the cell line and neurons, but has no effects on APP and sAPP-α. Since sAPP-α is produced by α-secretase cleavage of APP, the results suggest that α-secretase pathway of APP processing is not inhibited. Based on the fact that Aβ is produced via APP sequential proteolysis by β- and γ-secretases, the reduction of BACE1 and PSEN1 expressions in the cell line imply that β- and γ-secretase pathway of APP processing is down-regulated and furthermore, these data indicate that α4β2 nAChR activation-induced inhibition of Aβ production is derived from modulating β- and γ- secretase expression.

How are BACE1 and PSEN1 expressions down-regulated by activation of α4β2 nAChR? Several cell signal transduction pathways have been shown to regulate BACE1, and are known to be altered in AD. These signals include: PKC, ERK1/2, p25/CDK2, Cjun/JNK and intracellular calcium [25]. It is reported that nicotinic agonists activate ERK cascade via α4β2 nAChR in a concentration-dependent manner with an incubation period of 30 min in N1E-115 cells [26]. Another study provides the evidence that ERK pathway is inhibited when treated with nicotine for 2 h in SV-HUC-1 cells and Beas2B cells [27]. This result indicated that long time receptor activation of α4β2 nAChR exerted different effect on ERK pathway. In our study, ERK1 expression is reduced by treatment with nicotine for 8 h in SH-EP1-α4β2 nAChR-APP695 cells. The finding suggests that ERK1 pathway may play a role in BACE1 and PSEN1 reduction resulting from long period of α4β2 nAChR activation.

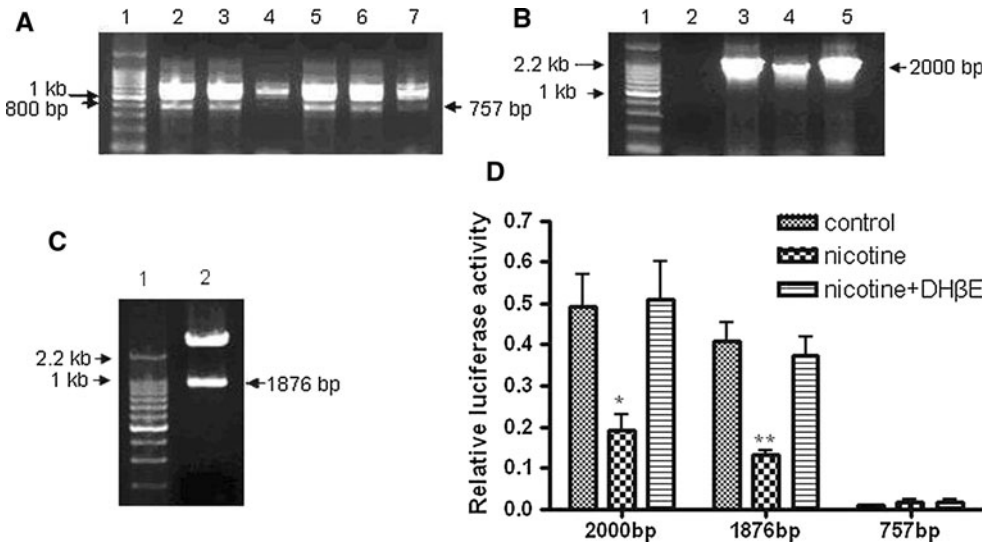

Fig. 6 Effect of nicotine on BACE1 promoter activity in SH-EP1-$\alpha4\beta2$ nAChR-APP695 cells after 8 h treatment. **a** Electrophoresis of BACE1-757 bp colony PCR product. *Lane 1* marker; *Lane 2–7* BACE1-757 bp colony PCR product. **b** Electrophoresis of BACE1-2000 colony PCR product. *Lane 1* marker; *Lane 2–5* colony PCR products. **c** Electrophoresis of double-digested pGL3-BACE1-1876. *Lane 1* marker; *Lane 2* double-digested pGL3-BACE1-1876. **d** BACE1-1876 bp and BACE1-2000 bp promoter activity in SH-EP1-$\alpha4\beta2$ nAChR-APP695 cell was decreased by nicotine (1 μM), The decrease was abolished by DHβE (1 μM). No promoter activity was detected in pGL3-BACE1-757 vector

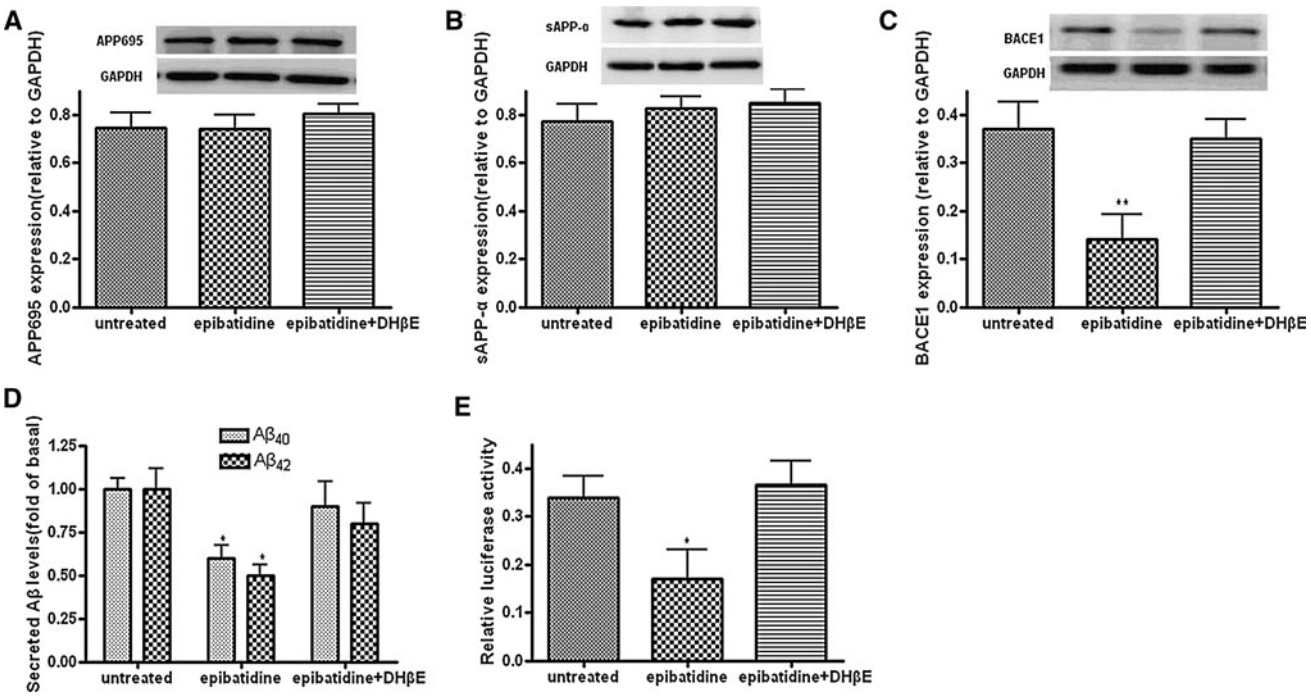

Fig. 7 Effects of $\alpha4\beta2$ nAChR activation on Aβ level and BACE1 transcription in hippocampal neurons. **a**, **b** APP695 and sAPP-α is not altered by treatment with epibatidine (0.1 μM) for 24 h. **c** Epibatidine (0.1 μM) decreases BACE1 expression in neurons, and the decrease is reversed by DHβE (1 μM). **d** Selective $\alpha4\beta2$ nAChR agonist epibatidine (0.1 μM) decreases $A\beta_{40}$ and $A\beta_{42}$ level after 24 h treatment, and the decrease on Aβ is abolished by co-treatment with antagonist DHβE (1 μM). **e** BACE1-2000 bp promoter activity in hippocampal neurons is attenuated by epibatidine (0.1 μM), and the decrease is abolished by DHβE (1 μM)

The MAPK family is comprised of three well-characterized subgroups: extracellular signal-regulated protein kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38-MAPK; all are involved in mediating cellular responses to extracellular stimuli. Rahman has reported that transcriptional activity of NFκB was prevented by inhibiting p38

MAPK activation in endothelial cells [28]. In present study, we found that nicotine down-regulated ERK1 and NFκB p65 subunit expression in the cells. Therefore, we think that prevention of NFκB expression may be induced by ERK1 down-regulation. The identification of several NFκB binding sites upstream of APP and BACE genes raises the possibility, yet to be demonstrated, of a role of NFκB in amyloidogenesis [29]. Recently, it has been reported that blockade of NFκB activation could reduce the amyloid pathology in Tg2576 mice. The age- and AD-associated increases in NFκB in brain may be significant contributors to increases in Aβ [30]. Our results suggest that inhibition of ERK1-NFκB pathway may contribute to down-regulation of BACE1 and PSEN1 induced by α4β2 nAChR activation.

Some transcription factors that control BACE1 mRNA expression respond to physiological or pathological stressors broadly implicated in AD pathogenesis [31, 32]. Since NFκB, as a transcription factor, has been implicated in the induction of BACE1 expression [10], we hypothesized that NFκB may influence BACE1 promoter activity. To test this hypothesis, we assessed BACE1 promoter activity in SH-EP1-α4β2 nAChR-APP695 cells using dual luciferase reporter gene assay. The results show that BACE1-1876 bp and BACE1-2000 bp promoter activities are decreased by nicotine, and the decreases are abolished by DHβE blockade. BACE1-2000 bp promoter activity in hippocampal neurons is also decreased by epibatidine, and the decrease is abolished by DHβE. The results indicate that BACE1 promoter activity is regulated by α4β2 nAChR, which may be one of the molecular mechanisms accounting for down-regulation of BACE1 and Aβ induced by nicotine. Why nicotine has no effect on PSEN1 promoter activity (data not shown) in the cell line needs to be further explored. Fluorescence detection is one of the most important methods for high throughput screening. Therefore, analysis of BACE1 promoter activity by dual-luciferase reporter assay may be useful for screening selective α4β2 nAChR agonists against AD in the cell line.

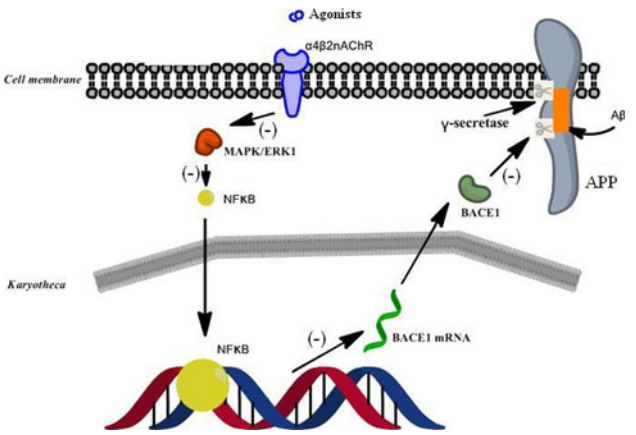

Fig. 8 The schematic drawing shows α4β2 nAChR agonists decrease Aβ by MAPK/ERK1-NFκB pathway

In conclusion, this article reports that activation of α4β2 nAChR decreases Aβ by regulating BACE1 transcription. Nicotine depresses BACE1 expression through MAPK and NFκB pathways. Our data provide new possible mechanism accounting for the beneficial effect of α4β2 nAChR activation on Aβ [Fig. 8]. Analysis of BACE1 promoter activity might be useful for drug screening as a high throughput method.

Acknowledgments This work was supported by the grant from the Shanghai Committee of Science and Technology, China (Grant No. 08431900600), the grant from the National Comprehensive Technology Platforms for Innovative Drug R&D, China (2009ZX09301–007), the national natural science foundation of China (No. 30572179, 30672443), and financial support from China postdoctoral science foundation(20090450709). We thank Dr. Ronald J. Lukas for SH-EP1 cell line.

References

1. Meyer-Luehmann M, Spires-Jones TL, Prada C et al (2008) Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. Nature 451:720–724
2. Koffie RM, Meyer-Luehmann M, Hashimoto T et al (2009) Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. Proc Natl Acad Sci USA 106:4012–4017
3. Masters CL, Simms G, Weinman NA et al (1985) Amyloid plaque core protein in Alzheimer diseaseand Down syndrome. Proc Natl Acad Sci USA 82(12):4245–4249
4. Turner PR, O'Connor K, Tate WP et al (2009) Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. Prog Neurobiol 70:1–32
5. Vassar R (2004) BACE1: the beta-secretase enzyme in Alzheimer's disease. J Mol Neurosci 23(1–2):105–114
6. Vassar R, Bennett BD, Babu-Khan S et al (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286(5440): 735–741
7. Yang LB, Lindholm K, Yan R et al (2003) Elevated beta-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. Nat Med 9:3–4
8. Zhao J, Fu Y, Yasvoina M et al (2007) Beta-site amyloid precursor protein cleaving enzyme 1 levels become elevated in neurons around amyloid plaques: implications for Alzheimer's disease pathogenesis. J Neurosci 27:3639–3649
9. Zhang XM, Cai Y, Xiong K et al (2010) β-Secretase-1 elevation in transgenic mouse models of Alzheimer's disease is associated with synaptic/axonal pathology and amyloidogenesis: implications for neuritic plaque development. Eur J Neurosci 30:2271–2283
10. Stockley JH, O'Neill C (2008) Understanding BACE1: essential protease for amyloid-beta production in Alzheimer's disease. Cell Mol Life Sci 65(20):3265–3289
11. Zhang LJ, Xiao Y, Qi XL et al (2010) Cholinesterase activity and mRNA level of nicotinic acetylcholine receptors (alpha4 and beta2 Subunits) in blood of elderly Chinese diagnosed as Alzheimer's disease. J Alzheimers Dis 19(3):849–858

12. Arneric SP, Arneric SP, Holladay M et al (2007) Neuronal nicotinic receptors: a perspective on two decades of drug discovery research. Biochem Pharmacol 74(8):1092–1101
13. Gotti C, Riganti L, Vailati S et al (2006) Brain neuronal nicotinic receptors as new targets for drug discovery. Curr Pharm Des 12(4):407–428
14. O'Neill MJ, Murray TK, Lakics V et al (2002) The role of neuronal nicotinic acetylcholine receptors in acute and chronic neurodegeneration. Curr Drug Targets CNS Neurol Disord 1:399–411
15. Cincotta SL, Yorek MS, Moschak TM et al (2008) Selective nicotinic acetylcholine receptor agonists: potential therapies for neuropsychiatric disorders with cognitive dysfunction. Curr Opin Investig Drugs 9(1):47–56
16. Nitsch RM, Slack BE, Wurtman RJ et al (1992) Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. Science 258(5080):304–307
17. Nie H, Li Z, Lukas RJ et al (2008) Construction of SH-EP1-alpha4beta2-hAPP695 cell line and effects of nicotinic agonists on Beta-amyloid in the cells. Cell Mol Neurobiol 28(1):103–112
18. Kaech S, Banker G (2006) Culturing hippocampal neurons. Nat Protoc 1(5):2406–2415
19. Ge YW, Ghosh C, Song W et al (2004) Mechanism of promoter activity of the Beta-amyloid precursor protein gene in different cell lines: identification of a specific 30 bp fragment in the proximal promoter region. J Neurochem 90(6):1432–1444
20. Caterina MH, Rakez K, Hui Z et al (2010) Loss of α7 Nicotinic Receptors Enhancesβ-Amyloid Oligomer Accumulation, Exacerbating Early-Stage Decline and Septohippocampal Pathology in a Mouse of Alzheimer's Disease. J Neurosci 30(7):2442–2453
21. Caccamo A, Fisher A, LaFerla FM (2009) M1 agonists as a potential disease-modifying therapy for Alzheimer's disease. Curr Alzheimer Res 6(2):112–117
22. Neugroschl J, Sano M (2010) Current treatment and recent clinical research in Alzheimer's disease. Mt Sinai J Med 77(1):3–16
23. Paterson D, Nordberg A et al (2000) Neuronal nicotinic receptors in the human brain. Prog Neurobiol 61:75–111
24. Cincotta SL, Yorek MS, Moschak TM et al (2008) Selective nicotinic acetylcholine receptor agonists: potential therapies for neuropsychiatric disorders with cognitive dysfunction. Curr Opin Investig Drugs 9:47–56
25. Tamagno E, Guglielmotto M, Aragno M et al (2008) Oxidative stress activates a positive feedback between the gamma- and beta-secretase cleavages of the Beta-amyloid precursor protein. J Neurochem 104(3):683–695
26. Tomizawa M, Casida JE (2002) Desnitro-imidacloprid activates the extracellular signal-regulated kinase cascade via the nicotinic receptor and intracellular calcium mobilization in N1E–115 cells. Toxicol Appl Pharmacol 184(3):180–186
27. Chen RJ, Ho YS, Guo HR et al (2010) Long-term nicotine exposure-induced chemoresistance is mediated by activation of Stat3 and downregulation of ERK1/2 via nAChR and beta-adrenoceptors in human bladder cancer cells. Toxicol Sci 115(1):118–130
28. Rahman A, Anwar KN, Minhajuddin M et al (2004) cAMP Targeting of p38 MAP Kinase Inhibits Thrombin-induced NF-{kappa}B Activation and ICAM-1 Expression in Endothelial Cells. Am J Physiol Lung Cell Mol Physiol 287(5):L1017–L1024
29. Memet S (2006) NF-kappaB functions in the nervous system: from development to disease. Biochem Pharmacol 72(9):1180–1195
30. Bourne KZ, Ferrari DC, Lange-Dohna C et al (2007) Differential regulation of BACE1 promoter activity by nuclear factor-kappaB in neurons and glia upon exposure to Beta-amyloid peptides. J Neurosci Res 85(6):1194–1204
31. Tamagno E, Guglielmotto M, Giliberto L et al (2009) JNK and ERK1/2 pathways have a dual opposite effect on the expression of BACE1. Neurobiol Aging 30(10):1563–1573
32. Virginie BP, Jean S, Steffen R et al (2008) NFkappaB-dependent control of BACE1 promoter transactivation by Abeta42. J Biol Chem 283(15):10037–10047