European Journal of Pharmacology 610 (2009) 37–41

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Molecular and Cellular Pharmacology

Involvement of protein trafficking in deprenyl-induced α-secretase activity regulation in PC12 cells

Hong-Qi Yang [a,c], Zhi-Kun Sun [a], Mao-Wen Ba [b], Jun Xu [a], Ying Xing [c,*]

[a] Department of Neurology, Henan Provincial People's Hospital, Zhengzhou 450003, Henan Province, People's Republic of China
[b] Department of Neurology, The Affiliated Yantai Yuhuangding Hospital of Qingdao University Medical College, Yantai 264000, Shandong Province, People's Republic of China
[c] Department of Physiology, Medical College of Zhengzhou University, Zhengzhou 450052, Henan Province, People's Republic of China

ARTICLE INFO

Article history:
Received 13 January 2009
Received in revised form 4 March 2009
Accepted 15 March 2009
Available online 24 March 2009

Keywords:
Alzheimer's disease
Deprenyl
ADAM10
Protein trafficking

ABSTRACT

Deprenyl is a selective B-type monoamine oxidase inhibitor and a neuroprotective agent that has been used to slow the progress of Alzheimer's disease for many years. We previously demonstrated that deprenyl could stem amyloid precursor protein processing (APP) toward the non-amyloidogenic pathway through mitogen activated protein kinase (MAPK) and protein kinase C (PKC)-dependent signaling pathways [Yang, H.Q., Ba, M.W., Ren, R.J., Zhang, Y.H., Ma, J.F., Pan, J., Lu, G.Q., Chen, S.D., 2007a. Mitogen activated protein kinase and protein kinase C mediated promotion of sAPPα by deprenyl. Neurochem. Int. 50, 74–82.]. The experiment here further showed that deprenyl could increase α-secretase activity in a dose-dependent manner in PC12 cells. Deprenyl increased α-secretase activity can be partially blocked by pretreatment with brefeldin A, an intracellular protein transport inhibitor, suggesting involvement of protein trafficking in deprenyl regulated α-secretase activity. In accordance with this, the experiment showed that brefeldin A also decreased sAPPα release induced by deprenyl. Deprenyl promoted ADAM10 transported to the membrane fraction, and this effect was blocked by pretreatment with brefeldin A. The immunocytochemistry staining revealed that deprenyl promoted colocalization of ADAM10 with PKCα and PKCε isoforms. These data suggest a novel pharmacological mechanism in which deprenyl increased α-secretase activity via protein trafficking related mechanism.

1. Introduction

Alzheimer's disease is a neurodegenerative disease characterized clinically by progressive impairments of memory and cognition. It is the most frequent form of dementia found in the elderly. Although hypotheses such as genetic predisposition, cholinergic hypofunction, beta amyloid cascade and cellular apoptosis have been proposed to explain the pathogenesis of Alzheimer's disease, detailed developmental mechanisms still remain to be clarified (Selkoe, 2001). Currently available drug therapies for Alzheimer's disease consist primarily of cholinesterase inhibitors (donepezil, galantamine, rivastigmine) and an N-methyl-D-aspartate receptor antagonist (memantine) approved by the FDA (Grossberg, 2005) and some neuroprotective agents. Deprenyl is a selective B-type monoamine oxidase inhibitor clinically used in Parkinson's disease (Chen et al., 2007). Also as a neuroprotective drug, deprenyl has been used to slow the progress of some neurodegenerative diseases such as Alzheimer's disease in the late stage of the disease (Wilcock et al., 2002; DeLaGarza, 2003). Although controversial, some clinical trials did indicate that deprenyl could alleviate some motor symptoms of Alzheimer's disease patients (Filip and Kolibas, 1999). But the detailed mechanism why deprenyl is effective is still poorly understood.

One of the hallmarks of Alzheimer's disease is the presence of senile plaques in the hippocampus, which are composed mainly of extracellular deposits of a 40–42(3) amino acid polypeptide known as beta amyloid, the Aβ. The Aβ peptide aggregates and forms deposits that are thought to lead to neuronal dysfunction (Selkoe, 2001). The Aβ is derived from a large transmembrane protein, the amyloid precursor protein (APP) by sequential proteolysis of two proteases, the β- and γ-secretase, at the N- and C-termini of the Aβ peptide respectively. Alternatively, APP can also be processed by α-secretase within the Aβ sequence and thus not only preclude the formation of Aβ peptide but also generate a soluble neurotrophic sAPPα (ectodomain shedding). In Alzheimer's disease patients' platelet and cerebrospinal fluid, β-secretase activity is increased and ADAM10 (a putative α-secretase candidate) activity is decreased, and the decrease in ADAM10 activity is in accordance with the disease progression (Colciaghi et al., 2004). While in hippocampus and cortex of severe Alzheimer's disease patients, the ADAM10 mRNA expression increased twofold, which may reflect the compensation mechanism in the latter stage of disease (Gatta et al., 2002). A recent study in our team indicated that deprenyl could increase the release of sAPPα, the product of α-secretase, through pathways dependent on mitogen activated protein kinase (MAPK) and protein kinase C (PKC) (Yang

* Corresponding author. Tel.: +86 371 66658377.
E-mail address: xingying_2008@163.com (Y. Xing).

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2009.03.047

et al., 2007a). But the precise mechanism how they act exactly to α-secretase is unclear. So here we detected the effect of deprenyl on α-secretase activity itself in PC12 cells and try to explore the possible related mechanisms.

2. Materials and methods

2.1. Reagents

Deprenyl, dimethyl sulphoxide (DMSO), phenylmethanesulfonyl fluoride (PMSF), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), brefeldin A, monoclonal mouse anti β-actin antibody and polyclonal rabbit anti-ADAM10 N-terminus antibody were provided by Sigma-Aldrich (St Louis, MO). Dulbecco's Modified Eagle Medium (DMEM), penicillin, streptomycin and fetal bovine serum (FBS) were purchased from Gibco (Carlsbad, CA). Monoclonal mouse anti-Alzheimer precursor protein antibody (6E10 clone) was obtained from Chemicon International Inc (Temecula, CA). Monoclonal mouse anti-PKCα and PKCε antibodies were provided by Santa-Cruz Biotechnology, Inc. (Santa Cruz, CA). 2′-Amino-3′-methoxyflavone (PD98059) and 2-[1-(3-Dimethylaminopropyl)-1H-indol-3-yl]-3-(1H-indol-3-yl)-maleimide (GF109203X) were purchased from Calbiochem (San Diego, CA). α-secretase activity detection kit was provided by R&D Systems Inc. (Minneapolis, MN). All other reagents are the highest grade available from Sigma-Aldrich unless otherwise indicated.

2.2. Cell culture and drug treatment

Partially differentiated rat pheochromocytoma PC12 cells were plated onto 100 mm culture dishes (Corning Incorporated, Corning, NY) in DMEM containing 10% heat-inactivated FBS, 1% penicillin and 1% streptomycin. Cells were grown at 37 °C in a humidified, 5% CO_2 environment. 24 h before drug treatment, the media were replaced with FBS-free media. Cells were then incubated with deprenyl (dissolved in DMSO) or vehicle for 2 h. Some cultures pretreated with various protein kinase inhibitors (all dissolved in DMSO) or brefeldin A (dissolved in ethanol) 30 min prior to deprenyl challenges were also carried out. At the indicated time point after drug treatment, the conditioned media were collected, concentrated with Centricon YM-30 filter units (Millipore, Bedford, MA) and frozen at −70 °C until further analysis. Cells were washed twice with cold phosphate buffered saline and lysed on ice with RIPA buffer containing 50 mM Tris–HCl (pH 8.0), 150 mM sodium chloride, 1.0% Igepal CA-630 (NP-40), 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate (SDS), supplemented with 2 mM PMSF. After centrifugation at 14 000 ×*g* for 30 min at 4 °C, the supernatants were transferred to new microtubes and stored at −70 °C. The protein concentration of each sample was quantified using the bicinchoninic acid (BCA) assay kit (Pierce Biotechnology, Rockford, IL).

For ADAM10 immuno-detection, cells were treated with lysis buffer containing 20 mM Tris–HC1 (pH 7.5), 2 mM EDTA, 2 mM EGTA, 5 mM DTT, 0.32 M sucrose and 2 mM PMSF on ice and centrifuged at 12,000 ×*g* for 30 min at 4 °C. The resulting supernatants were regarded as the cytosolic fractions. The pellets were incubated with the same lysis buffer, to which 1% Triton X-100 was added, on ice for 45 min. After centrifugation at 12,000 ×*g* for 20 min at 4 °C, the supernatant was collected for use as membrane fractions. After BCA quantification, 20 μg of protein mixed with 5× loading buffer and 20× reducing agent was boiled for 5 min and loaded onto a 7.5% SDS-PAGE.

2.3. Western blotting analysis

Twenty micrograms of protein mixed with 5× loading buffer (0.313 M Tris–HCl (pH 6.8) at 25 °C, 10% SDS, 0.05% bromophenol blue, 50% glycerol) and 20× reducing agent (2 M DTT) (Fermentas, Hanover, MD) was boiled for 5 min and loaded onto a 7.5% SDS-polyacrylamide electrophoresis gel. After electrophoresis, the protein was electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA). The membranes were saturated with 5% non-fat milk and incubated with primary antibodies (1:3000 for 6E10, 1:10,000 for β-actin, 1:1000 for ADAM10 in bovine serum album) at 4 °C overnight. After being washed for 30 min in Tris-buffered saline (TBS) with gentle agitation, membranes were incubated with horseradish peroxidase-conjugated anti-mouse/rabbit IgG secondary antibodies in non-fat milk at room temperature for 1 h. Signals were developed using ECL Western Blotting Detection kit (Amersham-Pharmacia Biotech, Little Chalfont, UK).

2.4. MTT assays

Cell viability was evaluated with MTT assays. Briefly, 24 h after plating 2×10^4 cells in each well of a 96 well plate, cells were incubated with drugs at a series of concentration. Twelve to 24 h later, the original media were replaced with media containing MTT at a final concentration of 0.5 g/L for 4 h. Then the media were discarded and DMSO was added for the colorimetric assay. Absorption was determined in a Tecan Sunrise Eliza-Reader (Switzerland) at $\lambda = 570/630$ nm after automatic subtraction of background signals. The results were expressed as a percentage of control group cells.

2.5. α-secretase activity detection

Following treatment with deprenyl for 2 h, cells were washed with cold phosphate buffered saline and incubated with extraction buffer for 10 min on ice. Equal amount of protein was added to 96-well plate, mixed with reaction buffer and substrate at room temperature in the dark with gentle agitation for 2 h. Signals were detected in a Tecan Sunrise Eliza-Reader (Switzerland) at an excitation wavelength of 350 nm and an emission wavelength of 500 nm after automatic subtraction of background signals. The results were expressed as a percentage of control groups.

2.6. Immunocytochemistry staining

After drug treatment, the media were discarded, washed twice with cold phosphate buffered saline and fixed with 4% polyformaldehyde at room temperature for 30 min. After being treated with 3% H_2O_2 and 0.1% Triton X-100 and then blocked with phosphate buffered saline containing 3% bovine serum albumin, cells were incubated with monoclonal mouse anti PKCα or PKCε antibodies (1:200 in bovine serum albumin) and polyclonal rabbit anti-ADAM10 antibody (1:200 in bovine serum albumin) overnight at 4 °C. Cells were washed 3 times with phosphate buffered saline and then incubated with TRITC-conjugated anti-mouse or FITC-conjugated anti-rabbit IgG secondary antibodies (Sigma-Aldrich, St Louis, MO). After being washed, the cells were then rinsed and coverslipped with a Dako antifade medium (Carpinteria, CA). The signals were detected by epifluorescence with an Axioplan-2 microscope. Negative controls were conducted with the same as those mentioned above except that primary antibodies were omitted.

2.7. Statistical analysis

Band intensities were quantified by densiometric analyses using an AxioCam digital camera (ZEISS, Germany) and the KS400 photo analysis system (Ver 3.0). Quantitative analysis of Western blots was performed by calculating the relative density of the immunoreactive bands and expressed as a percentage of control values. Data were presented as the mean ± S.D. Each procedure was performed in duplicate in three to five independent experiments. Statistical analyses were carried out using one-way ANOVA, followed by a

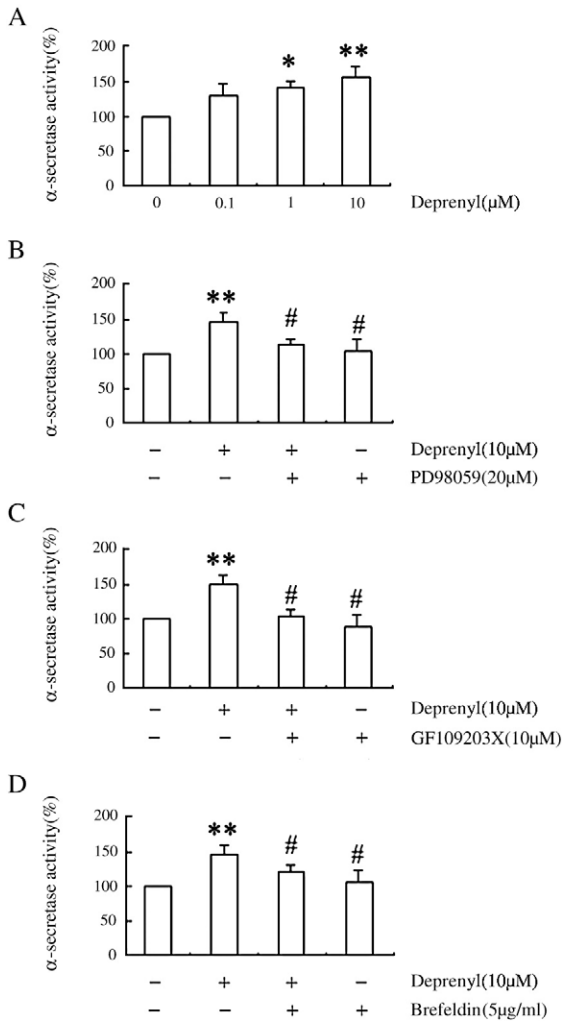

Fig. 1. Effect of deprenyl on α-secretase activity in various circumstances. After the PC12 cells were treated with different concentrations of deprenyl (A) or pretreated 30 min with 20 μM PD98059 (B), 10 μM GF109203X (C) or 5 μg/ml brefeldin A (D) and then with 10 μM deprenyl for 2 h, the cells were harvested and subjected to α-secretase activity detection. The results are representative of four independent experiments (*P<0.05, **P<0.01 vs. control group; #P<0.05 vs. 10 μM deprenyl group).

two-tailed Student's t-test. Multiple comparison tests were used when appropriate and P<0.05 was regarded as statistically significant.

3. Results

3.1. Deprenyl increased α-secretase activity and the possible related signaling pathways

As shown in Fig. 1A, deprenyl dose-dependently increased α-secretase activity as compared with the control group, with 10 μM deprenyl increased α-secretase activity almost 50%, significantly higher than the control group. We have previously shown that MAPK and PKC mediated increased sAPPα secretion by deprenyl (Yang et al., 2007a), so we detected the effects of PD98059 and GF109203X, inhibitors of MAPK and PKC, on α-secretase activity. In accordance with our previous study, both PD98059 and GF109203X partially blocked deprenyl's effect on α-secretase activities (Fig. 1B, C), meaning that the effect of deprenyl on α-secretase activity was mediated through MAPK and PKC signaling pathways. We next examined the effect of brefeldin A, a protein trafficking inhibitor, on deprenyl-induced α-secretase activity. The results were similar with

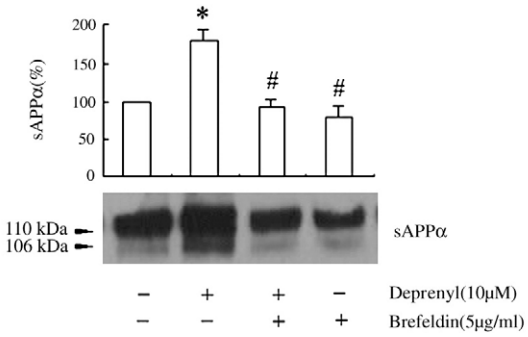

Fig. 2. Brefeldin A blocked deprenyl-induced sAPPα secretion. PC12 cells were pretreated with 5 μg/ml brefeldin A for 30 min and then incubated with 10 μM deprenyl for 2 h. Twenty micrograms of protein from the supernatant was run on a 7.5% SDS-PAGE for sAPPα detection with 6E10 antibody. Three independent experiments were performed in duplicate (*P<0.01 vs. control group; #P<0.05 vs. 10 μM deprenyl group).

those of PD98059 and GF109203X, implying that the protein trafficking related mechanism might also be involved in deprenyl's effect on α-secretase activity (Fig. 1D).

3.2. Brefeldin A antagonized deprenyl-induced sAPPα secretion

To further testify brefeldin A's effect on α-secretase activity, we collected the conditioned media after drug treatment and subjected to Western blot for sAPPα detection, using 6E10 antibody. This antibody recognizes the 1–16 amino acid sequence of human Aβ, a region absent from both β-secretase processed APP and the amyloid precursor-like protein 2(APLP2). Thus, 6E10 detects sAPPα that specifically derives from non-amyloidogenic, α-cleavage. The results indicated that deprenyl significantly promoted sAPPα release into the media as compared with control, but this effect was blocked by pretreatment of 5 μg/ml brefeldin A, suggesting that protein trafficking related mechanism might underlie the effect of deprenyl on sAPPα secretion. The results were shown in Fig. 2.

To exclude the possibility that the decreased sAPPα secretion after brefeldin A treatment might be mediated through its neurotoxicity to cells, the effect of brefeldin A on cell viability was also measured. The results indicated that at concentration of 0.1–10 μg/ml, brefeldin A didn't significantly affect cell viability (Fig. 3). This implied that the decreased sAPPα secretion after brefeldin A pretreatment was not induced by its neurotoxicity to cells, but may be mediated through its pharmacological properties.

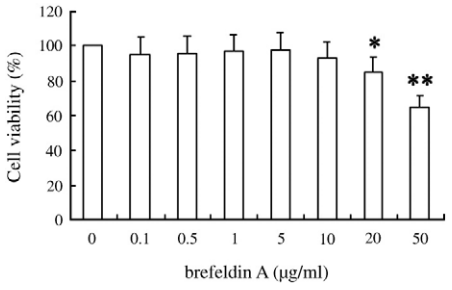

Fig. 3. Effect of brefeldin A on cell viability. PC12 cells were treated with the indicated concentrations (0.1 to 50 μg/ml) of brefeldin A for 24 h. MTT assays were carried out to detect cell survival in a Tecan Sunrise Eliza-Reader at λ = 570/630 nm after automatic subtraction of background signals. Results are expressed as a percentage of normal cells. The data shown are representative of five independent experiments (*P<0.05, **P<0.01 vs. control).

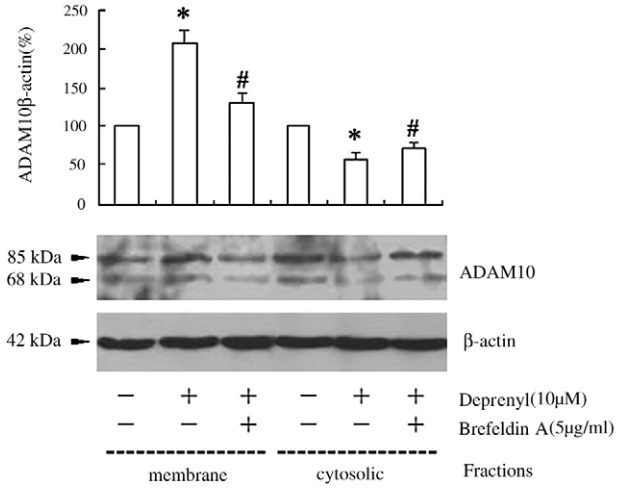

Fig. 4. Effect of deprenyl on ADAM10 subcellular distribution. PC12 cells were treated with vehicle alone or 10 μM deprenyl or pretreatment 30 min with 5 μg/ml brefeldin A and then with 10 μM deprenyl for 2 h. The cytosolic and membrane proteins were extracted separately for Western blot analysis. Three independent experiments were performed in duplicate (*P<0.01 vs. control group).

3.3. Effect of deprenyl on AMAM10 subcellular distribution

To further investigate the exact effect of deprenyl on α-secretase itself, the cytosolic and membrane fraction proteins were extracted separately after deprenyl treatment and then were subjected for ADAM10 detection. It was revealed in Fig. 4 that deprenyl increased the expression of both the enzymatically active form of ADAM10 (68 kDa) and the proenzyme form of ADAM10 (85 kDa) in the membrane fraction, in contrast to decreasing their expression in the cytosolic fraction. But this effect can be partially reversed by pretreatment of cells with 5 μg/ml brefeldin A. The results here indicated that deprenyl promoted ADAM10 transport from the cytosolic to the membrane fractions and implied that protein trafficking mechanism was responsible for deprenyl-induced α-secretase increasing.

3.4. Deprenyl promoted ADAM10 and PKC colocalization

We have previously shown that deprenyl activated PKC by promoting the PKCα and PKCε translocation. The experiment here showed that in the control group, more proteins (PKCα, PKCε and ADAM10) were labeled in the cytosolic fraction. Not only promoting membrane labeling for these proteins, deprenyl also facilitated the colocalization of PKCα and PKCε with ADAM10 (Fig. 5). The amount of both ADAM10 and PKC isoforms detectable in deprenyl treated cells was visibly higher than that detected in control group suggesting an enhanced deprenyl-induced trafficking of both ADAM10 and PKC isoforms towards the cell membranes. This experiment implies that deprenyl may promote the translocation of PKC and its substrate ADAM10 from the cytosolic to membrane fraction, which may facilitate their biological functions to be exerted. The increased trafficking to cellular membranes of ADAM10 was also evident in western blot analysis (Fig. 4).

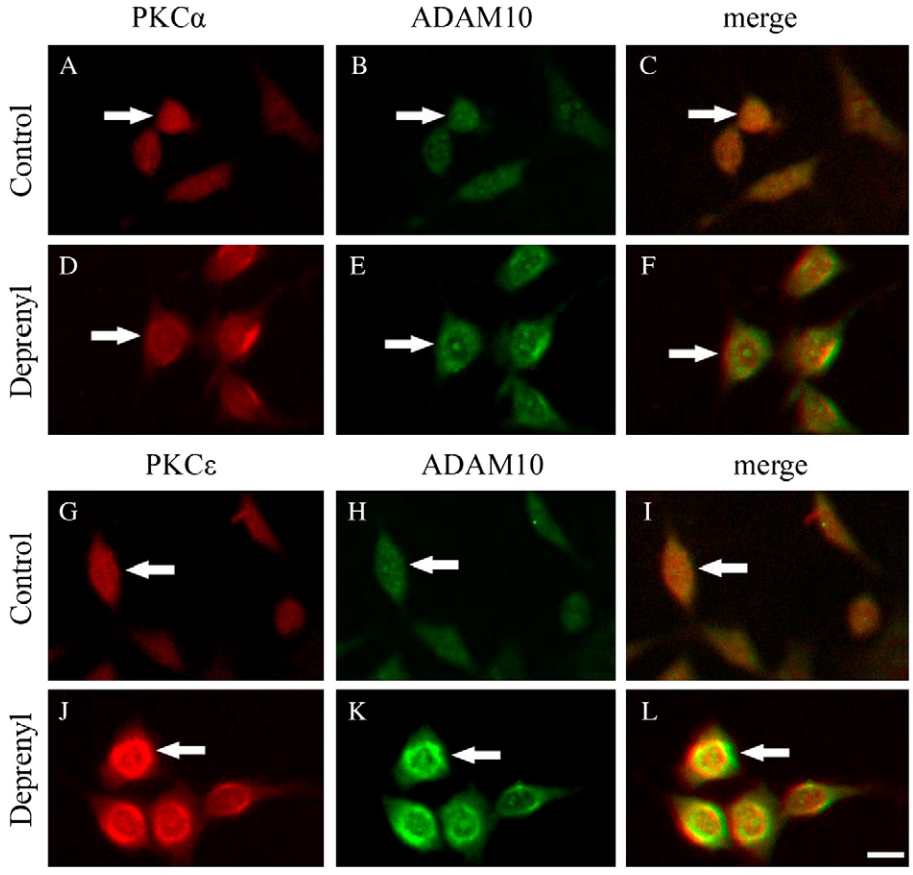

Fig. 5. Double immunofluorescence labeling for PKCα (A, D), PKCε (G, J) with ADAM 10 (B, E, H, K). PC12 cells were treated with vehicle alone (A–C; G–I) or 10 μM deprenyl (D–F; J–L) for 2 h and then subjected to immunocytochemistry staining. Staining for PKC isoforms (red) and ADAM 10 (green) was performed and colocalization was given by yellow fluorescence. Note the increased membrane labeling for PKCα (arrow in D), PKCε (arrow in J) and ADAM10 (arrows in E and K) as compared with the control group (arrows in A, G, B and H respectively). Scale bar represents 40 μm and is applicable to all photos. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

This study demonstrated that deprenyl increased α-secretase activity and that the effect of deprenyl on α-secretase activity could be partially blocked by protein trafficking inhibitor brefeldin A; Deprenyl promoted ADAM10 translocation and colocalization with PKCα and PKCε, the two main PKC isoforms involved in the pathogenesis of Alzheimer's disease.

We have previously shown that deprenyl increased sAPPα secretion in PC12 and SK-N-SH cells through MAPK and PKC-dependent manner (Yang et al., 2007a). In accordance with this, the experiment here indicated that deprenyl treatment for 2 h increased α-secretase activity about 50%, significantly higher than that of the control group, suggesting a positive correlation between sAPPα release and α-secretase activity detection. Also, the increased α-secretase activity by deprenyl could be blocked by pretreatment 30 min with MAPK and PKC inhibitors PD98059 and GF109203X respectively, implying that MAPK and PKC activations may be necessary for α-secretase activation after deprenyl treatment. Further, pretreatment of PC12 cells 30 min with 5 μg/ml brefeldin A, an inhibitor of intracellular protein transport, antagonized deprenyl-induced α-secretase activity, implying involvement of protein trafficking related mechanisms in deprenyl-induced α-secretase activation. This hypothesis is further supported by the experiment that brefeldin A also antagonized deprenyl-induced sAPPα secretion at the same time.

The detailed reason(s) why brefeldin A decreased deprenyl-induced α-secretase activity is not quite clear; In our opinion, it may be relate with the characteristics of α-secretase itself. Biotin-labeled experiments indicate that the enzymatically active form of ADAM10 is mainly localized in the plasma membrane while most of the proenzyme form is located intracellularly, most likely in the Golgi body (Buxbaum et al., 1998; Lammich et al., 1999). Deprenyl promoted ADAM10 translocation to the membrane fraction where it exists as an enzymatically form and facilitates the α-secretase functional activity to be exerted. Brefeldin A acts by blocking the trafficking of α-secretase from the Golgi body. Here, in the Golgi body it is localized as the premature form and needs to translocate to the plasma membrane where it exerts its biological function as the enzymatically active form. So brefeldin A treatment results in a reduction of α-secretase activity and the subsequent decreased sAPPα secretion. The brefeldin A experiment indirectly testified our above-mentioned hypothesis and may imply that ADAM10 trafficking is responsible for increased α-secretase activity.

ADAM 10 exists in two forms: premature and mature form. The former is the proenzyme form (80 kDa) which is latent and has little enzymatic activity. The latter (68 kDa) is derived from the former through cleavage of the prodomain motif by the proprotein convertase 7 (PC7) or furin (Anders et al., 2001; Endres et al., 2003; Lopez-Perez et al., 2001; Yang et al., 2007b; Zimmermann et al., 2004). The data also demonstrated that deprenyl increased the expression of both premature and mature forms of ADAM10 in the membrane where its enzymatic activity is higher than in the cytosolic fraction, thus directly explaining the increased α-secretase activity after deprenyl treatment.

In Alzheimer's disease patients, PKC immunoreactivity and isoform translocation are attenuated, and altered protein phosphorylation is correlated with decreased cognition and memory impairment (Wang et al., 1994). Studies have also shown that Aβ can cause a reduction of PKC isoenzyme levels and that Aβ can directly inhibit PKC activation (Lee et al., 2004). Thus the colocalization of PKCα and PKCε isoforms with ADAM10 further supports the hypothesis that deprenyl activates PKC and its substrate ADAM10 and that the translocation of both may be responsible for the increased α-secretase activity. Researchers have demonstrated decreased α-secretase activity in Alzheimer's disease patients as compared with control group (Colciaghi et al., 2002, 2004), so the activation of PKC and α-secretase may at least in part explain the efficacy of the drug in some Alzheimer's disease patients.

Taken together, the results here indicate that in PC12 cells, deprenyl increases α-secretase activity and promotes ADAM10 and PKCα and PKCε isoforms trafficking to the membrane fraction with the later probably suggesting a novel pharmacological mechanism in which deprenyl increases α-secretase activity via protein trafficking related mechanism.

Acknowledgements

This study was supported by grants from Henan Medical Technologies R&D Program (200703023) and Key Project of Science and Technology of Henan Province (0224630174).

References

Anders, A., Gilbert, S., Garten, W., Postina, R., Fahrenholz, F., 2001. Regulation of the α-secretase ADAM10 by its prodomain and proprotein convertases. FASEB J. 15, 1837–1839.

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 α converting enzyme is involved in regulated α-secretase cleavage of the Alzheimer's amyloid protein precursor. J. Biol. Chem. 273, 27765–27768.

Chen, J.J., Swope, D.M., Dashtipour, K., 2007. Comprehensive review of rasagiline, a second-generation monoamine oxidase inhibitor, for the treatment of Parkinson's disease. Clin. Ther. 29, 1825–1849.

Colciaghi, F., Borroni, B., Pastorino, L., Marcello, E., Zimmermann, M., Cattabeni, F., Padovani, A., Di Luca, M., 2002. α-secretase ADAM 10 as well as αAPPs is reduced in platelets and CSF of Alzheimer disease patients. Mol. Med. 8, 67–74.

Colciaghi, F., Marcello, E., Borroni, B., Zimmermann, M., Caltagirone, C., Cattabeni, F., Padovani, A., Di Luca, M., 2004. Platelet APP, ADAM 10 and BACE alternations in early stages of Alzheimer disease. Neurology 62, 498–501.

DeLaGarza, V.W., 2003. Pharmacologic treatment of Alzheimer's disease: an update. Am. Fam. Phys. 68, 1365–1372.

Endres, K., Anders, A., Kojro, E., Gilbert, S., Fahrenholz, F., Postina, R., 2003. Tumor necrosis factor-α converting enzyme is processed by proprotein-convertase to its mature form which is degraded upon phobol ester stimulation. Eur. J. Biochem. 270, 2386–2393.

Filip, V., Kolibas, E., 1999. Selegiline in the treatment of Alzheimer's disease: a long-term randomized placebo-controlled trial. Czech and Slovak Senile Dementia of Alzheimer Type Study Group. J. Psychiatry Neurosci. 24, 234–243.

Gatta, L.B., Albertini, A., Ravid, R., Finazzi, D., 2002. Levels of β-secretase BACE and α-secretase ADAM10 mRNAs in Alzheimer hippocampus. NeuroReport 13, 2031–2033.

Grossberg, G.T., 2005. Rationalizing therapeutic approaches in Alzheimer's disease. C.N.S. Spectr. 10, 17–21.

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

Lee, W., Boo, J.H., Jung, M.W., Park, S.D., Kim, Y.H., Kim, S.U., Mook-Jung, I., 2004. Amyloid beta peptide directly inhibits PKC activation. Mol. Cell. Neurosci. 26, 222–231.

Lopez-Perez, E., Zhang, Y., Frank, S.J., Creemers, J., Seidah, N., Checler, F., 2001. Constitutive α-secretase cleavage of the β-amyloid precursor protein in the furin-deficient LoVo cell line: involvement of the pro-hormone convertase 7 and the disintegrin metalloprotease ADAM 10. J. Neurochem. 76, 1532–1539.

Selkoe, D.J., 2001. Alzheimer's disease: genes, proteins, and therapy. Physiol. Rev. 81, 741–766.

Wang, H.Y., Pisano, M.R., Friedman, E., 1994. Attenuated protein kinase C activity and translocation in Alzheimer's disease brain. Neurobiol. Aging 15, 293–298.

Wilcock, G.K., Birks, J., Whitehead, A., Evans, S.J., 2002. The effect of selegiline in the treatment of people with Alzheimer's disease: a meta-analysis of published trials. Int. J. Geriatr. Psychiatry 17, 175–183.

Yang, H.Q., Ba, M.W., Ren, R.J., Zhang, Y.H., Ma, J.F., Pan, J., Lu, G.Q., Chen, S.D., 2007a. Mitogen activated protein kinase and protein kinase C mediated promotion of sAPPα by deprenyl. Neurochem. Int. 50, 74–82.

Yang, H.Q., Pan, J., Ba, M.W., Sun, Z.K., Ma, G.Z., Lu, G.Q., Xiao, Q., Chen, S.D., 2007b. New protein kinase C activator regulates amyloid precursor protein processing in vitro by increasing α-secretase activity. Eur. J. Neurosci. 26, 381–391.

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, 1489–1499.