Arch Pharm Res Vol 28, No 9, 1073-1078, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Mutant Presenilin 2 Increases Acetylcholinesterase Activity in Neuronal Cells

Hong Nga Nguyen, Dae Youn Hwang[1], Young Kyu Kim[1], Do Young Yoon[2], Jae Hwa Kim[2], Moon Soon Lee[3], Myung Koo Lee, Yeo Pyo Yun, Ki Wan Oh, and Jin Tae Hong

College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea, [1]National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul 122-704, Korea, [2]Lab of Cell Biology, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Korea, and [3]National Institute of Environmental Research, Incheon 404-780, Korea

(Received June 6, 2005)

A presenilin 2 mutation is believed to be involved in the development of Alzheimer's disease. In addition, transgenic mice with a presenilin 2 mutation have been reported to have learning and memory impairments. In this study, exposing PC12 cells expressing mutant presenilin 2 to 50 μM $A\beta_{25-35}$, 30 mM L-glutamate and 50 μM H_2O_2 caused a significant increase in acetylcholine esterase activity. An *in vivo* study revealed high levels of this enzyme activity in the mutant presenilin 2 transgenic brains compared with the wild type presenilin 2 transgenic and non-transgenic samples. These results suggest that a mutant presenilin 2-induced neurodegeneration in Alzheimer's disease might be involved in the increase in acetylcholinesterase activity. These findings might help in the development of an appropriate therapeutic intervention targeting mutant presenilin 2-induced Alzheimer's disease.

Key words: Neuronal cell, Presenilin 2, Acetylcholine esterase, Alzheimer's disease

INTRODUCTION

A presenilin 2 (PS2) mutation is believed to be involved in the development of Alzheimer's disease (AD) (Vito *et al.*, 1996; Vito-Wolozin *et al.*, 1996; Alves da Costa *et al.*, 2002; Tamotsu *et al.*, 2003). However, the mechanism for how PS2 is involved in AD is unclear. The learning and memory impairment observed in PS2 transgenic mice, particularly in mutant PS2 transgenic ones (Hwang *et al.*, 2002) strongly suggested the involvement of the PS2 gene in the neuronal degeneration in AD. It was also reported that the PS2 mutation, N141I, caused in increase in $A\beta_{42}$ production (Hwang *et al.*, 2002), and PC12 cells treated with $A\beta_{25-35}$ showed an increased proportion of apoptotic cell death (Song *et al.*, 2004). Moreover, a PS2 fragment, which consists of the 166 amino terminal aminoacid (the hydrophilic amino terminus and the first two transmembrane domains), was found to be sufficient for inhibiting DNA synthesis and inducing apoptosis (Janicki *et al.*, 1999). The overexpression of the wild type or mutant type (N141I) of the PS2 genes in neuronal cells causes a significant increase in the basal rate of cell death (Deng *et al.*, 1996; Janicki *et al.*, 1997; Araki *et al.*, 2001). Furthermore, cells expressing the N141I mutant PS2 were reported to have a significantly higher rate of apoptosis than the cells expressing the wild PS2 and the non-transfected cells (Mori *et al.*, 2002).

Several lines of evidence suggest that acetylcholine esterase (AChE) plays a role in AD. The presence of cortical AChE in the senile plaque and the promotion of the amyloid fiber assembly by AChE have been reported (Inestrosa *et al.*, 1996; Rees *et al.*, 2003). Moreover, AChE-Aβ complexes are more neurotoxic in culture than Aβ alone (Alvarez *et al.*, 1999). Besides the evidence showing that AChE activity is preserved in the aged normal cerebral cortex but significantly reduced in the entire neocortex and the hippocampus of AD brains in both the mild and moderate stages of the disease (Kuhl *et al.*, 1999), there are reports showing that the increased AChE activity around the plaque may be a direct consequence of amyloid deposition in AD (Saez-Valero *et al.*, 2002). This makes a strong case for an association

Correspondence to: Jin Tae Hong, College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea
Tel: 82-43-261-2813 Fax: 82-43-268-2732
E-mail: jinthong@chungbuk.ac.kr

between AChE and AD. However, the mechanism for how AChE is involved in AD is unclear. It was reported that AChE plays an important role in the process of neuroblastoma apoptosis (Yang *et al.*, 2002), and neuronal stress in AD may enhance the AChE activity (Li *et al.*, 2000). Several studies have shown that beta amyloid peptides can stimulate the expression of AChE in P19 cells (Sberna *et al.*, 1997), retinal cells (Melo *et al.*, 2003), N1E.115 neuroblastoma cells (Hu *et al.*, 2003), S42 cells (Zhang *et al.*, 2003) and even in neuronal and astrocyte cultures (Saez-Valero *et al.*, 2003; Fodero *et al.*, 2004).

Therefore, in order to further understand the role of PS2 and the relationship between this gene mutation and AD, this study compared the response of the normal PC12 cells, PC12 cells expressing wild PS2 and PC12 cells expressing the mutant PS2 under the apoptotic stimulation. AD is normally classified according to the age of onset (Rocchi *et al.*, 2003). Accordingly, this study examined PS2 transgenic mice at different ages. The main focus of this study was to determine the changes in the acetylcholine esterase activity in the three cell types treated with $A\beta_{25\text{-}35}$, L-glutamate and H_2O_2. In addition, the changes in the acetylcholine esterase activity in the brains from PS2 transgenic mice were examined to determine if these changes are related to neuronal cell death.

MATERIALS AND METHODS

Gene construction and stable transfection of mutant or wild PS2 into PC12 cells

The plasmids, pNSE-PS2mt and pNSE-PS2wt, were constructed by ligating the NSE (neuron-specific enolase) promoter-containing Hind III-EcoR1 fragment from pNSE-CAT and either the mutant PS2 (PS2mt) gene (N141I, Volga German Families) or the wild type PS2 (PS2wt) gene containing the Hind III-EcoR1 from plasmid pUHD-10-3. The pNSE-CAT and pUHD-10-3 were kind gifts from Dr. J. Gregor Sutcliffe at the Research Institute of Scripps and Dr. Tae-Wan Kim at the Columbia University, respectively. The resulting pNSE-PS2mt construct carried the 1.8 kb promoter fragment fused to a PS2mt fusion gene, as described elsewhere (Hwang *et al.*, 2002). PC12 cells are a rat cell line derived from a pheochomocytoma cells, and were maintained on tissue culture plastic in Dulbecco's modified Eagle medium (DMEM) and a F-12 nutrient (GIBCO BRL, Gaithersburg, MD, U.S.A.) supplemented with 10% with heat-inactivated horse serum (HS), 5% with fetal bovine serum (FBS) and penicillin (100 units/ml), streptomycin (100 units/mL) at 37°C under an atmosphere containing 5% CO_2 and 95% air. The cells were seeded on the culture plates and incubated until they reached 65-70% confluence. The culture media was changed 3 times per week. The constructed plasmid or plasmid containing the wild or mutant type PS2 or plasmid containing the NEO construct as a vector control were transfected into the PC12 cells using lipofectAMINE PLUS in OPTI-MEM according to manufacture's instructions (Invitrogen, Carlsbad, CA, U.S.A.), as previously described (Nguyen *et al.*, 2005). After 24 h, the culture medium was changed with the selection medium containing geneticin (G418 0.25 mg/mL) to select the stable colonies expressing PS2 (wild type or mutant type). Western blotting was carried out after three weeks to screen the cells expressing PS2 (wild type or mutant type). The stable transfected clones were selected according to their similar increased expression of the PS2-like relative holoproteins (54 kDa) and the N- and C-terminal maturation fragment (data not shown).

Mutant and wild type PS2 transgenic mice

Transgenic mice expressing the wild type PS2 and mutant PS2 described elsewhere (Hwang *et al.*, 2002) were used. The transgenic lines and age-matched control mice were handled in an accredited Korea FDA animal facility in accordance with the AAALAC International Animal Care guidelines. The mice were housed in cages under a strict light cycle (light on at 06:00 and off at 18:00). All the mice were given a standard irradiated chow diet (Purina Mills, St. Louis, MO) *ad libitum*. The mice were maintained in a specified pathogen-free state. All the pedigrees were hemizygous for the transgene.

Cell culture and treatment

The cells were cultured in poly D-lysine coated plastic dishes. The medium was changed every 48 h. The cells were seeded at a density of 0.5×10^6 cells/cm^2. Stock solutions of $A\beta_{25\text{-}35}$, L-glutamate and H_2O_2 were dissolved in de-ionized distilled water at 1 mM, 0.5 M and 10 mM, respectively. The stock solution (at -20°C) was diluted to the required concentrations immediately before use, and added to the culture medium. After treating the cells with the stimuli for 6 h, they were harvested for DNA and RNA isolation or to determine the AchE level.

Acetylcholine esterase assay

The Acetylcholine esterase assay was performed according to the Ellman method (Ellman *et al.*, 1961). Briefly, 5-10 μL of the sample was mixed with 200 ml of a reaction buffer (50 mM Tris-HCl pH 8.0 containing 0.01% DTNB, 0.02% acetylthiocholine, 0.1 mM iso-OMPA) in parallel with the corresponding samples mixed with 200 μL of the buffer without the substrate. The activity of the enzyme was determined after 10 min of incubation at 37°C. The optical density was measured at 405 nm, which corresponded to the quantity (nmole) of the acetylthiocholine that was hydrolyzed to thiocholine for 1 min per 1 mL

enzyme solution. The enzyme activity is expressed in units of nmole/min/mg protein.

Statistical analysis

The data was analyzed using one way analysis of variance followed by a Tukey's test as a post hoc test. P values <0.05 (marked with *) and p<0.001 (marked with **) were considered significant.

RESULTS

Increase in the AChE activity of the mutant PS2 transfected PC12 cells under apoptotic stimulating conditions

The dose-dependent apoptosis of the PC12 cells and PC12/Neo cells under the $A\beta_{25-35}$ treatment is reported elsewhere (Song *et al.*, 2004). In this study, the cultured cells expressing the PS2wt (PC12/PS2wt) and PS2mt (PC12/PS2mt) as well as the cells carrying the empty control vector (PC12/Neo) were exposed to the 50 μM $A\beta_{25-35}$, 30 mM L-glutamate or 50 μM H_2O_2 for 6 h and harvested for analysis. The results showed that the increases in AChE activity in the PC12/PS2wt and PC12/PS2mt were higher than that in the PC12/Neo under the same treatment conditions (Figs. 1A, B and C). However, there was no significant difference between the control samples, in which the enzyme activity was always significantly higher in the PC12/PS2mt cells than in the PC12/PS2wt cells. Compared with the PC12/Neo cells, the AChE activities of the PC12/PS2wt and PC12/PS2mt cells in the 50 μM $A\beta_{25-35}$ were significantly higher than that of the corresponding untreated cells (Fig. 1A). In the case of the 30 mM L-glutamate treatment, the increases in the AChE activity of the PC12/PS2wt and PC12/PS2mt cells were similar to the corresponding control cells (Approximately 1.2 fold increase, SE = ± 0.26 to PC12/PS2wt; SE = ± 0.37 to PC12/PS2mt) (Fig. 1B). Under the treatment condition of 50 mM H_2O_2, the AChE activity of the PC12/PS2wt cells normalized to the corresponding untreated samples was approximately 1.2 times higher than that the PC12/Neo cells (SE = ± 0.16 to PC12/PS2wt, SE = ± 0.26 to PC12/Neo), while the AChE activity in the PC12/PS2mt cells normalized to the corresponding untreated samples was approximately 1.4 times higher than that in the PC12/Neo cells (SE = ± 0.24 to PC12/PS2mt) (Fig. 1C).

Increase AChE activities of the PS2 transgenic mice brain tissues

The AChE activity was examined in the brain tissues PS2 (wild type/mutant type) transgenic mice as *in vivo* study model in order to further investigate the involvement

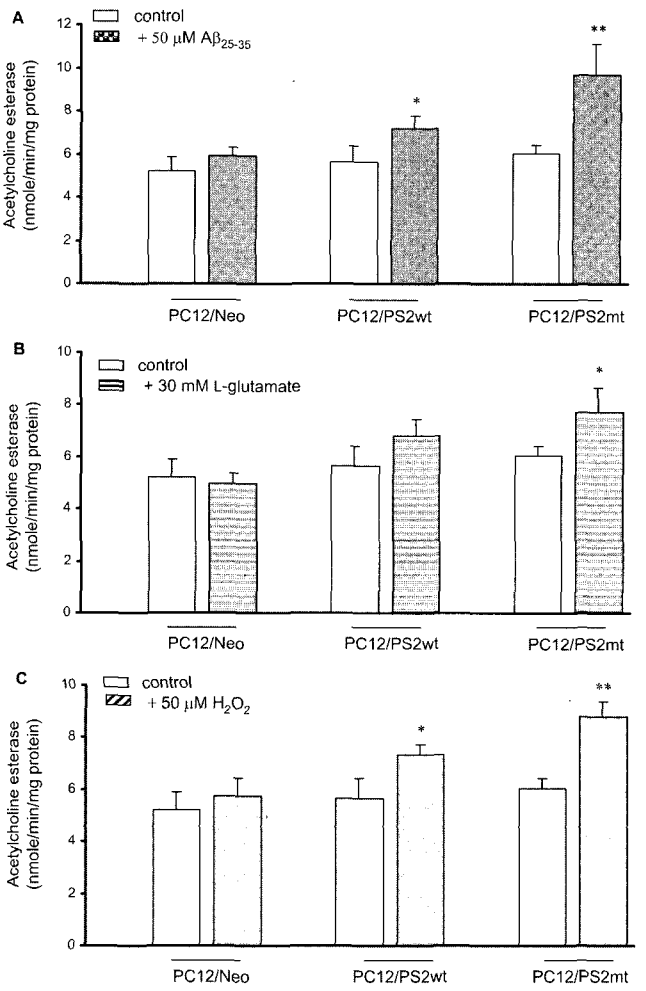

Fig. 1. Acetylcholine esterase activities of three cell types: PC12/Neo, PC12/PS2wt, PC12/PS2mt treated with 50 μM $A\beta_{25-35}$ (A), 30 mM L-glutamate (B), 50 μM H_2O_2 (C). The cells were cultured until they reached 80%-90% confluence. The cells were then exposed to the stimuli for 6 h, in parallel with the untreated cells (control samples), and harvested for AChE extract. The data is represented as a mean ± SD (bars) of three independent experiments carried out in duplicate (n=6). Significant differences versus the corresponding control samples were assessed using one way analysis of variance followed by a Tukey's post hoc test (*p<0.05, ** p<0.001).

of the mutant PS2 gene (N141I) in the potential of neuronal degeneration. PS2 transgenic mice (Tg-wt and Tg-mt) and non transgenic mice (non-Tg) at 5 different ages were used: 2, 6 (young mice), 12, 18, 24 months (old mice). There was a significant increase in the enzyme activity in the older mouse brain tissues, including the cortex and hippocampus, of the Tg-wt and Tg-mt mice (Figs. 2A and B). The AChE activities in the Tg-wt and Tg-mt at 24 month old mice was approximately 1.5 to 2 times higher than that in the non-Tg samples at the same age (Figs. 2A and B).

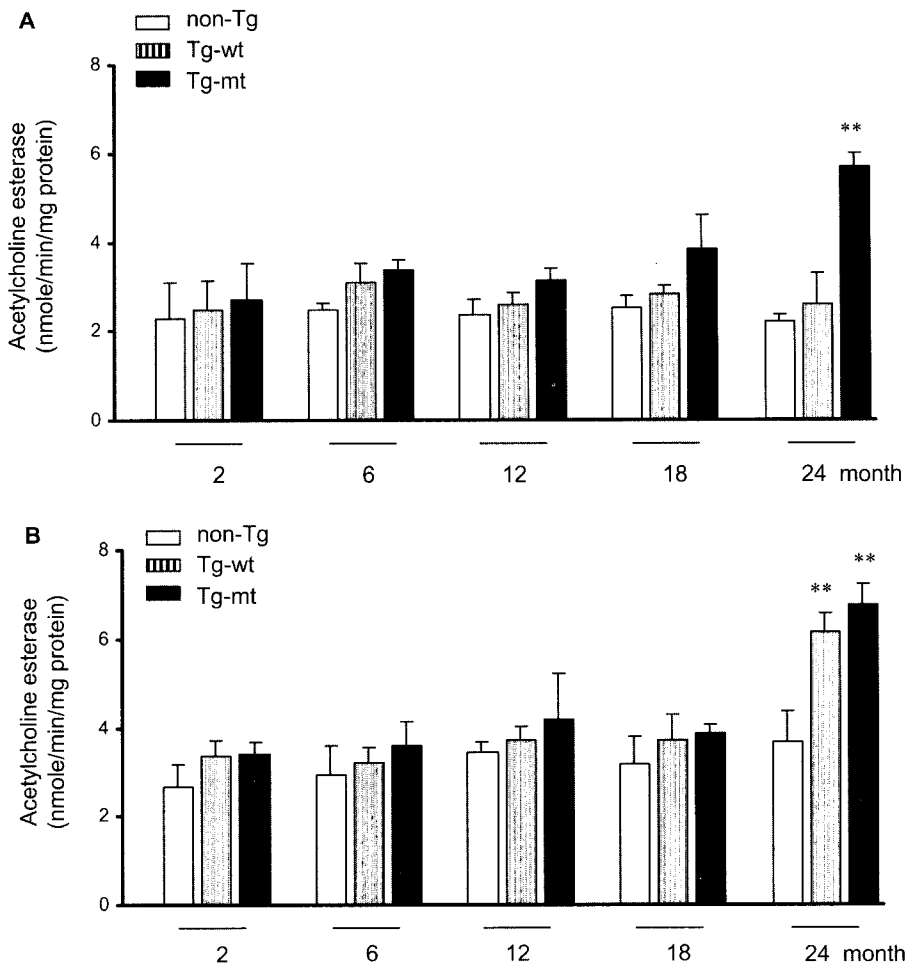

Fig. 2. Acetylcholine esterase activities of the mouse brain tissues. The brain of non-transgenic mice, wild type PS2 transgenic mice and mutant PS2 transgenic mice at 2, 6, 12, 18, 24 months were separated into the cortex (A) and hippocampus (B) in order to determine the enzyme activity assay. The data is represented as a mean ± SD (bars) at least three animals ($n \geq 3$). Significant differences versus the non-Tg samples were assessed using a one way analysis of variance followed by a Tukey's post hoc test (*$p<0.05$, ** $p<0.001$).

DISCUSSION

The learning and memory impairment of PS2 transgenic mice, particularly in the case of mutant PS2 transgenic mice, correlated with the increase in PS2 wild type (PS2wt) and PS2 mutant type (PS2mt) expression in the cortex and hippocampus of the transgenic mice described in a previous study (Hwang *et al.*, 2002). In another in vitro study, western blot analysis showed that the levels of the PS2-like protein and that of the C and N terminal PS2 fragments were higher in all three PS2wt transfected and PS2mt transfected PC12 cell lines than in the PC12 and the empty vector transfected PC12 cells. 50 μM $A\beta_{25-35}$, 30 mM L-glutamate or 50 μM H_2O_2 caused the apoptotic cell death of these cells in a time dependent manner 24 h to 72 h after the stimuli (Lee *et al.*, 2005). This study further investigated how the mutant PS2 (N141I) influences the AChE activity in both the *in vitro* and *in vivo* AD models and whether or not these changes are related to neuronal cell death.

The results showed no significant change in the AChE activity in the PC12/Neo cells under apoptotic stimulation, whereas the AChE activity increased in the apoptotic stimulated PC12/PS2wt cells, suggested the involvement of the PS2 gene in the change in AChE activity. Moreover, the results showing that the AChE activity enhancements were remarkably higher in the treated PC12/PS2mt cells than in the treated PC12/PS2wt cells suggests that a mutation of PS2 affects the susceptibility of AChE activation in the cells in the apoptotic pathway. In addition, AChE expression was reported to increase the number of apoptotic neuroblastoma cells after a long-term culture (Lei *et al.*, 2002), and AChE activity was reported to increases around the plaque (Saez-Valero *et al.*, 2002). Therefore, AChE might be an exacerbating factor in neuronal apoptosis and may participate not only in the

normal development of mammalian neuronal cells but also in the capacity to respond to external insults. Hence, the PS2 mutation may induce neuronal apoptosis by enhancing the AChE activity. The clear increase in AChE activity in the Tg-mt brains even without stimulation, particularly in the older mouse brains, suggests that PS2 may play a role in controlling AChE activation, and a mutation of PS2 may further enhance AChE activation in older mouse brains. Acetylcholine, which is a necessary neurotransmitter in synapse system, is digested through the catalytic action of AChE. Therefore, the enhancement of AChE activity in the cells expressing the mutant PS2 can decrease the neuro-transmittance capacity in cells, which may be involved in the memory deficiencies associated with AD. On the other hand, it was reported that neuronal stress in AD may cause an increase in AChE expression and activity through a molecular cascade that is mediated *via* secreted beta-APP-induced microglial activation (Li *et al.*, 2000). The AChE purified from bovine and human brains as well as mouse recombinant AChE have all been demonstrated to convert the alpha-helical, inert and wild form of the $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides as well as the two mutant fragments ($A\beta_{12-28}$ Val18→Ala and Glu→Gln) into the beta-pleated Aβ fibrils, which help promote the neurodegeneration in AD brains (Shen, 2004). Overall, there was a relationship between oxidative stress and the increase in AChE activity. Either there is a deficiency in the catalytic function of this enzyme to remove excess acetylcholine or the enzyme enhances APP processing leading to an increase in the neurotoxic Aβ peptide level.

In conclusion, the mutant PS2 clearly increases the AChE activity, which might be implicated in the Aβ aggregation in AD. These findings might useful for the development of an appropriate therapeutic intervention for targeting mutant PS2-induced AD cases.

ACKNOWLEDGEMENTS

This work was supported by a grant (R05-2002-000-00644-0) from the Basic Research Program of the Korea Science and Engineering Foundation and Research fund from the Korea Research Institute of Bioscience and Biotechnology (2003-2004).

REFERENCES

Alvarez, A., Alarcon, R., Opazo, C., Campos, E. O., Munoz, F.J., Calderon, F.H., Dajas, F., Gentry, M. K., Doctor, B. P., De Mello, F. G., and Inestrosa, N. C., Stable complexes involving acetylcholinesterase and amyloid beta peptide change the biochemical properties of the enzyme and increase the neurotoxicity of Alzheimer's fibrils. *J. Neurosci.*, 18, 3213-3223 (1999).

Alves da Costa, C., Paitel, E., Mattson, M. P., Amson, R., Telerman, A., Ancolio, K., and Checler, F., Wild-type and mutated presenilins 2 trigger p53-dependent apoptosis and down-regulate presenilin 1 expression in HEK293 human cells and in murine neurons. *Proc. Natl. Acad. Sci. U.S.A.*, 99, 4043-4048 (2002).

Araki, W., Yuasa, K., Takeda, S., Takeda, K., Shirotani, K., Takahashi, K., and Tabira, T., Pro apoptotic effect of presenilin 2 (PS2) overexpression is associated with down regulation of Bcl2 in cultured neurons. *J. Neurochem.*, 79, 1161-1168 (2001).

Deng, G., Pike, C. J., and Cotman, C. W., Alzheimer's associated presenilin2 confers increased sensitivity to apoptosis in PC12 cells. *FEBS Lett.*, 397, 50-54 (1996).

Ellman, G. L., Courtney, K. D., Andres, V. Jr., and Featherstone, R. M., A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, 7, 88-95 (1961).

Fodero, L. R., Mok, S. S., Losic, D., Martin, L. L., Aguilar, M. I., Barrow, C. J., Livett, B. G., and Small, D. H., Alpha7-nicotinic acetylcholine receptors mediate an Abeta (1-42)-induced increase in the level of acetylcholinesterase in primary cortical neurones. *J. Neurochem.*, 88,1186-1193 (2004).

Hu, W., Gray, N. W., and Brimijoin, S., Amyloid-beta increases acetylcholinesterase expression in neuroblastoma cells by reducing enzyme degradation. *J. Neurochem.*, 86, 470-478 (2003).

Hwang, D. Y., Chae, K. R., Kang, T. S., Hwang, J. H., Lim, C. H., Kang, H. K., Goo, J. S., Lee, M. R., Lim, H. J., Min, S. H., Cho, J. Y., Hong, J. T., Song, C. W., Paik, S. G., Cho, J. S., and Kim, Y. K., Alterations in behavior, amyloid beta-42, caspase-3, and Cox-2 in mutant PS2 transgenic mouse model of Alzheimer's disease. *FASEB J.*, 16, 805-813 (2002).

Inestrosa, N. C., Alvarez, A., Perez, C. A., Moreno, R. D., Vicente, M., Linker, C., Casaneuva, O. I., Soto, C., and Garrido, J., Acetylcholinesterase accelerates assembly of amyloid beta peptides into Alzheimer's fibrils: Possible role of the periferal site of the enzyme. *Neuron*, 16, 881-891 (1996).

Janicki, S. M. and Monterio, M. J., Presenilin overexpression arrests cells in the G1 phase of cell cycle. Arrest potentiated by the Alzheimer's disease PS2 (N141I) mutant. *Am. J. Pathol.*,155, 135-144 (1999).

Janicki, S. and Montaeiro, M. J., Increase apoptosis arising from increased expression of the Alzheimer's disease-associated presenilin 2 mutation (N141I). *J. Cell Biol.*, 139, 485-495 (1997).

Kuhl, D. E., Koeppe, R. A., Minoshima, S., Snyder, S. E., Ficaro, E. P., Foster, N. L., Frey, K. A., and Kilbourn, M. R., *In vivo* mapping of cerebral acetylcholinesterase activity in aging and Alzheimer's disease. *Neurology*, 52, 691-699 (1999).

Lee, S. Y., Hwang D. Y., Kim, Y. K., Lee, J. W., Shin, I. C., Oh, K. W., Lee, M. K., Lim, J. S., Yoon, D. Y., Hwang, S. J., and Hong, J. T., PS2 mutation increases neuronal cell vulnerability to neurotoxicants through activation of caspase-3 by enhancing of ryanodine receptor-mediated calcium release. *FASEB J.*, in press (2005).

Lei, Y., Heng, Y. H., and Xue, J. Z., Increase expression of intranuclear AChE involved in apoptosis of SK-N-SH cells. *Neurosci. Res.*, 42, 261-268 (2002).

Li, Y., Liu, L., Kang, J., Sheng, J. G., Barger, S. W., Mrak, R. E., and Griffin, W. S., Neuronal-glial interactions mediated by interleukin-1 enhance neuronal acetylcholinesterase activity and mRNA expression. *J. Neurosci.*, 20,149-155 (2000).

Melo, J. B., Agostinho, P., and Oliveira, C. R., Involvement of oxidative stress in the enhancement of acetylcholinesterase activity induced by amyloid beta-peptide. *Neurosci. Res.*, 45,117-127 (2003).

Mori, M., Nakagami, H., Morishita, R., Mitsuda, N., Yamamoto, K., Yoshimura, S., Ohkubo, N., Sato, N., Ogihara, T., and Kaneda, Y., N141I mutant presenilin 2 gene enhances neuronal cell death and decrease bcl2 expression. *Life Sci.*, 70, 2567-2580 (2002).

Nguyen, H. N., Lee, S. Y., Hwang, D. Y., Kim, Y. K., Yuk, D. Y., Lee, J. S., and Hong, J. T., Decrease in NF-kappaB, AP-1 and SP-1 activities in neuronal cells expressing presenilin 2. *Neuroreport*, 16, 731-735 (2005).

Rees, T., Hammond, P. I., Soreq, H., Younkin, S., and Brimijoin, S., Acetylcholinesterase promotes beta-amyloid plaques in cerebral cortex. *Neurobiol. Aging*, 24, 777-787 (2003).

Rocchi, A., Pellegrini, S., Siciliano, G., and Murri, L., Causative and susceptibility genes for Alzheimer's disease: A review. *Brain Res. Bull.*, 61, 1-24 (2003).

Saez-Valero, J., Fodero, L. R., White, A. R., Barrow, C. J., Small, D. H., Acetylcholinesterase is increased in mouse neuronal and astrocyte cultures after treatment with beta-amyloid peptides. *Brain Res.*, 965, 283-286 (2003).

Sberna, G., Saez-Valero, J., Beyreuther, K., Masters, C. L., and Small, D. H., The amyloid beta-protein of Alzheimer's disease increases acetylcholinesterase expression by increasing intracellular calcium in embryonal carcinoma P19 cells. *J. Neurochem.*, 69, 1177-1184 (1997).

Shen, Z. X., Brain cholinesterase: The molecular and cellular basis of Alzheimer's disease. *Med. Hypotheses*, 63, 308-321 (2004).

Song, Y. S., Park, H. J., Kim, S. Y., Lee, S. H., Yoo, H. S., Lee, H. S., Lee, M. K., Oh, K. W., Kang, S. K., Lee, S. E., and Hong, J. T., Protective role of Bcl-2 on beta-amyloid-induced cell death of differentiated PC12 cells: Reduction of NF-kappaB and p38 MAP kinase activation. *Neurosci. Res.*, 49, 69-80 (2004).

Tamotsu, H. G., Atsushi, T., Yoshihisa, W., Naoyuki, I., Takashi, M., and Takeshi, T., A unifying model for functional difference and redundancy of presenilin-1 and -2 in cell apoptosis and differentiation. *Gene*, 34257, 1-9 (2003).

Vito, P., Lacana, E., and D'Adamio, L., Interfering with apoptosis: Ca-binding protein ALG-2 and Alzheimer's disease gene ALG-3. *Science*, 271, 521-525 (1996).

Vito, P., Wolozin, B., Ganjei, J. K., Iwasaki, K., Lacana, E., and D'Adamio, L., Requirement of the familial Alzheimer's disease gene PS2 for apoptosis. Opposing effect of ALG-3. *J. Biol. Chem.*, 271, 31025-31028 (1996).

Yang, L., He, H. Y., Zhang, X. J., Increased expression of intranuclear AChE involved in apoptosis of SK-N-SH cells. *Neurosci. Res.*, 42, 261-268 (2002).

Zhang, H. Y., Brimijoin, S., and Tang, X. C., Apoptosis induced by beta-amyloid 25-35 in acetylcholinesterase-overexpressing neuroblastoma cells. *Acta Pharmacol. Sin.*, 24, 853-858 (2003).