Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 458 (2003) 235–241
www.elsevier.com/locate/ejphar

Propentofylline protects β-amyloid protein-induced apoptosis in cultured rat hippocampal neurons

Yoshiki Koriyama*, Kenzo Chiba, Tetsuro Mohri

Department of Biodynamics, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Ishikawa 920-1181, Japan

Received 18 July 2002; received in revised form 11 November 2002; accepted 15 November 2002

Abstract

β-Amyloid protein 1–42 (β42) can induce apoptosis in the cultured hippocampal neurons, suggesting that it plays an important role in causing neurodegeneration in Alzheimer's disease. Recently, propentofylline, a synthetic xanthine derivative, has been reported to depress ischemic degeneration of hippocampal neurons in gerbils. The present study investigated whether or not propentofylline affected the β42-induced apoptosis of hippocampal neurons, and if so, which type of signaling machinery works in the neuroprotective action of propentofylline. Addition of propentofylline markedly attenuated the β42-induced cell death of rat hippocampal neurons. The amyloid protein certainly induced apoptosis in the cultured hippocampal cells revealed by nuclear condensation, caspase-3 activation and an increase of Bax. Intriguingly, propentofylline blocked both the apoptotic features induced by β42 and further induced an anti-apoptotic protein, Bcl-2, during a short time of incubation. The neuroprotective action of propentofylline was comparably replaced with dibutyryl cAMP (dbcAMP) and was completely suppressed by a low concentration of specific protein kinase A (PKA) inhibitor. Taken altogether, the data strongly suggest that the protection of propentofylline on the β42-induced neurotoxicity is caused by enhancing anti-apoptotic action through cAMP–PKA system. Propentofylline as a therapeutic agent to Alzheimer's disease is discussed.

Keywords: β-Amyloid protein; Hippocampal neuron; Propentofylline; cAMP; Protein kinase A; Apoptosis

1. Introduction

β-Amyloid protein, the major component of senile plaques, plays a critical role in the neuropathology of the brain in Alzheimer's disease. β-Amyloid protein is mainly composed of two molecular species of spontaneously aggregating peptide of 40 and 42 (β40 and β42) amino acid residues, and has been reported to be neurotoxic to hippocampal and cortical neurons (Pike et al., 1991; Iwatsubo et al., 1994; Tamaoka et al., 1994). However, the mechanism of β42-induced neurotoxicity is still unclear. In culture systems, β42 caused the cell death of various neurons through an apoptotic (Cotman and Anderson, 1995; Estus et al., 1997; Guo et al., 1999) or a necrotic mechanism (Behl et al., 1994; Gschwind and Huber, 1995).

Propentofylline is a novel xanthine derivative with pharmacological effects different from those of the classical methylxanthines theophylline and caffeine. It has been developed as a treatment for dementia (Saletu et al., 1990; Marcusson et al., 1997). Propentofylline depresses activation of microglial cells and astrocytes which is associated with neuronal damage during ischemic injury of gerbil brain (DeLeo et al., 1988; Schubert et al., 1994). Our previous paper (Koriyama et al., 2000) demonstrated that propentofylline attenuated cell death induced by β42 and remarkably elevated cAMP level in hippocampal neurons in a dose-dependent manner. Recently, Parvathenani et al. (2000) showed that cAMP analog delayed β-amyloid 25–35 (a synthetic active fragment of amyloid protein, β25–35)-induced neurotoxicity of rat cortical neurons, although there is a question whether or not a native β42 and this β25–35 induced the same neurotoxicity. Therefore, the present study aims to answer this question: Does propentofylline have a direct neuroprotective effect on the β42-induced cytotoxicity of cultured rat hippocampal cells with a similar mechanism of cAMP? Our results show that propentofylline prevents the β42-induced cell death via a

* Corresponding author. Department of Molecular Neurobiology, Graduate School of Medicine, University of Kanazawa, 13-1 Takara-machi, Kanazawa, Ishikawa 920-8640, Japan. Tel.: +81-76-265-2451; fax: +81-76-234-4235.
E-mail address: koriyama@med.kanazawa-u.ac.jp (Y. Koriyama).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02789-9

cAMP system by enhancing anti-apoptotic action in hippocampal neurons.

2. Materials and methods

2.1. Animal care and use

Female Wistar rats (Japan SLC, Japan) were used according to the Japanese Pharmacological Society's Animal Guiding Principles for the Care and Use of Laboratory Animals.

2.2. Cell culture and treatment with β42 or drugs

Primary hippocampal cells were isolated from 17- to 18-day-old fetal rats (Wistar) and cultured in 48-well culture plates, unless otherwise specified, with Dulbecco's modified Eagle medium (DMEM) and 10% of fetal calf serum for 2 days. Then the cells were incubated with 10 μM cytosine-β-D-arabino-franoside for 24 h to halt cell division and select neuronal cells. Cell layers were washed with calcium- and magnesium-free phosphate-buffered saline (PBS (−)) and cultured with serum-free DMEM to treat cells with β42 and/or drugs for up to 8 days as specified in the Results. β42 was purchased from the American Peptide Company (California, USA). The peptide was dissolved in PBS (−) at 200 μM and stored at 37 °C for a week before use to promote its aggregation. Propentofylline was purchased from Hoechst Marion Roussel (Tokyo, Japan) and dissolved in dimethyl sulfoxide (DMSO) at 10 mg/ml. Caspase inhibitor, *N*-acetyl-Asp-Glu-Val-Asp-aldehyde (Ac-DEVD-CHO, Peptide Institute, Japan), protein kinase A (PKA) inhibitors, *N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89) and *N*-[2-(*N*-formyl-*p*-chlorocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-85) (Seikagaku Kogyo, Japan) were all dissolved in DMSO.

2.3. Hoechst33258 staining

Hippocampal neurons cultured in glass-bottom dishes were washed twice with PBS (−) and fixed with 0.1% glutaraldehyde in PBS (−) at room temperature for 1 h. After fixation, the dishes were washed and exposed to 100 μg/ml Hoechst33258 (Wako) in PBS (−) at room temperature for 5 min. Then, the neurons were observed with a fluorescence microscopy.

2.4. Cell viability

Two determinations of lactate dehydrogenase (LDH) released into the medium and ability of cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were used to evaluate cell viability. To determine the activity of LDH released, 0.25 ml of medium was subjected to the conventional absorption assay at 340 nm for each well after treatment of cells with specified drugs as described previously (Takadera et al., 1993). Data (ΔA/min) were expressed relative to each control. To determine the ability of cells to reduce MTT, 20 μl of solution of 2.75 mg/ml MTT in PBS (−) was added to each well. The plates were incubated at 37 °C for 25 min before 0.2 ml/well of 0.04 N HCl dissolved in isopropanol was added to them to solubilize the reaction product formazan. Absorption at 550 nm of each sample solution was measured with a microplate reader (ImmunoMini NJ-2300, InterMed) for 96-well plate. Data were expressed relative to each control.

2.5. Caspase-3 activity assay

At 24 h treatment of β42 in 35-mm dishes after culture for selection, as described for cells of culture plates, each dish of cell layers was rinsed with cold PBS (−) and harvested with a rubber policeman into 250 μl of Tris buffer (50 mM Tris, 1 mM EDTA, 1 mM dithiothreitol, pH 7.5) to homogenize in an injection syringe with 25G needle. Each cell homogenate was sonicated and centrifuged for 1 h at 15,000 rpm. Samples of the supernatant (30 μg protein per sample) were incubated with a fluorogenic substrate, Ac-DEVD-7-amino-4-methylcoumarin (AMC) (20 μM), specific for caspase-3 at 37 °C for 1 h. Ac-DEVD-AMC was purchased from the Peptide Institute and dissolved in DMSO at 10 mM. The reaction was stopped by addition of 2.5 mM monoiodoacetic acid. Cleavage of the substrate by caspase-3 was measured by the fluorescence of the product, AMC, using a Hitachi 850 fluorescence spectrophotometer (excitation at 380 nm and emission at 460 nm). Protein content of the samples for various assays was determined according to the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.6. Western blot analysis of Bcl-2 and Bax

Hippocampal cell extracts were prepared by washing the cells attached to 35-mm dishes three times with ice-cold PBS after treatment, as specified in the Results, and lysing them in a buffer solution (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.2 mM phenyl methyl sulfonyl fluoride, 0.5% NP-40, protease inhibitor cocktail (Sigma)). Protein content of samples for various assays was determined using bovine serum albumin as standard. Each sample of 15 μg protein supernatants obtained by centrifugation of the extracts at $1000 \times g$ for 10 min was boiled with twofold volumes of buffer solution (0.02% bromophenol blue, 3% sodium dodecyl sulfate (SDS), 2-mercaptoethanol, 30% glycerol, 30 mM Tris–HCl) for 5 min and subjected to electrophoresis on 12.5% SDS–polyacrylamide gel. After the proteins were transferred onto the nitrocellulose membrane (Amersham International, UK),

they were incubated in blocking solution (3% bovine serum albumin, 0.1% NP-40 in PBS) at room temperature for 30 min and then probed with the anti-mouse Bcl-2 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and anti-rabbit Bax antibody (Santa Cruz Biotechnology) in blocking solution at 4 °C overnight. The membrane was washed five times in 10 mM Tris–buffer (pH 8.0) containing 0.05% Tween 20, probed with the secondary antibody (alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G (IgG) or anti-rabbit IgG in blocking solution at 4 °C for 1 h and washed again in the 10 mM Tris–buffer. Detection of the signal was performed with a detection kit, 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium phosphatase substrate system (Kirkegaard Perry Laboratories, USA). To confirm the amount of protein visually on acrylamide gel, we performed staining for protein by rapid stain Coomassie brilliant blue kit (Nacalai Tesque, Kyoto).

3. Results

3.1. Propentofylline prevents β42-induced apoptosis of hippocampal neurons

In our culture system, β42-induced cell death of rat hippocampal neurons was estimated by MTT reduction (Fig. 1A) and LDH release (Fig. 1B) assays. Twenty micromolars of β42 induced a 70% decrease of MTT reduction and 200% increase of LDH release as compared with control values for 7 days. Most of the cells lost their fibrous networks and specific cell shape. In the presence of 20 μg/ml propentofylline, β42 neurotoxicity was dramatically suppressed in both assays (Fig. 1). Propentofylline itself did not affect the cell viability of hippocampal cells. The addition of propentofylline at lower concentration than 20 μg/ml had no effect on either basal level of control and β42-induced neurotoxicity (data not shown).

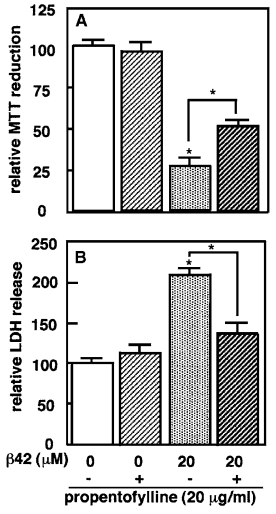

Fig. 1. Prevention of β42 neurotoxicity by propentofylline in hippocampal neurons. Cultures were incubated with or without 20 μg/ml propentofylline for 1 h before treatment with or without β42 for 7 days as indicated. Values represent the mean ± S.E.M. ($n = 5$) of MTT reduction (A) and LDH release (B) assays in percentage of control. *$P < 0.01$ vs. control or β42 only. ANOVA, Student's *t*-test.

To examine whether or not the β42-induced cell death is apoptotic, we stained the cells with Hoechst33258. In comparison with the control (Fig. 2A) and propentofylline alone (Fig. 2B), β42 (20 μM) brought about apoptotic cell death with nuclear chromatin condensation, as shown in Fig. 2C. Adding propentofylline (20 μg/ml) to the medium clearly suppressed the nuclear condensation induced by β42 (Fig. 2D).

3.2. Propentofylline prevents β42-induced caspase-3 activation of hippocampal neurons

Activation of caspases is now well accepted to be responsible for causing apoptotic cell death (Matsuzawa and Ichijo, 2001). In particular, caspase-3 is a major executioner of the apoptotic signals. We therefore evaluated the β42 toxicity with a caspase-3 inhibitor of Ac-DEVD-CHO. β42 induced a 30% decrease in MTT reduction after 2 days (Fig. 3). In the presence of caspase-3 inhibitor (10 μM), a small but significant suppression of β42 induced a decrease in MTT reduction. The caspase-3 inhibitor itself did not affect cell viability. A similar suppression of β42 neurotoxicity by Ac-DEVD-CHO (10 μM) could be seen after 5 days of culture in the LDH release assay (data not shown). As the β42-induced cell death was involved in the activation of caspase-3, we next measured the caspase-3 activity in our culture system. Caspase-3 activity of rat hippocampal neurons was remarkably elevated (about 2.2-fold) 24 h after treatment of 20 μM β42 (Fig. 4). Addition of propentofylline (20 μg/ml) to the medium mostly, but not fully, blocked the β42-induced activation of caspase-3. Dibutyryl cAMP (100 μM) also significantly blocked the caspase-3 activation by β42. In our previous study, addition of dbcAMP dose-dependently blocked the increase of LDH release induced by β42 (Koriyama et al., 2000).

3.3. Propentofylline modulates apoptosis-related proteins Bcl-2 and Bax protein

Bax and Bcl-2 are found in various cell types as pro- or anti-apoptotic members that initiate or hinder the caspase cascade. In particular, Bcl-2 has a central role in preventing adaptors from activating caspases. We therefore investigated the effect of propentofylline and β42 on the cellular level of Bcl-2 in hippocampal neurons. In this 6-h culture, propentofylline (20 μg/ml) itself induced a 24%

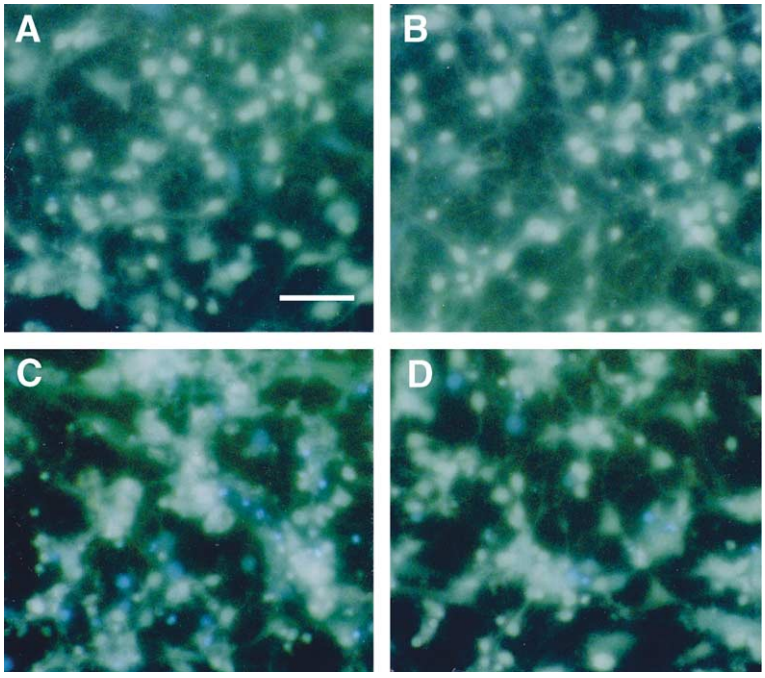

Fig. 2. Protective effect of propentofylline on nuclear condensation induced by β42. Hippocampal neurons were treated with (B, D) or without (A, C) 20 μg/ml propentofylline for 1 h before treatment with (C, D) or without (A, B) 20 μM β42 for 2 days. Cell nuclei were stained with Hoechst33258 and observed under fluorescence microscope. Scale bar = 50 μm.

increase in Bcl-2 levels compared with the control value, revealed by a 26 kDa protein in Western blotting (Fig. 5). Conversely, β42 itself reduced the level of Bcl-2 by half. Propentofylline (20 μg/ml) significantly depressed the β42-induced reduction of Bcl-2. In contrast to Bcl-2, β42 induced a 50% increase of Bax level after 6 h of culture (Fig. 6). Propentofylline actually suppressed the β42-induced Bax elevation revealed by a 23 kDa protein band in Western blotting, although propentofylline itself did not affect the level of Bax as shown in the Bcl-2.

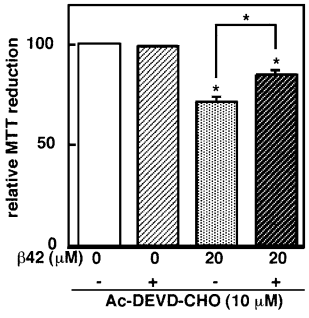

Fig. 3. Prevention of β42 neurotoxicity by caspase-3 inhibitor. Hippocampal neurons were incubated with or without a caspase inhibitor, Ac-DEVD-CHO, for 1 h and further incubated with or without β42 for 2 days as indicated. Values represent the mean ± S.E.M. ($n = 5–6$) of MTT reduction assays in percentage of control. *$P < 0.01$ vs. control or β42 only. ANOVA, Student's *t*-test.

3.4. Protein kinase A inhibitor blocks protective action of propentofylline

In our previous paper (Koriyama et al., 2000), propentofylline prevented the β42 neurotoxicity of rat hippo-

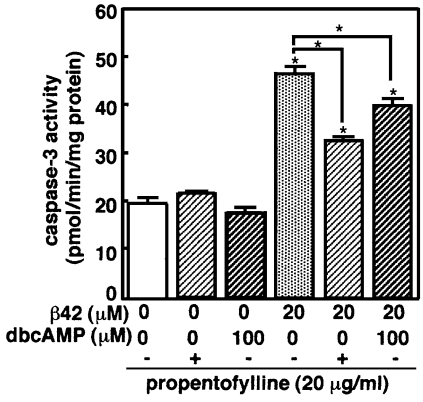

Fig. 4. Prevention of β42-induced caspase-3 activation by propentofylline. Hippocampal neurons were treated with or without propentofylline and dbcAMP for 1 h and further incubated with or without β42 for 24 h as indicated. Cells were lysed with Tris buffer, and 30 μg of protein for each sample was incubated with a fluorogenic substrate, Ac-DEVD-MCA (20 μM). Cleavage of the substrate emitted a fluorescent signal, which was measured by a fluorometer (excitation at 380 nm, emission at 460 nm). Details of the method are described in Materials and methods. Values represent the mean ± S.E.M. ($n = 3$). *$P < 0.01$ vs. control or β42 only. ANOVA, Student's *t*-test.

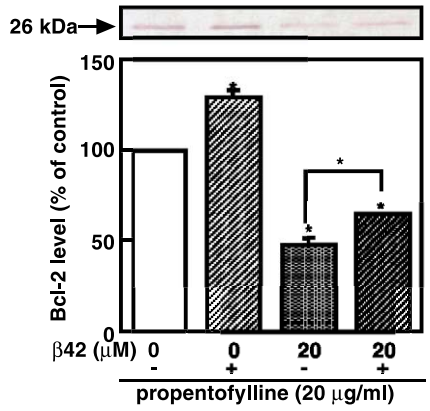

Fig. 5. Protective action of propentofylline on β42-induced Bcl-2 reduction. Cultures were incubated with or without 20 μg/ml propentofylline and/or β42 for 6 h. The supernatant of the extract of the cultures (15 μg protein/lane) was subjected to Western blot analysis. Insertions show a 26-kDa band of Bcl-2 protein. Details of the method are described in Materials and methods. Values represent the mean ± S.E.M. of triplicate experiments. *$P<0.01$ vs. control or β42 only. ANOVA, Student's *t*-test.

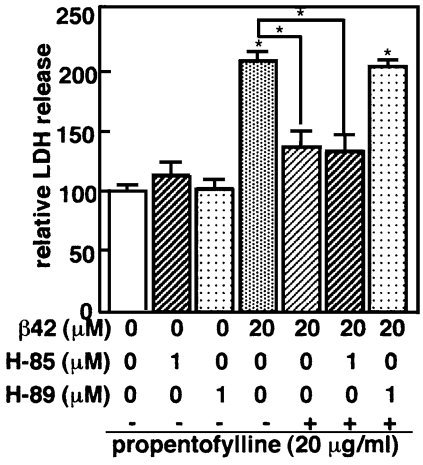

Fig. 7. Cancellation of the protective action of propentofylline on β42 neurotoxicity with PKA inhibitor. Hippocampal neurons were incubated with or without propentofylline and PKA inhibitors (1 μM) for 1 h and further incubated with or without β42 for 7 days as indicated. Values represent the mean ± S.E.M. ($n=5$) of LDH release assay in percent of control. *$P<0.01$ vs. control or β42 only. ANOVA, Student's *t*-test.

campal cells, accompanied with elevated intracellular levels of cAMP. In this study, dbcAMP suppressed the caspase-3 activation induced by β42 (Fig. 4). To explain the role of cAMP in the neuroprotection signal of propentofylline on the β42-induced degenerative neurons, we tested β42 neurotoxicity of cultured rat hippocampal neurons in the presence of propentofylline and PKA inhibitor simultaneously. H-89 (1 μM), a specific PKA inhibitor, almost completely blocked the neuroprotection of propentofylline on β42-induced cell death revealed by LDH release assay after 7 days, whereas H-85, a less specific PKA inhibitor, showed no effect on the propentofylline action (Fig. 7). Such PKA inhibitors alone never affected the cell viability.

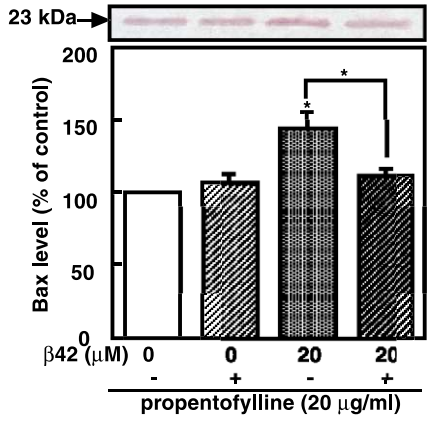

Fig. 6. Protective action of propentofylline on β42-induced Bax elevation. Cultures were incubated with or without 20 μg/ml propentofylline and/or β42 for 6 h. The supernatant of the extract of the cultures (15 μg protein/lane) was subjected to Western blot analysis. Insertions show a 23-kDa band of Bax protein. Values represent the mean ± S.E.M. of triplicate experiments. *$P<0.01$ vs. control or β42 only. ANOVA, Student's *t*-test.

4. Discussion

4.1. Propentofylline suppresses β42-induced apoptosis of cultured rat hippocampal neurons

The role of amyloid protein in Alzheimer's disease is not yet fully understood. Although it is clear that deposits of insoluble amyloid protein are found in senile plaques in the brains of people with Alzheimer's disease, particularly in the hippocampus, studies of primary and clonal neuronal cells with amyloid protein suggest two types of cell death: apoptosis (Forloni et al., 1993; Loo et al., 1993) and necrosis. Our present data demonstrated that hippocampal neurons underwent extensive apoptosis after treatment with β42. β42 induced various apoptotic features such as chromatin condensation, caspase-3 activation, a decrease in the amount of Bcl-2 protein and an increase in the amount of Bax protein. Bax is a Bcl-2 homologue that counteracts the protective action of Bcl-2. The protective action of Bcl-2 needs a concomitant depression of Bax level (Oltvai et al., 1993; Yin et al., 1994). In our culture system, the opposite effect of β42 on Bax and Bcl-2 was the case. Propentofylline, a synthetic xanthine derivative, suppressed all such apoptotic indicators induced by β42 from the rat-cultured hippocampal neurons. Propentofylline was originally reported to cause inhibition of both phosphodiesterase and adenosine uptake (Nagata et al., 1985; Ohkubo et al., 1991; Fredholm et al., 1992; Meskini et al., 1994). A neuroprotective action of propentofylline has also been shown in neurons and glial cells (Andine et al., 1990; Shinoda et

al., 1990; Banati et al., 1994; Park and Rudolphi, 1994). Propentofylline (20 μg/ml) consistently prevented cell death, chromatin condensation and caspase-3 activation. Additionally, propentofylline induced a significant increase and decrease of Bcl-2 and Bax, respectively. The enhancement of anti-apoptotic Bcl-2 and attenuation of pro-apoptotic Bax evoked by propentofylline were transient (6 h of incubation). The rapid and transient induction of Bcl-2, concomitant with a decrease of Bax, may explain the limited depression of caspase-3 activation by β42.

4.2. Neuroprotective action of propentofylline via cAMP–PKA system

In a previous paper (Koriyama et al., 2000), propentofylline dose-dependently increased intracellular levels of cAMP in the hippocampal cells. The level of cAMP elevated by 20 μg/ml of propentofylline was about twice that of control (86.5 ± 3.8 pmol/mg protein of propentofylline treatment vs. 44.2 ± 1.9 pmol/mg protein of basal control level). This concentration of propentofylline in the potency of elevating cAMP was compatible with 100 μM of isobutylmethylxanthine (IBMX), a non-specific phosphodiesterase inhibitor. Actually, IBMX, dbcAMP and forskolin, an adenylate cyclase activator, could all potentially protect the β42 neurotoxicity of rat hippocampal neurons (data not shown). An increase of intracellular levels of cAMP or dbcAMP promotes cell survival or delays cell death induced by various conditions such as sialoglycopeptide, low potassium concentration and serum deprivation (Kobayashi and Shinozawa, 1997; Moran et al., 1999; Desire et al., 2000; Bayatti and Engele, 2001). In the present study, propentofylline (20 μg/ml) and dbcAMP (100 μM) could effectively suppress caspase-3 activation by β42. The neuroprotective action of propentofylline on β42-induced cell death was completely blocked by H-89 but not by H-85. These data strongly indicate that the protective action of propentofylline is mediated by a cAMP–PKA system. The protective action of cAMP may implicate a phosphorylation process of cAMP response element binding protein (CREB) by PKA. Recently, Tong et al. (2001) reported that β42 decreased CREB phosphorylation in cultured cortical neurons. Interestingly enough, cAMP elevated Bcl-2 levels via phosphorylation of CREB by insulin growth factor-1 in PC 12 cells (Pugazhenthi et al., 1999). Bcl-2 elevation by transforming growth factor-β1 could also prevent the amyloid protein neurotoxicity in rat hippocampal neurons (Prehn et al., 1996). Bad, however, is the fact that another Bcl-2 family member which has a pro-apoptotic action was known to be phosphorylated and inactivated by PKA (Harada et al., 1999). In Alzheimer's disease-affected brains, there are alterations of the level of Bcl-2 family proteins (Kitamura et al., 1999). Therefore, it is concluded that β42 apoptosis in cultured hippocampal neurons is prevented by propentofylline, which is capable of activating a cAMP–PKA system, depressing the caspase cascade and modifying Bcl-2 family proteins. In contrast to other agents which increase the intracellular level of cAMP, propentofylline can pass through the blood–brain barrier (Yamada et al., 1998). Thus, the development of a specific therapy by propentofylline that targets a cAMP–PKA system and Bcl-2 family proteins is expected to allow more sensitive treatment for Alzheimer's disease in the future.

Acknowledgements

We wish to thank Dr. S. Kato for his critical comments and also thank Ms. T. Urano and Dr. M. Devadas for their assistance. This study was supported in part by the grant of the special research fund of Hokuriku University to Y.K.

References

Andine, P., Rudolphi, K.A., Fredholm, B.B., Hagberg, H., 1990. Effect of propentofylline (HWA 285) on extracellular purines and excitatory amino acids in CA1 of rat hippocampus during transient ischaemia. Br. J. Pharmacol. 100, 814–818.

Banati, R.B., Schubert, P., Rothe, G., Gehrmann, J., Rudolphi, K., Valet, G., Kreutzberg, G.W., 1994. Modulation of intracellular formation of reactive oxygen intermediates in peritoneal macrophages and microglia/brain macrophages by propentofylline. J. Cereb. Blood Flow Metab. 14, 145–149.

Bayatti, N., Engele, J., 2001. Cyclic AMP modulates the response of central nervous system glia to fibroblast growth factor-2 by redirecting signalling pathways. J. Neurochem. 78, 972–980.

Behl, C., Davis, J.B., Lesley, R., Schubert, D., 1994. Hydrogen peroxide mediates amyloid beta protein toxicity. Cell 77, 817–827.

Cotman, C.W., Anderson, A.J., 1995. A potential role for apoptosis in neurodegeneration and Alzheimer's disease. Mol. Neurobiol. 10, 19–45.

DeLeo, J., Schubert, P., Kreutzberg, G.W., 1988. Propentofylline (HWA 285) protects hippocampal neurons of Mongolian gerbils against ischemic damage in the presence of an adenosine antagonist. Neurosci. Lett. 84, 307–311.

Desire, L., Courtois, Y., Jeanny, J.C., 2000. Endogenous and exogenous fibroblast growth factor 2 support survival of chick retinal neurons by control of neuronal bcl-x(L) and bcl-2 expression through a fibroblast growth factor receptor 1- and ERK-dependent pathway. J. Neurochem. 75, 151–163.

Estus, S., Tucker, H.M., van-Rooyen, C., Wright, S., Brigham, E.F., Wogulis, M., Rydel, R.E., 1997. Aggregated amyloid-beta protein induces cortical neuronal apoptosis and concomitant "apoptotic" pattern of gene induction. J. Neurosci. 17, 7736–7745.

Forloni, G., Chiesa, R., Smiroldo, S., Verga, L., Salmona, M., Tagliavini, F., Angeretti, N., 1993. Apoptosis mediated neurotoxicity induced by chronic application of beta amyloid fragment 25–35. NeuroReport 4, 523–526.

Fredholm, B.B., Fastbom, J., Kvanta, A., Gerwins, P., Parkinson, F., 1992. Further evidence that propentofylline(HWA 285) influences both adenosine receptors and adenosine transport. Fundam. Clin. Pharmacol. 6, 99–111.

Gschwind, M., Huber, G., 1995. Apoptotic cell death induced by beta-amyloid 1–42 peptide is cell type dependent. J. Neurochem. 65, 292–300.

Guo, Q., Sebastian, L., Sopher, B.L., Miller, M.W., Ware, C.B., Martin, G.M., Mattson, M.P., 1999. Increased vulnerability of hippocampal neurons from presenilin-1 mutant knock-in mice to amyloid beta-pep-

tide toxicity: central roles of superoxide production and caspase activation. J. Neurochem. 72, 1019–1029.
Harada, H., Becknell, B., Wilm, M., Mann, M., Huang, L.J., Taylor, S.S., Scott, J.D., Korsmeyer, S.J., 1999. Phosphorylation and inactivation of BAD by mitochondria-anchored protein kinase A. Mol. Cell 3, 413–422.
Iwatsubo, T., Odaka, A., Suzuki, N., Mizusawa, H., Nukina, N., Ihara, Y., 1994. Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). Neuron 13, 45–53.
Kitamura, Y., Taniguchi, T., Shimohama, S., 1999. Apoptotic cell death in neurons and glial cells: implications for Alzheimer's disease. Jpn. J. Pharmacol. 79, 1–5.
Kobayashi, Y., Shinozawa, T., 1997. Effect of dibutyryl cAMP and several reagents on apoptosis in PC12 cells induced by a sialoglycopeptide from bovine brain. Brain Res. 778, 309–317.
Koriyama, Y., Yamazaki, M., Chiba, K., Mohri, T., 2000. Evaluation of neurotoxicity of Alzheimer's amyloid beta protein (beta42) in cultured hippocampal cells and its prevention by propentofylline. Jpn. J. Pharmacol. 82, 301–306.
Loo, D.T., Copani, A., Pike, C.J., Whittemore, E.R., Walencewicz, A.J., Cotman, C.W., 1993. Apoptosis is induced by beta-amyloid in cultured central nervous system neurons. Proc. Natl. Acad. Sci. U. S. A. 90, 7951–7955.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Marcusson, J., Rother, M., Kittner, B., Rossner, M., Smith, R.J., Babic, T., Folnegovic-Smalc, V., Moller, H.J., Labs, K.H., 1997. A 12-month, randomized, placebo-controlled trial of propentofylline (HWA 285) in patients with dementia according to DSM III-R. The European Propentofylline Study Group. Dement. Geriatr. Cogn. Disord. 8, 320–328.
Matsuzawa, A., Ichijo, H., 2001. Molecular mechanisms of the decision between life and death: regulation of apoptosis by apoptosis signal-regulating kinase 1. J. Biochem. 130, 1–8.
Meskini, N., Nemoz, G., Okyayuz-Baklouti, I., Lagarde, M., Prigent, A.F., 1994. Phosphodiesterase inhibitory profile of some related xanthine derivatives pharmacologically active on the peripheral microcirculation. Biochem. Pharmacol. 47, 781–788.
Moran, J., Itoh, T., Reddy, U.R., Chen, M., Alnemri, E.S., Pleasure, D., 1999. Caspase-3 expression by cerebellar granule neurons is regulated by calcium and cyclic AMP. J. Neurochem. 73, 568–577.
Nagata, K., Ogawa, T., Omosu, M., Fujimoto, K., Hayashi, S., 1985. In vitro and in vivo inhibitory effects of propentofylline on cyclic AMP phosphodiesterase activity. Arzneimittel-Forschung 35, 1034–1036.
Ohkubo, T., Mitsumoto, Y., Mohri, T., 1991. Characterization of the uptake of adenosine by cultured rat hippocampal cells and inhibition of the uptake by xanthine derivatives. Neurosci. Lett. 13, 275–278.
Oltvai, Z.N., Milliman, C.L., Korsmeyer, S.J., 1993. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 74, 609–619.
Park, C.K., Rudolphi, K.A., 1994. Antiischemic effects of propentofylline (HWA 285) against focal cerebral infarction in rats. Neurosci. Lett. 178, 235–238.
Parvathenani, L.K., Calandra, V., Roberts, S.B., Posmantur, R., 2000. cAMP delays beta-amyloid (25–35) induced cell death in rat cortical neurons. NeuroReport 11, 2293–2297.
Pike, C.J., Walencewicz, A.J., Glabe, C.G., Cotman, C.W., 1991. In vitro aging of beta-amyloid protein causes peptide aggregation and neurotoxicity. Brain Res. 563, 311–314.
Prehn, J.H., Bindokas, V.P., Jordan, J., Galindo, M.F., Ghadge, G.D., Roos, R.P., Boise, L.H., Thompson, C.B., Krajewski, S., Reed, J.C., Miller, R.J., 1996. Protective effect of transforming growth factor-beta 1 on beta-amyloid neurotoxicity in rat hippocampal neurons. Mol. Pharmacol. 49, 319–328.
Pugazhenthi, S., Miller, E., Sable, C., Young, P., Heidenreich, K.A., Boxer, L.M., Reusch, J.E., 1999. Insulin-like growth factor-I induces bcl-2 promoter through the transcription factor cAMP-response element-binding protein. J. Biol. Chem. 274, 27529–27535.
Saletu, B., Moller, H.J., Grunberger, J., Deutsch, H., Rossner, M., 1990. Propentofylline in adult-onset cognitive disorders: double-blind, placebo-controlled, clinical, psychometric and brain mapping studies. Neuropsychobiology 24, 173–184.
Schubert, P., Rudolphi, K.A., Fredholm, B.B., Nakamura, Y., 1994. Modulation of nerve and glial function by adenosine—role in the development of ischemic damage. Int. J. Biochem. 26, 1227–1236.
Shinoda, I., Furukawa, Y., Furukawa, S., 1990. Stimulation of nerve growth factor synthesis/secretion by propentofylline in cultured mouse astroglial cells. Biochem. Pharmacol. 39, 1813–1816.
Takadera, T., Sakura, N., Mohri, T., Hashimoto, T., 1993. Toxic effect of a beta-amyloid peptide (beta 22–35) on the hippocampal neuron and its prevention. Neurosci. Lett. 161, 41–44.
Tamaoka, A., Odaka, A., Ishibashi, Y., Usami, M., Sahara, N., Suzuki, N., Nukina, N., Mizusawa, H., Shoji, S., Kanazawa, I., Mori, H., 1994. APP717 missense mutation affects the ratio of amyloid beta protein species(Abeta1-42/43 and Abeta1-40) in familial Alzheimer's disease brain. J. Biol. Chem. 269, 32721–32724.
Tong, L., Thornton, P.L., Balazs, R., Cotman, C.W., 2001. Beta-amyloid-(1–42) impairs activity-dependent cAMP-response element-binding protein signaling in neurons at concentrations in which cell survival is not compromised. J. Biol. Chem. 276, 17301–17306.
Yamada, K., Tanaka, T., Senzaki, K., Kameyama, T., Nabeshima, T., 1998. Propentofylline improves learning and memory deficits in rats induced by beta-amyloid protein-(1–40). Eur. J. Pharmacol. 349, 15–22.
Yin, X.M., Oltvai, Z.N., Korsmeyer, S.J., 1994. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. Nature 369, 321–323.