Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 520 (2005) 12–21

www.elsevier.com/locate/ejphar

Blockade of 5-HT_3 receptor with MDL72222 and Y25130 reduces β-amyloid protein (25–35)-induced neurotoxicity in cultured rat cortical neurons

Ju Yeon Ban, Yeon Hee Seong *

College of Veterinary Medicine and Research Institute of Herbal Medicine, Chungbuk National University, 12, Gaesin-dong, Heungduk-Gu, Cheongju, Chungbuk 361-763, Republic of Korea

Received 15 July 2005; accepted 19 July 2005
Available online 8 September 2005

Abstract

The present study was performed to examine neuroprotective effects of 5-hydroxytryptamine (5-HT)$_3$ receptor antagonists against β-amyloid protein (25–35)-, a synthetic 25–35 amyloid peptide, induced neurotoxicity using cultured rat cortical neurons. β-Amyloid protein (25–35) produced a concentration-dependent reduction of cell viability, which was significantly reduced by (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*] cyclohepten-5,10-imine (MK-801), an *N*-methyl-D-aspartate (NMDA) receptor antagonist, verapamil, an L-type Ca^{2+} channel blocker, and N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor. The 5-HT_3 receptor antagonists, tropanyl-3,5-dichlorobenzoate (MDL72222, 0.1–10 μM) and *N*-(1-azabicyclo[2.2.2.]oct-3-yl)-6-chloro-4-ethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide hydrochloride (Y25130, 0.05–5 μM), decreased the β-amyloid protein (25–35) (10 μM)-induced neuronal cell death as assessed by a colorimetric 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay and the number of apoptotic nuclei, evidenced by Hoechst 33342 staining. MDL72222 and Y25130 inhibited the β-amyloid protein (25–35) (10 μM)-induced elevation of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) and glutamate release, generation of reactive oxygen species, and caspase-3 activity. These neuroprotective effects of MDL72222 (10 μM) and Y25130 (5 μM) were completely blocked by the simultaneous treatment with 100 μM 1-phenylbiguanide, a 5-HT_3 receptor agonist, indicating that the protective effects of these compounds were due to 5-HT_3 receptor blockade. These results suggest that the activation of the 5-HT_3 receptor may be partially involved in β-amyloid protein-induced neurotoxicity, by membrane depolarization for Ca^{2+} influx. Therefore, the blockade of 5-HT_3 receptor with MDL72222 and Y25130, may ameliorate the β-amyloid protein-induced neurotoxicity by interfering with the increase of $[Ca^{2+}]_c$, and then by inhibiting glutamate release, generation of reactive oxygen species and caspase-3 activity.

Keywords: 5-HT_3 receptor antagonist; MDL72222; Y25130; 5-HT_3 receptor; β-Amyloid protein; Neurotoxicity; Cortical neuron; Neuroprotection

1. Introduction

Alzheimer's disease is characterized by neuronal loss and extracellular senile plaque, whose major constituent is β-amyloid protein, a 39–43 amino acid peptide derived from amyloid precursor protein (Ivins et al., 1999). Both in vitro (Iversen et al., 1995) and in vivo (Chen et al., 1994) studies have reported the toxic effects of β-amyloid protein or β-amyloid peptide fragments suggesting an important role for β-amyloid protein in the pathogenesis of Alzheimer's disease. In cultures, β-amyloid protein can directly induce neuronal cell death (Ueda et al., 1994) and can render neurons vulnerable to excitotoxicity (Koh et al., 1990) and oxidative insults (Goodman and Mattson, 1994). The mechanisms underlying β-amyloid protein-neurotoxicity are complex but may involve *N*-methyl-D-

* Corresponding author. Tel.: +82 43 261 2968; fax: +82 43 267 3150.
E-mail address: vepharm@chungbuk.ac.kr (Y.H. Seong).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.07.021

aspartate (NMDA) receptor, a glutamate receptor subtype, modulation induced by glutamate release, sustained elevations of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), and oxidative stresses (Forloni, 1993; Gray and Patel, 1995; Ueda et al., 1997; Ekinci et al., 2000). NMDA receptor acts either as a selective substrate of β-amyloid protein binding or as a mediator of β-amyloid protein-triggered glutamate excitotocixity (Harkany et al., 1999). NMDA receptor is a ligand-gated/voltage-sensitive cation channel, especially highly permeable to Ca^{2+}. Extensive elevation of the $[Ca^{2+}]_i$ may lead directly to cellular dysfunction, overexcitation or death (Horn et al., 1999). Therefore, Ca^{2+} influx through NMDA receptor activation by β-amyloid protein may be a critical role in β-amyloid protein-induced neurotoxicity. Formation of reactive oxygen species is also believed to be involved in the pathogenesis of neurodegenerative disorders (Olanow, 1993). Several lines of evidence support the involvement of oxidative stress as an active factor in β-amyloid protein-mediated neuropathology, by triggering or facilitating neurodegeneration through a wide range of molecular events that disturb neuronal homeostasis (Ekinci et al., 2000).

The 5-hydroxytryptamine $(5\text{-HT})_3$ receptor is the only 5-HT-activated ligand-gated ion channel that increases intracellular cation ions, such as Ca^{2+} as well as Na^+ and K^+, by its activation. The stimulation of the receptor induces neuronal depolarization and excitation (Derkach et al., 1989; Maricq et al., 1991). 5-HT_3 receptors are concentrated in limbic and cortical areas thought to be involved with learning and memory (Kilpatrick et al., 1987; Waeber et al., 1989). Antagonists at the 5-HT_3 receptor have been considered as potential cognitive enhancers for the treatment of dementia (Altman and Normile, 1988; Gower, 1992). Studies in rodents have demonstrated that 5-HT_3 receptor antagonists such as ondansetron and SEC-579 can enhance cognitive function (Barnes et al., 1990; Costal and Naylor, 1994; Hodges and Fletcher, 1995) and central acetylcholine release (Robinson, 1983; Barnes et al., 1989). Moreover, ondansetron was reported to attenuate the deficit in various learned behaviors caused by systemically administered scopolamine (Carey et al., 1992; Carli et al., 1997). In addition, it was also demonstrated that the blockade of 5-HT_3 receptors plays a neuroprotective role in ischemia-induced damage (Kagami-ishi et al., 1992). Therefore, it seems reasonable to explore neuroprotective effects of 5-HT_3 receptor antagonists against β-amyloid protein-induced apoptotic death and to study associated potential underlying mechanisms. The current study aims at determining whether blockade of 5-HT_3 receptor with tropanyl-3,5-dichlorobenzoate (MDL72222) and *N*-(1-azabicyclo[2.2.2.]oct-3-yl)-6-chloro-4-ethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide hydrochloride (Y25130) is able to protect the neuronal cells against β-amyloid protein (25–35)-induced neurotoxicity in cultured rat cortical neurons.

2. Materials and methods

2.1. Chemicals (reagents) and physiological solution

β-Amyloid protein (25–35) was purchased from Bachem (Bubendorf, Switzerland). MDL72222 and Y25130 and 1-phenylbiguanide hydrochloride were purchased from Tocris Cookson Inc. (St. Ballwin, MO, USA). (5*R*,10*S*)-(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*] cyclohepten-5,10-imine (MK-801) and N^G-nitro-L-arginine methyl ester (L-NAME) were purchased from RBI (Natick, MA, USA). Verapamil, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), *o*-phthaldialdehyde, 2-mercaptoethanol, Dulbecco's modified Eagle's medium (DMEM), Joklik-modified MEM, poly-L-lysine and amino acids for HPLC standard were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hoechst 33342 dye, fluo-4 AM and 2′,7′-dichlorodihydrofluorescin diacetate (H_2DCF-DA) were purchased from Molecular Probes Inc. (Eugene, OR, USA). Fetal bovine serum was purchased from Gibco (Logan, UT, USA). PRO-PREP protein extraction solution was purchased from iNtRON Biothechnology Inc. (Seoul, Korea). Western Lightening™ chemiluminescence reagent was purchased from Perkin Elmer Life Sciences Inc. (Boston, MA, USA). Anti-caspase 3 (rabbit polyclonal IgG) and horse-radish peroxidase conjugated anti-rabbit IgG were purchased from Upstate Biotechnology (Lake Placid, NY, USA). All other chemicals used were of the highest grade available.

β-Amyloid protein (25–35) stock solution of 2 mM was prepared in sterile distilled water, stored at −20 °C, and incubated for more than 2 days at 37 °C to aggregate before use. MDL72222 was dissolved in dimethylsulfoxide (DMSO) with the concentration of 10 mM and further diluted with experimental buffers. The final concentration of DMSO was 0.1%, which did not affect cell viability (99.8 ±2.1%). Y25130, 1-phenylbiguanide, MK-801, verapamil and L-NAME were solubilized in experimental buffers. For every experiment, MDL72222 (0.1–10 μM), Y25130 (0.05–5 μM), 1-phenylbiguanide (100 μM), MK-801 (10 μM), verapamil (10 μM) and L-NAME (1 mM) or their vehicle were applied 15 min prior to the treatment with β-amyloid protein (25–35) and were present in the medium during the incubation period with β-amyloid protein (25–35). For some experiments, a HEPES-buffered solution (incubation buffer) containing 8.6 mM HEPES, 154 mM NaCl, 5.6 mM KCl and 2.3 mM $CaCl_2$ at pH 7.4 was used.

2.2. Animals

Specific pathogen-free pregnant Sprague–Dawley (SD) rats (Daehan Biolink Co. Ltd., Chungbuk, Korea) were housed in an environmentally controlled room with temperature of 23±2 °C, relative humidity of 55±5%, and a 12-h light/dark cycle, and food and water were available ad libitum. The procedures involving experimental animals adhered to the 'Guide Principles in the Use of Animals in