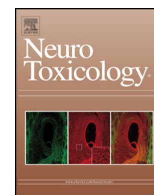
NeuroToxicology 52 (2016) 114–126

Contents lists available at ScienceDirect

NeuroToxicology

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



Involvement of autophagy upregulation in 3,4-methylenedioxymethamphetamine ('ecstasy')-induced serotonergic neurotoxicity

I-Hsun Li [a], Kuo-Hsing Ma [b], Tzu-Jen Kao [c,d], Yang-Yi Lin [b], Shao-Ju Weng [b], Ting-Yin Yen [b], Lih-Chi Chen [e], Yuahn-Sieh Huang [b,*]

[a] Department of Pharmacy Practice, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan
[b] Department of Biology and Anatomy, National Defense Medical Center, Taipei, Taiwan
[c] Graduate Institute of Neural Regenerative Medicine, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan
[d] Center for Neurotrauma and Neuroregeneration, Taipei Medical University, Taipei, Taiwan
[e] Department of Pharmacy, Taipei City Hospital, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 27 November 2014
Received in revised form 16 November 2015
Accepted 16 November 2015
Available online 21 November 2015

Keywords:
Autophagy
MDMA
LC3B
3-Methyladenine
Tryptophan hydroxylase
Serotonergic neuron

ABSTRACT

It has been suggested that autophagy plays pathogenetic roles in cerebral ischemia, brain trauma, and neurodegenerative disorders. 3,4-Methylenedioxymethamphetamine (MDMA or ecstasy) is an illicit drug that causes long-term serotonergic neurotoxicity in the brain. Apoptosis and necrosis have been implicated in MDMA-induced neurotoxicity, but the role of autophagy in MDMA-elicited serotonergic toxicity has not been investigated. The present study aimed to examine the contribution of autophagy to neurotoxicity in serotonergic neurons in *in vitro* and *in vivo* animal models challenged with MDMA. Here, we demonstrated that in cultured rat serotonergic neurons, MDMA exposure induced LC3B-densely stained autophagosome formation, accompanying by a decrease in neurite outgrowth. Autophagy inhibitor 3-methyladenine (3-MA) significantly attenuated MDMA-induced autophagosome accumulation, and ameliorated MDMA-triggered serotonergic neurite damage and neuron death. In contrast, enhanced autophagy flux by rapamycin or impaired autophagosome clearance by bafilomycin A1 led to more autophagosome accumulation in serotonergic neurons and aggravated neurite degeneration. In addition, MDMA-induced autophagy activation in cultured serotonergic neurons might be mediated by serotonin transporter (SERT). In an *in vivo* animal model administered MDMA, neuroimaging showed that 3-MA protected the serotonin system against MDMA-induced downregulation of SERT evaluated by animal-PET with 4-[^{18}F]-ADAM, a SERT radioligand. Taken together, our results demonstrated that MDMA triggers upregulation of autophagy in serotonergic neurons, which appears to be detrimental to neuronal growth.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Autophagy is a highly conserved, regulated process by which intracellular constituents are delivered to lysosomes for degradation. Depending on the mode of delivery, there are three different types of autophagy: macroautophagy, chaperone-mediated autophagy, and microautophagy. Macroautophagy, simply referred to hereafter as autophagy, has been extensively investigated (Ghavami et al., 2014; Nixon, 2013). Upon initiation of autophagy, a small vesicular sac ("phagophore") elongates and subsequently encloses a portion of cytoplasm, which results in formation of an autophagosome. This is a multistep process in which the formation of an autophagosome necessitates the sequential modification of microtubule-associated protein 1 light chain 3 (LC3). After

Abbreviations: MDMA, 3,4-methylenedioxymethamphetamine; LC3, microtubule-associated protein 1 light chain 3; SERT, serotonin transporter; TPH, tryptophan hydroxylase; SSRIs, selective serotonin reuptake inhibitors; 5-HT, 5-hydroxytryptamine (serotonin); 3-MA, 3-methyladenine; PI3K, phosphatidylinositol 3-kinase; PE, phosphatidylethanolamine; ROS, reactive oxygen species; CNS, central nervous system; PET/SPECT, positron emission computed tomography/single photo emission computed tomography; VOIs, volumes of interest; SURs, specific uptake ratios.

* Corresponding author at: Department of Biology and Anatomy, National Defense Medical Center, Taipei, No.161, Sec. 6, Min-Chuan E. Rd., Neihu 114, Taipei, Taiwan, ROC. Tel.: +886 2 87923100x18735; fax: +886 2 87923159.
E-mail address: anatoman2001@yahoo.com.tw (Y.-S. Huang).

http://dx.doi.org/10.1016/j.neuro.2015.11.009
0161-813X/© 2015 Elsevier Inc. All rights reserved.

synthesis, the C-terminal of LC3 is first cleaved by a cysteine protease, ATG4, to produce LC3-I with a molecular weight (MW) of 16 kDa. The product is localized diffusely throughout the entire cytoplasm. Upon induction of autophagy, LC3 is lipidated. A fraction of LC3-I is transferred to phosphatidylethanolamine (PE) to produce a new molecule, LC3-PE conjugate (also known as LC3-II), with an apparent MW of 14 kDa. LC3-II is associated with the autophagosome and thus the amount of LC3-II and the formation of LC3 puncta are thought to be a faithful marker of the autophagosome (Kesidou et al., 2013; Yang et al., 2013). The autophagosome then fuses with a lysosome to form an autolysosome, leading to degradation of the enclosed materials by acid hydrolases. The basal level of autophagy helps to control the cellular quality of proteins and organelles, and protects cells from protein aggregation or damaged organelles (Yang et al., 2013).

Autophagy can be activated in response to various cellular and environmental stress conditions to promote cell survival (e.g., starvation, oxidative stress), or to act as a mode of cell death (e.g., cerebral ischemia) (Abounit et al., 2012; Baehrecke, 2005; Din et al., 2012). Autophagic cell death (also called type II programmed cell death) is characterized by the massive accumulation of autophagic vacuoles in the cytoplasm of cells as they die (Din et al., 2012; Shen and Codogno, 2011). Defective autophagy has been connected to many human diseases including cancer, myopathies, and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (Ghavami et al., 2014; Kwon et al., 2011; Lee, 2012; Nixon and Yang, 2012).

3,4-Methylenedioxymethamphetamine (MDMA or Ecstasy), which is a central nervous system (CNS) psychostimulant, is one of the most popular recreational drugs that is abused by adolescents. Because of its stimulant properties, repeated or high-dose MDMA/ecstasy use can produce a variety of neurological disorders including cognitive impairments and mood disturbances (Kirilly, 2010). A combination of MDMA, its metabolites, oxidative stress and hyperthermia contributes to MDMA's neurotoxicity (Barbosa et al., 2014a; Mueller et al., 2013; Puerta et al., 2010). In the CNS, MDMA is toxic to serotonergic neurons in rodent and human, and also damage to dopaminergic system in mice (O'Shea et al., 2001). Particularly, acute and sub-acute administration of MDMA to rodents can produces long-lasting serotonin and dopamine depletion, which is accompanied by decreases in the density of the serotonin transporter (SERT), dopamine transporters, monoamine oxidase, tyrosine hydroxylase and tryptophan hydroxylase (TPH), a rate-limiting enzyme in 5-HT biosynthesis, and degeneration of striatal serotonergic and dopaminergic terminals (Kish et al., 2010; Leonardi and Azmitia, 1994; Xie et al., 2006). In addition, MDMA is toxic to other brain regions, including the cerebral cortex, thalamus, and striatum (Capela et al., 2007a). The mechanism underlying the neurotoxicity of MDMA is very complex and not fully understood. Oxidative stress, excitotoxicity, and mitochondrial dysfunction have been implicated in MDMA-induced neurotoxicity (Barbosa et al., 2014b; Puerta et al., 2010; Zhou et al., 2003).

MDMA also appears to induce apoptosis by increasing the expression of the pro-apoptotic protein Bax and inhibition of the anti-apoptotic protein Bcl-2 in the rat hippocampus (Soleimani Asl et al., 2012). Studies *in vitro* shown that MDMA, and related amphetamines, induce neuronal apoptosis in cortical and cerebellar granule neurons (Capela et al., 2007a; Jimenez et al., 2004; Stumm et al., 1999). However, the pathophysiological role of autophagy in MDMA-induced neurotoxicity is unknown. The aim of this study was to investigate the effect of MDMA on autophagy in rat serotonergic neurons. We observed a robust increase of autophagy in primary rat serotonergic neurons after MDMA treatment, which paralleled neurite shortening and serotonergic neuron death. Inhibition of autophagy with 3-methyladenine (3-MA) significantly prevented serotonergic neurotoxicity by MDMA *in vitro* and *in vivo*, suggesting that excessive autophagy is a contributing factor to MDMA-induced neurotoxicity.

2. Materials and methods

2.1. Antibodies and reagents

The following antibodies were used: rabbit anti-LC3B (Sigma Chemical, St. Louis, MO), mouse anti-SERT (Millipore, Temecula, CA), mouse anti-TPH (Sigma), mouse anti-MAP2 (Sigma), mouse anti-β-actin (Sigma), FITC-conjugated goat anti-rabbit IgG (Sigma), and Texas red-conjugated goat anti-mouse IgG (Vector Laboratories, Burlingame, CA). 3-Methyladenine, rapamycin, bafilomycin A1 (Sigma) and Torin-1 (Tocris Bioscience, Ellisville, MO) were used in our autophagy flux studies. Citalopram was obtained from Abcam (Cambridge, United Kingdom). MDMA (purity, 98%) was obtained from the Investigation Bureau of Taiwan.

2.2. Primary culture of serotonergic neurons from embryonic rat brain stems

Primary cultured brain stem and serotonergic neurons were established from embryonic Sprague–Dawley rats according to a published procedure with modifications (Kivell et al., 2000). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the National Defense Medical Center (Taipei, Taiwan). E14 embryos were removed from the uterus and their brain stems were removed. Meninges and blood vessels were carefully removed. The brain stems were placed in ice-cold calcium and magnesium-free Hank's balanced saline solution (HBSS, Invitrogen, Carlsbad, CA), minced, digested with 0.0625% trypsin and 0.05% DNase for 10 min at 37 °C, suspended in DMEM with 10% fetal bovine serum (FBS) to inactivate the trypsin, and pelleted by centrifugation at 1000 rpm for 10 min. After suspending the cell pellet in medium and adjusting the cell density to 1×10^6 cells/ml, neurons were seeded on coverslips or culture dishes precoated with poly-D-lysine and cultured with growth medium (1:1 mixture of DMEM/F-12 and neurobasal medium [Invitrogen], 5% FBS, supplemented with 0.5 mM L-glutamine, 1% penicillin–streptomycin, and 2% B-27 serum-free supplement [Invitrogen]). On the third day post-plating, non-neuronal cell proliferation was suppressed by treatment with 5 μM cytosine-D-arabinofuranoside for 24 h. On the fifth day of culture, the cells were used for the experiment. Serotonergic neurons were identified by positive staining with anti-TPH antibody.

2.3. Primary rat cortical neuron culture

Rat primary cortical neuron culture was performed according to a published procedure with modifications (Kindlundh-Hogberg et al., 2010). E18 Sprague–Dawley rats were removed from the uterus and their cortex were removed. Meninges and blood vessels were carefully removed. The cerebral cortex was placed in ice-cold calcium and magnesium-free Hank's balanced saline solution (HBSS), minced, digested with 0.0625% trypsin and 0.05% DNase for 10 min at 37 °C, suspended in DMEM with 10% FBS to inactivate the trypsin, and pelleted by centrifugation at 1000 rpm for 10 min. After suspending the cell pellet in medium and adjusting the cell density to 1×10^6 cells/ml, neurons were seeded on coverslips or culture dishes precoated with poly-D-lysine and cultured with neurobasal medium (Invitrogen), supplemented with 0.5 mM L-glutamine, 1% penicillin-streptomycin and 2% B-27 serum-free supplement (Invitrogen). On the third day post-plating, non-neuronal cell proliferation was suppressed by treatment with

Download English Version:

https://daneshyari.com/en/article/5854667

Download Persian Version:

https://daneshyari.com/article/5854667

Daneshyari.com