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URB597 IMPROVES COGNITIVE IMPAIRMENT INDUCED BY CHRONIC CEREBRAL HYPOPERFUSION BY INHIBITING mTOR-DEPENDENT AUTOPHAGY

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Abstract—Chronic cerebral hypoperfusion (CCH) is associated with various ischemic cerebrovascular diseases that are characterized by cognitive impairment. The role of autophagy in cognitive dysfunction under conditions of CCH is poorly understood. To address this issue, the present study investigated the effect of the fatty acid amide hydrolase (FAAH) inhibitor URB597 on autophagy and cognition in a CCH model as well as the underlying mechanisms. Cognitive function was evaluated with the Morris water maze and by assessing long-term potentiation (LTP). The expression of autophagy-related proteins and mammalian target of rapamycin (mTOR) signaling pathway components was evaluated by immunofluorescence and western blot analyses, and ultrastructural changes were examined by transmission electron microscopy (EM). URB597 improved cognitive impairment by inhibiting CCH-induced autophagy, which was associated with mTOR signaling. Moreover, the ultrastructural deterioration resulting from CCH was improved by chronic treatment with URB597. These findings indicate that URB597 modulates autophagy in an mTOR-dependent manner, and mitigates neuronal damage and cognitive deterioration caused by CCH.

Key words: autophagy, chronic cerebral hypoperfusion, cognitive impairment, endocannabinoid system, mTOR signaling.

INTRODUCTION

Chronic cerebral hypoperfusion (CCH) is a vascular risk factor for vascular injury, blood–brain barrier dysfunction, neurodegeneration, apoptosis, and neuroinflammation, which contribute to cognitive impairment (Su et al., 2015; Choi et al., 2016; Wang et al., 2016). CCH-associated cognitive dysfunction is common in aging-related central nervous system (CNS) disorders such as vascular dementia (VD), Alzheimer's disease (AD), and ischemic cerebral disease (Petersen, 2011; Mufson et al., 2012; Eshkoor et al., 2015). Collectively, these place an economic burden on society (Ferri et al., 2005; Tricco et al., 2012). There are currently no effective measures for the prevention and treatment of CCH.

Autophagy is a cellular process for the degradation of damaged proteins, aged organelles, and unnecessary components in which a double-membrane vesicle known as the autophagosome sequesters cytosolic cargo for ultimate delivery to the lysosome (Choi et al., 2013). Autophagy is essential for maintaining cellular integrity and homeostasis (Shintani and Klionsky, 2004; Ravikumar et al., 2010; Galluzzi et al., 2014), but excessive or impaired autophagy can lead to cell death; it has also been implicated in various pathologies, including cancer, inflammation, muscular disorder, neurodegenerative diseases, and neonatal brain injury as well as in aging (Ravikumar et al., 2010; Choi et al., 2013; Navone et al., 2015; Kiriyama and Nochi, 2015; Shibutani et al., 2015; Xie et al., 2016; Zhong et al., 2016). In AD, defective autophagy leads to neuronal injury and cognitive impairment (Komatsu et al., 2006; Kiriyama and Nochi, 2015). However, the role of autophagy in cerebral ischemia remains controversial: increased autophagy was shown to contribute to ischemia-induced neuronal damage (Rami et al., 2008; Liu et al., 2015a), while some studies suggest that autophagy protects neurons against ischemia-induced injury (Qiu et al., 2016; Srivastava et al., 2016). The role of autophagy in cognitive impairment under conditions of CCH remains poorly understood.

The endocannabinoid system (ECS) comprises cannabinoid receptor (CB)1 and CB2, theirs ligands (e.g., anandamide and 2-arachidonylglycerol), and

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Abbreviations: 3-MA, 3-methyladenine; ACSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; BCCAO, bilateral common carotid artery occlusion; CCH, chronic cerebral hypoperfusion; CNS, central nervous system; ECS, endocannabinoid system; EM, electron microscopy; FAAH, fatty acid amide hydrolase; fEPSP, field excitatory postsynaptic potential; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; i.c.v., intracerebroventricular injection; i.p., intraperitoneal injection; LC, light chain; LTP, long-term potentiation; mTOR, mammalian target of rapamycin; PARP, poly-ADP ribose polymerase; PI3K, phosphoinositide 3-kinase; TBS, theta-burst stimulation; VD, vascular dementia.

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endogenous enzymes including fatty acid amide hydrolase (FAAH) (Kano et al., 2009; Caltana et al., 2015; Su et al., 2015), and modulates synaptic transmission and plasticity, neuronal development and metabolism, and inflammation in pathophysiological processes such as ischemic stroke, AD, VD, brain trauma, neonatal hypoxia–ischemia, and aging (Marsicano and Lafenetre, 2009; Xu and Chen, 2015; Iannotti et al., 2016). ECS function can be altered by cannabinoid receptor antagonists/agonists and inhibition of synthesizing/hydrolyzing enzymes (Fernandez-Ruiz et al., 2015). FAAH inhibitors and nonselective synthetic cannabinoid receptor agonists have been shown to protect against ischemic neuronal damage, neuroinflammation, and cognitive impairment (Leker et al., 2003; Schmidt et al., 2012; England et al., 2015; Fernandez-Ruiz et al., 2015; Su et al., 2015), while some evidence suggests that upregulation of CB1 by URB597 (URB) or WIN55,212-2 treatment impairs long-term potentiation (LTP) and leads to learning and memory deficits (Basavarajappa et al., 2014; Hasanein and Teimuri Far, 2015; Panlilio et al., 2016). This involves changes in mammalian target of rapamycin (mTOR) signaling, which is a pathway associated with autophagy (Puighermanal et al., 2009; Choi et al., 2013); however, the mechanistic link between the ECS system and autophagy is unclear. To address this issue, the present study investigated whether URB can improve CCH-induced cognitive impairment and the role of autophagy in this process.

EXPERIMENTAL PROCEDURES

Animals and CCH model

Male Sprague–Dawley rats (1 month old) weighing 140–160 g were obtained from Shanghai Sippr-BK Laboratory Animals (Shanghai, China) and were housed three per cage at constant temperature (23 °C ± 1 °C) and humidity (60%) on a 12:12-h light/dark cycle, with free access to food and water. All procedures were carried out in accordance with guidelines published by the National Institutes of Health (NIH) Policies on the Care and Use of Laboratory Animals. CCH was induced by bilateral common carotid artery occlusion (BCCAO) (Hai et al., 2003). Rats were anesthetized with urethane (25%, 1 ml/100 g), and a midline cervical incision was performed to expose bilateral common carotid arteries, which were carefully separated from vagal nerves and vessels. The arteries were double-ligated with 4–0 surgical sutures and the incision was sutured. Sham-operated animals were not subjected to carotid artery ligation. The CCH phase was 12 weeks.

Drug administration and treatment groups

The FAAH inhibitor URB (Cayman Chemicals, Tallinn, Estonia) was administered by intraperitoneal injection (i.p.) at 0.3 mg/kg/day (Su et al., 2015), and the autophagy inhibitor 3-methyladenine (3-MA; Sigma, St. Louis, MO, USA) was delivered by intracerebroventricular injection (i.c.v.) at 5 μl/day (600 nmol, diluted in 0.9% saline to a final volume of 5 μl) using a 25-μl Hamilton syringe (Sigma) at a rate of 0.5 μl/min (Yan et al., 2011; Jin et al., 2016). Animals were randomly divided into four groups (n = 20 each) and received one of the following treatments daily for 12 weeks: (1) sham (treated with an equal volume of vehicle); (2) BCCAO (treated with an equal volume of vehicle); (3) BCCAO + URB (0.3 mg/kg/day, i.p.); and (4) BCCAO + URB + 3-MA (0.3 mg/kg/day, i.p. and 5 μl/day, i.c.v.). The rats in this study received the drugs treatment after receiving BCCAO (Su et al., 2016).

Physiological parameters

Basal parameters of blood pressure and body temperature were monitored as previously described (Suchomelova et al., 2015). The blood pressure was tested in conscious rats using a rat tail artery blood pressure monitor (CODA, Kent Scientific Corporation, USA), and the body temperature was measured repeatedly using a digital thermometer with rectal probe (Geneva, Ohio, USA).

Morris water maze

Spatial memory was tested with the Morris water maze. A circular pool with a diameter and height of 1.8 m and 60 cm, respectively, was filled with water (25 °C ± 1 °C) to a level of 35 cm and divided into four quadrants. Visual cues were fixed to the room's walls. A platform with a diameter of 9 cm was placed 1 cm below the surface of water in the middle of the southwest quadrant. For 4 consecutive days, rats (n = 10 per group) were trained four times daily starting in one of the four quadrants. In each trial, rats were given 60 s to swim in the pool to search for the platform. Swimming was tracked using a video camera connected to a computer, and the distance, speed, and latency to reach the hidden platform were recorded. If a rat did not find the hidden platform within 60 s, it was guided to and allowed to remain on the platform for 15 s. On day 5, the probe trial performance was assessed by removing the platform and allowing the rat to swim freely for 60 s. The time spent in the target quadrant and the number of platform crossings were recorded.

Electrophysiological recordings

Rats (n = 5 for each group) were anesthetized with urethane (25%, 1 ml/100 g). The brain was rapidly dissected and transferred to ice-cold artificial cerebrospinal fluid (ACSF) composed 125 mM NaCl, 25 mM $NaHCO_3$, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, 1.25 mM NaH_2PO_4, and 10 d-glucose saturated with 95% O_2 and 5% CO_2 (pH 7.3). Transverse 400-μm hippocampal slices were cut and allowed to recover in ice-cold oxygenated ACSF (95% O_2 and 5% CO_2) at room temperature at least for 60 min prior to recordings. Individual slices were placed in a recording chamber and were perfused with oxygenated ACSF (30 °C ± 1 °C) at a rate of 3 ml/min. The stimulating electrode was placed at the Schaffer collateral to induce LTP; the recording electrode was placed in the stratum radiatum