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DOPAMINE INDUCES GLUTAMATE ACCUMULATION IN ASTROCYTES TO DISRUPT NEURONAL FUNCTION LEADING TO PATHOGENESIS OF MINIMAL HEPATIC ENCEPHALOPATHY

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Abstract—Minimal hepatic encephalopathy (MHE) is induced by elevated intracranial dopamine (DA). Glutamate (Glu) toxicity is known to be involved in many neurological disorders. In this study, we investigated whether DA increased Glu levels and collaborated with Glu to impair memory. We found that DA upregulated TAAR1, leading to reduced EAAT2 expression and Glu clearance in primary cortical astrocytes (PCAs). High DA increased TAAR1 expression, and high Glu increased AMPAR expression, inducing the activation of CaN/NFAT signaling and a decrease in the production of BDNF (Brain Derived Nerve Growth Factor)/NT3 (neurotrophin-3) in primary cortical neurons (PCNs). DA activated TAAR1 to downregulate EAAT2 and increase extracellular Glu levels in MHE. Additionally, DA together with Glu caused decreased production of neuronal BDNF/NT3 and memory impairment through the activation of CaN/NFAT signaling in MHE. From these findings, we conclude that DA increases Glu levels via interaction with TAAR1 and disruption of EAAT2 signaling in astrocytes, and DA interacting with TAAR1 and Glu interacting with AMPAR synergistically decreased the production of BDNF by activation of CaN/NFAT signaling to impair memory in MHE rats.

Key words: minimal hepatic encephalopathy, dopamine, glutamate, Brain-Derived Nerve Growth Factor, neurotrophin-3.

INTRODUCTION

Minimal hepatic encephalopathy (MHE) (Dhiman and Chawla, 2009) refers mainly to subtle changes in cognitive function observable by electrophysiological parameters such as electroencephalograms (EEGs) and brainstem auditory-evoked potentials (BAEP) (Tan et al., 2009). In our previous study, cognitive impairment in MHE was confirmed to be attributable to an elevation of dopamine (DA) (Ding et al., 2013). However, the deeper mechanism underlying the effects of DA on the pathogenesis of MHE remains unclear.

DA, norepinephrine, and serotonin, known as common biogenic amines, act as neurotransmitters and can interact with a new family of GPCRs now referred to as trace amine-associated receptors (TAARs) (Borowsky et al., 2001; Lindemann et al., 2005). Astrocytes are responsible for clearing approximately 90% of extracellular glutamate (Glu) from the synaptic cleft via excitatory amino acid transporter 2 (EAAT2) (Anderson and Swanson, 2000). Consequently, malfunctions of EAAT1 and EAAT2 may lead to aberrant Glu accumulation and neuron injury known as excitotoxicity. Additionally, cytokines, chemokines, biogenic and trace amines, neurotransmitters, and pharmacological agents induce intracellular signaling cascades in astrocytes through the activation of membrane and cytosolic receptors (Fraser et al., 1994; Porter and McCarthy, 1997; Liu et al., 2004). It has been reported that molecular alterations in astrocyte TAAR1 levels correspond to changes in astrocyte EAAT2 levels and function (Cisneros and Ghorpade, 2014). Methamphetamine (Meth), known as a TAAR1 agonist, modulates Glu clearance abilities (Cisneros and Ghorpade, 2014). To our knowledge, it has not yet been documented that DA exposure of astrocytes leads to the expression and function of TAAR1 and decreases EAAT2 levels and function in astrocytes. We hypothesized that DA may result in excitotoxic accumulation of extracellular Glu.

Glu is the predominant excitatory neurotransmitter in the mammalian CNS; however, excessive activation of Glu receptors can cause excitotoxicity (Choi, 1992). Glu toxicity is known to be involved in many neurological disorders, including Alzheimer's disease, ischemic stroke, Parkinson's disease, epilepsy, and depression (Coyle and Puttfarcken, 1993; Simonian and Coyle, 1996; Mao et al., 2015). Glu-induced excitotoxicity also plays an important role in the pathogenesis of DA-induced MHE diseases. However, the molecular basis of this role remains elusive. We hypothesized that DA may increase Glu level and collaborate with Glu to impair memory.

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In this study, we investigated whether DA caused elevation of Glu in MHE and the underlying mechanism for the synergistic induction of memory loss by DA and Glu. We found that DA regulated EAAT2 and TAAR1 expression and function. Further, DA exposure led to a reduction in Glu clearance in astrocytes, which was prevented by TAAR1 RNA interference (RNAi). Finally, we demonstrated that DA and Glu synergistically activated calcineurin (CaN)/nuclear factor of activated T cell (NFAT) signaling to decrease the production of BDNF/NT3 in neurons. Sensitization of TAAR1 by DA and overexcitation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPAR) by elevated Glu co-signal to activate CaN. Based on these findings, we propose that astrocyte responses to DA are mediated via TAAR1 activation followed by a decrease in Glu clearance abilities, and the combined toxicity of DA and Glu results in synergistic effects on the activation of CaN/NFAT and reduction of BDNF/NT3 levels. In summary, this study identified the elevation of Glu along with DA that may play a key role in cognitive decline in MHE.

EXPERIMENTAL PROCEDURES

MHE models and treatments

A total of 40 Sprague–Dawley rats weighing 220–250 g were obtained from the Experimental Animal Center of the Chinese Academy of Sciences in Shanghai for use in this study. All experiments were carried out in accordance with the guidelines laid down by the Ethics Committee of the Affiliated Hospital of Wenzhou Medical University regarding the care and use of animals for experimental procedures. Before experimentation, all animals underwent 2 behavioral tests: the Y-maze (YM) and the water-finding task (WFT). Normal values for these behavioral tests were obtained. Rats were then randomly divided into 2 groups: a control group (n = 20) and a thioacetamide (TAA) group (n = 30). Liver cirrhosis was induced by intraperitoneal (IP) injection of TAA (200 mg/kg in normal saline, Sigma–Aldrich) twice per week for 8 weeks. TAA-treated rats were diagnosed with hepatic encephalopathy (HE) based on the following symptoms: later development of decreased motor activity, lethargy, and eventual progression to coma. TAA-treated rats with no HE symptoms were again subjected to behavioral tests to confirm whether MHE had developed. If TAA-treated rats met the following alternative criteria for MHE, they were included in the MHE group: (a) values of YM lower than mean ± 1.96·SD; (b) values of WFT more than mean ± 1.96·SD (Albrecht et al., 2000).

Y-maze

Rats were individually placed at the end of an arm and allowed to explore the maze freely for 8 min. Total arm entries and spontaneous alternation percentage (SA%) were measured. SA% was defined as the ratio of arm choices that differed from the previous 2 choices (successful choices) to total choices during the run (total entries minus 2 because the first 2 entries could not be evaluated) (Yamada et al., 2005).

Water-finding task

A rat was placed at the near-right corner of the apparatus and allowed to explore it freely for 3 min. Rats were omitted from the analysis when they could not find the tube within the 3-min exploration. After the training session, rats were deprived of water for 24 h. In the trial session, rats were again individually placed at the same corner of the apparatus and allowed to find and drink the water in the alcove. The elapsed times until the entry into the alcove (entry latency, EL), until the touching/sniffing/licking of the water tube (contacting latency, CL), and until the initiation of drinking from the water tube (drinking latency, DL) were measured (Kawasumi et al., 2004).

Microdialysis

A microdialysis probe (BAS MD-2204, 4-mm membrane) was stereotaxically inserted into the anterior cingulate cortex (from −2.7 mm anterior to the bregma to 1.3 mm posterior to the bregma). Glutamate (60 μmol/10 μl in artificial cerebrospinal fluid) was perfused at 2 μl/min using a microinjection pump (BeeHive BAS, USA). The microdialysis samples were continuously collected into microvials collected every 20 min. Three samples were collected as baseline values at the end of a 2-h equilibration period. The concentrations of glutamate in the microdialysis samples were determined using a CMA 600 Analyzer (Solna, Sweden). Level changes for all measured chemicals were expressed as percent relative to the mean baseline value (Ye et al., 2011).

DA-treated rats models and treatments

Rats were anesthetized with intramuscular xylazine (16 mg/kg) followed by ketamine (100 mg/kg). Intracerebroventricular (ICV) injection of DA hydrochloride (10 μg/3 μl in saline) was stereotaxically performed in the left lateral ventricles of the rats 3 times at 7-day intervals (anterior–posterior, +0.3 mm; lateral, 1.0 mm; horizontal, 3.0 mm from the bregma) (n = 15). At 24 h after the injection, rats underwent YM and WFT tests. Then the rats were anesthetized with intramuscular xylazine (16 mg/kg); the blood was drawn from the aorta abdominalis, and cerebral cortex tissues were collected.

Evaluation of Glu uptake

Extracellular glutamate levels were measured by a fluorimetric method using the Amplex Red Glutamic Acid assay kit (Invitrogen). After 12 h of DA treatment, PCA culture medium was replaced by Hepes buffer containing 500 μM glutamate. At each time point, 50 μl of the supernatant was transferred into 96-well microplates and then mixed with 50 μl substrate mixture (100 mM Amplex Red, 0.25 U/ml horseradish peroxidase, 0.08 U/ml L-glutamate oxidase, 0.5 U/ml glutamate pyruvate transaminase, and 200 μl alanine)