Glutamate affects the production of epoxyeicosanoids within the brain: The up-regulation of brain CYP2J through the MAPK-CREB signaling pathway

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ABSTRACT

Glutamate is the major excitatory neurotransmitter in the brain, and chronic glutamate excitotoxicity has been thought to be involved in numerous neurodegenerative diseases. We investigated the effects of glutamate at concentrations lower than the usual extrasynaptic concentrations on the production of epoxyeicosanoids mediated by brain CYP2J. Glutamate increased CYP2J2 mRNA levels in astrocytes in a dose-dependent manner, while an antagonist of the metabotropic glutamate receptor subtype 5 (mGlu5 receptor) attenuated the glutamate-induced increases in CYP2J2 levels by glutamate. Glutamate increased the binding of cAMP response element-binding protein (CREB) with the CYP2J2 promoter, and the inhibition of the MAPK signaling pathway (ERK1/2, p38, and JNK) decreased the binding of CREB with the CYP2J2 promoter following the glutamate treatment. CREB activated the CYP2J2 promoter located at −1522 to −1317 bp, and CREB overexpression significantly increased CYP2J2 mRNA levels. The CYP2J2 and mGlu5 mRNA levels were higher in the frontal cortex, hippocampus, cerebellum, and brainstem in adult rats that received a subcutaneous injection of monosodium L-glutamate at 1, 3, 5, and 7 days of age. The data from the partial least-squares-discriminant analysis showed the epoxyeicosanoid profile of the hippocampus from the cerebellum, brain stem, and frontal cortex. The sum of the epoxyeicosatrienoic acids (EETs) and dihydroxyeicosatrienoic acids (DHETs) was increased by 1.16-fold, 1.18-fold, and 1.19-fold in the frontal cortex, cerebellum, and brain stem, respectively, in rats treated with monosodium L-glutamate compared with the control group. The results suggest that brain CYP2J levels and CYP2J-mediated epoxyeicosanoid production can be regulated by extrasynaptic glutamate. The glutamate receptors expressed in astrocytes may mediate the regulation of drug-metabolizing enzymes and the metabolome of endogenous substances by glutamate.

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1. Introduction

Arachidonic acid (AA), a polyunsaturated fatty acid present in mammalian cell membranes, is metabolized by cyclooxygenases (COX) (Funk, 2001; Simmons et al., 2004; Smith et al., 2000), lipoxygenases (LOX) (Brash, 1999; Funk, 2001; Kuhn et al., 2005) and cytochrome P450 (CYP) (Capdevila and Falck, 2002; Fleming, 2008; McGiff and Quilley, 1999; Roman, 2002; Spector et al., 2004;

Zeldin, 2001). Disordered AA cascades have been found in neuropsychiatric diseases. The CYP2 family, including CYP2C8 and CYP2J2, is the predominant AA epoxygenase, and the epoxidation of AA produces four regioisomeric *cis*-epoxyeicosa-trienoic acids (5,6-, 8,9-, 11,12-, and 14,15-EET) (Konkel and Schunck, 2011). Bioactive EETs have a substantial anti-inflamma-tory (Node et al., 1999) action both in vitro and in vivo and are metabolized by soluble epoxide hydrolase (sEH) to less-active dihydroxyeicosatrienoic acids (DHETs) (Fleming, 2001; Graves et al., 2013). The inhibitors of sEH, which raise endogenous EET levels, provide protection from stroke, cerebral ischemia, pain and other medical conditions (Imig and Hammock, 2009; Shen, 2010). EETs can prevent the impairment of cellular structure and promote anti-inflammatory reactions in various cell types. A previous study reported that 14,15-EET levels in cerebrospinal fluid were elevated in aneurysmal subarachnoid hemorrhage patients compared to controls (Siler et al., 2015). Mice lacking sEH had elevated 14,15-EET and were protected from the delayed decrease in microvascu-lar cortical perfusion after subarachnoid hemorrhage compared to wild type mice.

CYP2J2 is one of the main CYP isoforms expressed in the human brainand represents approximately 20% of the total CYP transcripts (Dutheil et al., 2009). Immunohistochemistry staining showed that CYP2J2 proteins were higher in the glial cells in the frontal cortex, hippocampus, and cerebellum, although CYP2J2 was also observed in neurons. CYP2C8 was present at lower levels, and other CYP2C isoforms, including CYP2C9, CYP2C18, and CYP2C19, were not observed in the human brain. CYP2J2 could be the main enzyme responsible for the production of EETs.

Glutamate is the major excitatory neurotransmitter in the brain and exerts its action through glutamate receptors localized in the cellular membranes of neurons and glia cells. Chronic glutamate excitotoxicity has been thought to be involved in numerous neurodegenerative diseases, including amyotrophic lateral sclero-sis, Alzheimer's disease and Huntington's disease (Lewerenz and Maher, 2015). A previous study showed that the short stimulation of glutamatergic neurotransmission induced a small but significant loss of membrane cholesterol via cellular mobilization of CYP46A1 towards the plasma membrane (Sodero et al., 2012). The impact of chronic glutamate excitotoxicity on drug-metabolizing enzymes and the related metabolism of endogenous substance remains unclear.

In the present study, we investigated the regulatory mechanism of brain CYP2J by glutamate and the changes in epoxyeicosanoid production in rats. Rat CYP2J3 was the major isoform among the CYP2J subfamily which shares high amino acid sequence identity with human CYP2J2 (> 70%) (Xu et al., 2013). This work adds to the current knowledge on the regulation of drug-metabolizing enzymes within the brain by neurotransmitters.

2. Materials and methods

2.1. Animals and treatment

Adult Wistar rats (250–300 g) obtained from the Experimental Animal Center (Hubei, China) were kept in a room under a controlled temperature (22–25 °C), on a 12 h artificial light/dark cycle, with free access to food and water. All animal care and experimental procedures were approved by the Animal Care Committee of Wuhan University and conducted in accordance with the Chinese guidelines for the care and use of laboratory animals. After a 2-week accommodation period in our facilities, the animals were bred by randomly housing two females in a cage with one male rat. On the day of parturition, the litters were adjusted to 2 or 4 male pups per cage. Age-matched pups were randomly divided into the following 2 groups: the monosodium L-glutamate group

and the control group. At 1, 3, 5, and 7 days of age, rats were subcutaneously injected with either monosodium L-glutamate (4 mg/g BW; Cat. 6106-04-3, Sigma-Aldrich, St. Louis, MO, USA) or an equivalent amount of saline. Previous study has shown the activation of MAPK signaling pathway in rat hippocampus at $6 \sim 72$ h after the last injection of monosodium L-glutamate (Ortuno-Sahagun et al., 2014) Monosodium L-glutamate treatment increased the N-methyl-D-aspartate receptors (NMDAs) and glial cell number in hippocampus from the rats treated with the same regimen used in present study (Beas-Zarate et al., 2001; Beas-Zarate et al., 2002). The pups were weaned at 25 days of age. At 10 weeks after the Injection, the rats were sacrificed and the brains were quickly removed. The samples for LC–MS/MS analysis were immediately frozen and stored at −80 °C. The tissues for mRNA detection were stored in RNAlater according to the manufacturer's protocol (Ambion, Austin, TX).

2.2. Cell culture and treatment

Human glioma U251 cells (China Centre for Type Culture Collection, CCTCC, Wuhan, China) were exposed to a range of glutamate concentrations (10–50 μM) for 24 h. To investigate the involvement of metabotropic glutamate receptor subtype 5 (mGlu5 receptor) in the regulation of CYP2J2 levels by glutamate, U251 cells were pre-treated with or without the selective mGluR5 antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), for 30 min before the cells were incubated with 50 μM of glutamate for 24 h. To investigate the involvement of the MAPK signaling pathway in the regulation of CYP2J2 levels by glutamate, U251 cells were pre-treated with or without specific inhibitors of JNK (SP600125, Cat. S5567, Sigma-Aldrich, St Louis, MO, USA), p38 (SB202190, Cat. S8307, Sigma-Aldrich, St Louis, MO, USA), or ERK (U0126, Cat. U120, Sigma-Aldrich, St Louis, MO, USA) for 30 min before the cells were incubated with 50 μM of glutamate for 8 h. To investigate the effects of the MAPK signaling pathway on the binding of cAMP response element-binding protein (CREB) with the *CYP2J2* promoter following glutamate treatment, U251 cells were pre-treated with or without specific inhibitors of JNK, p38, or ERK for 30 min before the cells were incubated with 50 μM of glutamate for 24 h.

2.3. Real-time RT-PCR

Total RNA was isolated using TRIzol reagent (Cat. 108952, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. cDNA was synthesized using a cDNA synthesis kit (TOYOBO, Osaka, Japan). All real-time PCR reactions with SYBR Green (Cat. K1621, TOYOBO, Osaka, Japan) were performed on a CFX connect real-time PCR detection system (Bio-Rad, Hercules, CA, USA). GAPDH was used for the normalization of relative expression levels. Primers are listed in Supplementary Table S1.

2.4. Immunoblotting

The total proteins (20 μg) from the cultured cells were separated by SDS-polyacrylamide gel electrophoresis (4% stacking and 10% separating gels) and were then transferred overnight onto PVDF membranes. The membranes were incubated for 2 h with monoclonal rabbit anti-human CREB antibody (Cat. ab32515,1:800, Abcam, Cambridge, UK), monoclonal rabbit anti-human phospho-CREB (S133) antibody (Cat. ab32096, 1:800, Abcam, Cambridge, UK), monoclonal rabbitanti-human ERK1/2 antibody (Cat. 4695, 1:3000, Cell Signaling Technology, Beverly, MA, USA), monoclonal rabbit anti-human phospho-ERK1/2 antibody (Cat. 4370, 1:3000, Cell Signaling Technology, Beverly, MA, USA), monoclonal rabbit anti-human p38 MAPK antibody (Cat. 8690, 1:3000, Cell Signaling