NeuroToxicology 34 (2013) 212–218

Contents lists available at SciVerse ScienceDirect

NeuroToxicology

Methamphetamine regulation of sulfotransferase 1A1 and 2A1 expression in rat brain sections

Tianyan Zhou [a,c], Chaoqun Huang [b], Yue Chen [b], Jiaojiao Xu [a], Preeti Devaraya Shanbhag [b], Guangping Chen [b,*]

[a] State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing, China
[b] Department of Physiological Sciences, Oklahoma State University, Stillwater, OK, United States
[c] Department of Pharmaceutics, School of Pharmaceutical Sciences, Peking University Medical Health Center, Beijing, China

ARTICLE INFO

Article history:
Received 19 July 2012
Accepted 21 September 2012
Available online 29 September 2012

Keywords:
Sulfotransferase
Methamphetamine
Gene regulation
Rat brain sections
rSULT1A1
rSULT2A1

ABSTRACT

Sulfotransferase catalyzed sulfation regulates the biological activities of various neurotransmitters/hormones and detoxifies xenobiotics. Rat sulfotransferase rSULT1A1 catalyzes the sulfation of neurotransmitters and xenobiotic phenolic compounds. rSULT2A1 catalyzes the sulfation of hydroxysteroids and xenobiotic alcoholic compounds. In this work, Western blot and real-time RT-PCR were used to investigate the effect of methamphetamine on rSULT1A1 and rSULT2A1 protein and mRNA expression in rat cerebellum, frontal cortex, hippocampus, and striatum. After 1-day treatment, significant induction of rSULT1A1 was observed only in the cerebellum; rSULT2A1 was induced significantly in the cerebellum, frontal cortex, and hippocampus. After 7 days of exposure, rSULT1A1 was induced in the cerebellum, frontal cortex, and hippocampus, while rSULT2A1 was induced significantly in all four regions. Western blot results agreed with the real-time RT-PCR results, suggesting that the induction occurred at the gene transcriptional level. Results indicate that rSULT1A1 and rSULT2A1 are expressed in rat frontal cortex, cerebellum, striatum, and hippocampus. rSULT1A1 and rSULT2A1are inducible by methamphetamine in rat brain sections in a time dependable manner. rSULT2A1 is more inducible than rSULT1A1 by methamphetamine in rat brain sections. Induction activity of methamphetamine is in the order of cerebellum > frontal cortex, hippocampus > striatum. These results suggest that the physiological functions of rSULT1A1 and rSULT2A1 in different brain regions can be affected by methamphetamine.

1. Introduction

Cytosolic sulfotransferases (SULTs) catalyze the sulfation of a wide range of hydroxyl-containing compounds (Bojarova and Williams, 2008; Chapman et al., 2004; Gamage et al., 2006; Hempel et al., 2007; Kauffman, 2004; Rath et al., 2004; Runge-Morris and Kocarek, 2005; Wang and James, 2006). Various bio-signaling molecules including neurotransmitters, hydroxysteroids, and glucocorticoids, are regulated by sulfation. Sulfation usually leads to the inactivation of biological signaling molecules, as the sulfated forms are usually unable to bind to receptors. Sulfation of xenobiotics usually leads to detoxification, as sulfated xenobiotics are readily excreted. Although SULTs are most highly expressed in the liver, they are present in many tissues, including the small intestine, brain, adrenal gland, kidney, skin, blood platelets and nucleated blood cells (Alnouti and Klaassen, 2006; Nowell and Falany, 2006; Richard et al., 2001). SULTs are divided into two major subfamilies, phenol SULTs (SULT1) and hydroxysteroid SULTs (SULT2) (Blanchard et al., 2004). Several of the SULTs have been identified in human brain tissues, and the phenol SULTs have been detected both in neurons and glial cells in several different brain regions, such as the cerebellum, hippocampus, stratium, and so on (Alnouti and Klaassen, 2006; Richard et al., 2001; Salman et al., 2009; Vietri et al., 2003; Whittemore et al., 1986; Yasuda et al., 2007; Zou et al., 1990). rSULT1A1 shows a very broad distribution and broad substrate specificity (Duffel, 1994; Riches et al., 2007). It is one of the major SULT1 enzymes. It is responsible for the sulfation of neurotransmitters and xenobiotic phenolic compounds. Hydroxysteroid SULTs (SULT2, including SULT2A and SULT2B subfamilies) have also been identified in the brain, catalyzing the sulfation of hydroxyl-containing neurosteroids such as androsterone and allopregnanolone and xenobiotic alcohol compounds (Shimizu et al., 2003; Shimizu and Tamura, 2002).

* Corresponding author at: 264 McElroy Hall, Oklahoma State University, Stillwater, OK, 74078, United States. Tel.: +1 405 744 2349; fax: +1 405 744 8263.
E-mail address: guangping.chen@okstate.edu (G. Chen).

0161-813X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuro.2012.09.010

SULT2A1 is one of the major SULT2 enzymes (Fang et al., 2007; Liu et al., 2006). It is also widely distributed and responsible for the sulfation both xenobiotics and hydroxysteroids. rSULT1A1 and rSULT2A1, two of the major SULTs, representing the two major subfamilies, are selected for the studies.

Methamphetamine (METH) is one of the most abused psychostimulants in the world (Brackins et al., 2011). METH administration has been shown to produce long-term decreases in numerous measures of both dopaminergic and serotonergic function such as enzyme activity, monoamine content and monoamine transmitters in experimental animals as well as in human (Gold et al., 2009; Guilarte et al., 2003; Kish, 2008; Kita et al., 2003; Krasnova and Cadet, 2009; Quinton and Yamamoto, 2006). The pharmacological and toxic effect of METH is also dependent on treatment methods. Acute administration of METH produces locomotor activation (Gaytan et al., 1998), and repeated administration produces a progressive sensitization of this behavioral activation until stereotyped behavior comes to predominate the behavioral repertoire (Robinson and Berridge, 1993). Chronic or intermittent METH abuse may create temporary or permanent disturbances in the dopaminergic systems of the brain that may predispose individuals to parkinsonism. METH toxicity is frequently reported as a potential model of drug induced parkinsonism (Gerlach and Riederer, 1996; Guilarte, 2001; Kita et al., 2003; Tolwani et al., 1999).

Similar to cytochrome P450s (CYP), most SULTs are regulated by hormones that remain under the control of the central nervous system (CNS). METH treatment is known to cause the change of dopamine in the CNS. Dopamine is one of the most important endogenous neurotransmitters as well as an important endogenous substrate of SULTs (Lu et al., 2005; Yasuda et al., 2007, 2009). It has been demonstrated that the expression of CYPs can be regulated by dopamine and other psychostimulants through the dopamine receptor-linked signaling pathway by changing hormone levels *in vivo* (Konstandi et al., 2008; Wójcikowski, 2004; Wójcikowski et al., 2007, 2008). Psychostimulant regulation of SULTs is not well studied. To the best of our knowledge, there are only two reports on METH regulation of SULTs. One study used a microarray method to screen a series of various candidate genes after a single-dose METH treatment (4.0 mg/kg) in rats (Niculescu et al., 2000). This report showed that the treatment induced rat brain rSULT1A1 mRNA 4.3-fold in the amygdale. Later, our results indicated that METH induced rSULT1A1, rSULT2A1 and rSULT1E1 protein expression both in the rat liver and brain (Zhou et al., 2010). In this study, the effect of short- and long-term treatment of male rats with METH on rSULT1A1 and rSULT2A1 expressions in the cerebellum, frontal cortex, hippocampus, and striatum was studied. Identifying regulation of rSULTs by METH in brain sections will assist in understanding the functions of SULTs in the brain as well as the toxic effects of MEHT on the brain.

2. Materials and methods

2.1. Materials

Methamphetamine was purchased from Sigma–Aldrich (St. Louis, MO). SDS-polyacrylamide gel electrophoresis reagents were obtained from Bio-Rad (Hercules, CA). Western blot chemiluminescence reagent kits (Super Signal West Pico Stable Peroxide and Super Signal West Pico Luminol/Enhancer solutions) were purchased from Pierce Chemical (Rockford, IL). PVDF membranes used for Western blotting analyses were purchased from Millipore Corporation (Bedford, MA). TRI REAGENT for total RNA extraction was purchased from MRC (Cincinnati, OH). M-MLV reverse transcriptase was obtained from Promega (Madison, WI). qPCR MasterMix Plus with SYBR® Green I dNTP was purchased from Eurogentec (San Diego, CA). Rabbit anti-rat SULT1A1was provided by Dr. David Ringer (American Cancer Society). Rabbit anti-STa (rSULT2A1) was provided by Dr. Michael W. Duffel (Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa city, IA). Protein assay reagent was purchased from Bio-Rad (Hercules, CA). All other reagents and chemicals were of the highest analytical grade available.

2.2. Animals and drug treatment

Male Sprague-Dawley rats (Harlan, Indianapolis, IN), 10–11 weeks old and 200–300 g body weight were used in this investigation. Rats were housed in a temperature- and humidity-controlled room and supplied with rodent chow and water for at least 1 week before use. Rats with each treatment were divided into 4 groups with four in each group. METH was dissolved in sterilized saline and administrated by intraperitoneal injection at 1, 5 and 20 mg/kg/day (single dose administration) for a 1-day treatment, and 0, 0.2, 1, 5 mg/kg/day for a 7-day treatment, respectively. The corresponding group of control rats received only sterilized saline. Animals did not show obvious signs/symptoms of METH intoxication or toxicity under these treatment conditions. The rats were sacrificed 24 h after the final drug treatment. The cerebellum, frontal cortex, hippocampus, and striatum of the rat brain were collected, washed with sterilized ice-cold NaCl (0.9%, w/v) solution, and snap-frozen. Samples were stored at −80 °C until use.

2.3. Cytosol preparation

The cerebellum, frontal cortex, hippocampus and striatum of the rat brain were homogenized in 50 mM Tris buffer containing 0.25 M sucrose, 3 mM β-mercaptoethanol and 0.02% (v/v) Tween-20, pH 7.4. All homogenates were centrifuged at 100,000 × *g* for 1 h at 4 °C. Cytosol aliquots were collected and preserved at −80 °C for Western blot analysis.

2.4. Western blot analysis

Cytosol protein from the frontal cortex, cerebellum, hippocampus and striatum of the rat brain (40 μg) was used in a 12% polyacrylamide gel in an electrophoresis system (Novex, San Diego, CA). After running at 200 V, the protein bands were transferred overnight at 35 V onto a PVDF membrane. Themembranes were blocked for 1 h by 5% (w/v) nonfat dry milk in Tris-buffered saline (TBS). For these cytosol proteins from different brain regions, membranes were incubated with either rabbit anti-rat AST-IV (rSULT1A1), or rabbit anti-rat STa (rSULT2A1) (1:1000) overnight in TBST (50 mM Tris, pH 7.5, 150 mM NaCl, and 0.05% (v/v) Tween 20) containing 5% (w/v) nonfat dry milk on a shaker at 4 °C. After incubation, all membranes were washed with TBST for 3× 10 min and incubated in secondary antibody (Horseradish peroxidase-conjugated Immuno-Pure goat anti-rabbit IgG (H + L) at 1:8000 dilutions in the same buffer for at least 1 h). The membranes were washed with TBST for 3× 10 min. The fluorescent bands were developed with 3 ml of substrate containing the same volume of each Super Signal West Pico Luminol Enhancer solution and Super Signal West Pico Stable Peroxidase solution at room temperature for 5 min. The fluorescence image was obtained using a VersaDoc IMAGING SYSTEM 5000MP (BIO-RAD, Hercules, CA). The densitometric quantification of protein bands was obtained using Quantity One 4.6.5 software of VersaDoc imaging system.

2.5. Quantitative real-time PCR

Total RNA was extracted from different regions of the brain using TRI REAGENT from MRC according to supplier's guidelines.