Neuroscience 320 (2016) 230–238

INVOLVEMENT OF DORSAL STRIATAL α1-CONTAINING $GABA_A$ RECEPTORS IN METHAMPHETAMINE-ASSOCIATED REWARDING MEMORIES

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Abstract—Rewarding memories induced by addictive drugs may contribute to persistent drug-seeking behaviors, which is an important contributing factor to drug addiction. However, the biological mechanisms underlying drug-associated rewarding memories have not yet been fully understood, especially the new synthetic drugs, such as amphetamine-type stimulants (ATS). In this study, using the rat-conditioned place preference (CPP) model, a classic animal model for the reward-associated effects of addictive drugs, we found that the expression level of $GABA_A$ α1 subunits was significantly decreased in the dorsal striatum (Dstr) after conditioned methamphetamine (METH) pairing, and no significant differences were observed in the other four rewarding memory-associated areas (medial prefrontal cortex (mPFC), nucleus accumbens (NAc), amygdala (Amy), and dorsal hippocampus (DH)). Intra-Dstr injection of either the $GABA_A$ receptor agonist muscimol or the specific α1$GABA_A$ receptor-preferring benzodiazepine (BDZ) agonist zolpidem significantly abolished METH CPP formation. Thus, this study extends previous findings by showing that $GABA_A$ receptors, particularly the α1-containing $GABA_A$ receptors, may be strongly implicated in METH-associated rewarding memories. This work provides us with a new perspective on the goal of treating ATS addiction.

Key words: methamphetamine, $GABA_A$α1, dorsal striatum, rewarding memories, conditioned place preference.

INTRODUCTION

Amphetamine-type stimulants (ATS) comprise a class of psychoactive substances including amphetamine, methamphetamine (METH), 3,4-methylenedioxymetham phetamine, and 3,4-methylenedioxyamphetamine, among which METH is the most prevalent abuse drug and can produce both physical and psychological harm to its users(Burns, 2014). METH leads to significant medical consequences, including psychosis, dependence, cognitive disorders, overdose and death (Batki and Harris, 2004; Curran et al., 2004). The long-term use of METH results in addiction, which is characterized by a loss of control over drug intake despite adverse consequences. Currently, there are as yet no effective approaches for the treatment of METH addiction, and behavioral or psychological therapies have demonstrated limited efficacy (Shearer and Gowing, 2004). Therefore, understanding the neurobiological mechanisms of METH addiction is of crucial importance. Most traditional researches on METH dependence in both animals and humans focus on the meso-corticolimbic dopamine system. Nevertheless, the GABA (gamma-aminobutyric acid)-ergic system is attracting more and more attention for its important role in the development and manifestation of addiction (Addolorato et al., 2012; Kumar et al., 2013).

GABA is a major neurotransmitter that exerts inhibitory effects on the central nervous system. GABA receptors are the major components of the GABA-ergic system, including the ionotropic receptors ($GABA_A$ and $GABA_C$) and the metabotropic receptors ($GABA_B$), and the ionotropic $GABA_A$ receptors are the first identified and best studied. Currently, $GABA_A$ receptors have already become important targets for the treatment of insomnia and are also suggested to play important roles in anxiety, memory deficit, and drug addiction (Tan et al., 2011). $GABA_A$ receptor modulators are reported to interfere with the medical consequences of ATS. For example, animal experiments have demonstrated that mice pre-treated with diazepam, a $GABA_A$ receptor agonist, exhibit decreased reactivity to ATS (Panhelainen et al., 2011). In contrast, the $GABA_A$ receptor antagonist bicuculline could increase animal activities in response to ATS injection (Enomoto et al., 2011). Clinical research has also shown that taking alprazolam, a benzodiazepine (BDZ) drug, before ATS, would weaken the effects of ATS and compete with ATS-induced desire (Rush et al., 2004).

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Abbreviations: βCCt, β-carboline-3-carboxylate-t-butyl ester; Amy, amygdala; ANOVA, analysis of variance; ATS, amphetamine-type stimulants; BDZ, benzodiazepine; CPP, conditioned place preference; DH, dorsal hippocampus; DMSO, dimethylsulfoxide; Dstr, dorsal striatum; GABA, gamma-aminobutyric acid; METH, methamphetamine; mPFC, medial prefrontal cortex; NAc, nucleus accumbens.

http://dx.doi.org/10.1016/j.neuroscience.2016.02.001
0306-4522/

Therefore, the $GABA_A$ receptors may also be potential targets for the treatment of ATS addiction.

$GABA_A$ receptors are composed of five protein subunits that belong to different classes, including α1–6, β1–3, γ1–3, δ, ε, θ1–3, π, and ρ1–3, and the different subunits vary in function. Recent studies have reported that the activation of the $GABA_A$ α1 subunit was the main factor in the $GABA_A$ receptor's inhibitory effects on memory (Makaron et al., 2013; Soto et al., 2013). Therefore, we hypothesize that $GABA_A$ receptors, especially the $GABA_A$ receptors that contain α1 subunits, are involved in the rewarding memories induced by METH addiction. In this study, utilizing METH, we tested this hypothesis in the rat-conditioned place preference (CPP) model by examining the $GABA_A$ α1 subunit expression changes in different brain regions, especially addiction-related limbic structures, e.g., the medial prefrontal cortex (mPFC), nucleus accumbens (NAc), dorsal striatum (Dstr), amygdala (Amy), and dorsal hippocampus (DH), and determining the role of the $GABA_A$ α1 subunit in the induction of METH addiction.

EXPERIMENTAL AND PROCEDURES

Animals

Sprague–Dawley male rats weighting 220–300 g (aging 50-60 days) were obtained from the Laboratory Animal Center, Chinese Academy of Sciences (Shanghai, China). Rats were housed 2–3 per cage and maintained on a 12-h light/dark cycle with access to food and water *ad libitum*. All experimental procedures in this manuscript were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996) and approved by the Institutional Animal Care and Use Committee of Chinese Academy of Sciences (Shanghai, China).

To minimize the basal stress, saline-treated rats and METH-treated rats were housed in different cages, and the rats were moved to the testing room 24 h before behavioral tests, and handled twice a day for 4 days before behavioral tests.

Drugs and antibodies

METH was obtained from the China academy of military medical science. Muscimol was supplied by Invitrogen. Bicuculline and β-carboline-3-carboxylate-t-butyl ester (βCCt) were supplied by Sigma Aldrich. Zolpidem were supplied by Medchemexpress Co., Ltd. The antibodies of anti-$GABA_A$α1, anti-$GABA_A$α2 and anti-$GABA_A$β2 were purchased from Millipore and diluted 1:1000 for Western blot analysis. Anti-Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) was purchased from Bio Legend, Inc and diluted 1:20,000 for Western blot analysis.

CPP

The CPP apparatus (Jiliang Software and Instruments, Shanghai, China) was divided into two equal-sized compartments [40 cm (length) × 40 cm (width) × 60 cm (height)] separated by a removable board (10 × 10 cm), which allowed rats free access to each compartment. Two compartments were distinguished by visual and tactile cues: one was a black and white horizontal striped wall with an iron wire floor, whereas the other was a black and white vertical striped wall with a steel bar floor. These distinctive tactile and visual stimuli served as the conditioning.

The place conditioning procedure used in this experiment included four phases: habituation, preconditioning, conditioning, and testing. In the habituation phase, the rats were allowed to freely explore the entire apparatus for 30 min. In the preconditioning phase, the rats were allowed to freely explore the entire apparatus for 15 min. The time spent in each compartment was recorded in the preconditioning phase, and rats showing a strong unconditioned aversion (one compartment > 720 s) for either compartment were eliminated from the study. Conditioning occurred over the next 8 d. The dose of MEHT (1 mg/kg, i.p.) in the Conditioning phase was established from a dose-dependent study conducted by DeMarco et al. (2009), where METH (1 mg/kg) produced a reliable and consistent CPP, and the doses of 1, 2.5, 5 and 10 mg/kg did not produce a dose-dependent change in place preference. As higher doses did not result in greater place preference changes, we used a dose of 1 mg/kg in this study. This dose was consistent with previous studies (Xu et al., 2006; Yang et al., 2008; Herrold et al., 2009; Voigt et al., 2011; Subiah et al., 2012), confirming that METH is a reward-related addictive drug. On the first day of the conditioning phase, the rats were injected with either METH (1 mg/kg, i.p.) or saline (1 mL/kg, i.p.) and then confined to the non-preferred compartment in a counterbalanced manner for 45 min. This compartment will be referred to as the "drug treatment-paired compartment." On the second day, the rats were injected with saline (1 mL/kg, i.p.) and then confined to the opposite compartment from the first day for 45 min. This procedure was repeated four times in the conditioning phase. This conditioning protocol has been successfully used in the past to reliably produce METH CPP (Subiah et al., 2012). The testing phase occurred 24 h after the conditioning trial, and all rats were allowed to freely explore the entire apparatus for 15 min; the amount of time spent in each compartment was recorded. The CPP score represents the time in the drug treatment-paired compartment during the testing phase minus the time in that compartment during the preconditioning phase.

Unconditioned METH injection (unpaired), on the other hand, refers to the same drug injection (saline or METH) paradigm, but without pairing with compartment.

Subcellular fractionation

For Western blotting analysis, rats were anesthetized and killed by decapitation immediately (0 h) or 1 h after MEHT CPP on the seventh day of CPP conditioning. Coronal brain sections (1 mm thick) were obtained using a rat brain slicer (Braintree Scientific, Massachusetts, USA). Both sides of the Dstr were punched from brain slices using a blunt end, 17-gauge syringe needle (1 mm inner diameter). The homogenate was centrifuged at 1000×*g* for 10 min, and the supernatant was collected.