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Short communication

Chicago sky blue 6B, a vesicular glutamate transporters inhibitor, attenuates methamphetamine-induced hyperactivity and behavioral sensitization in mice

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HIGHLIGHTS

- CSB6B, a VGLUTs inhibitor, attenuated acute METH-induced hyperactivity.
- CSB6B attenuated development of METH-induced behavioral sensitization.
- CSB6B attenuated expression of behavioral sensitization to METH.
- VGLUTs were involved in METH dependence.
- VGLUTs may be a new target against METH dependence.

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ABSTRACT

Several lines of evidence demonstrate that glutamatergic system plays an important role in drug addiction. The present study was designed to investigate the effects of Chicago sky blue 6B (CSB6B), a vesicular glutamate transporters (VGLUTs) inhibitor, on methamphetamine (METH)-induced behaviors in mice. Mice were induced behavioral sensitization to METH by subcutaneous injection of 1 mg/kg METH once daily for 7 days and then challenged with 1 mg/kg METH in 14th day. Intracerebroventricular administration of CSB6B (7.5 μg) 2.5 h prior to METH was to observe its effects on METH -induced behavioral sensitization. Our results showed that the expressions of behavioral sensitization were significantly attenuated by intracerebroventricular administration of CSB6B 2.5 h prior to METH either during the development period or before methamphetamine challenge in mice, while CSB6B itself had no effect on locomotor activity. Meanwhile, pretreatment of CSB6B also attenuated hyperactivity caused by a single injection of METH in mice. These results demonstrated that CSB6B, a VGLUTs inhibitor, attenuated acute METH-induced hyperactivity and chronic METH-induced behavioral sensitization, which indicated that VGLUTs were involved in the effect of chronic METH-induced behavioral sensitization and may be a new target against the addiction of METH.

1. Introduction

Methamphetamine (METH) is a highly addictive psychostimulant that dramatically causes behavioral abnormalities in humans [1,2] and behavioral sensitization and conditioned place preference in rodents [3]. Although these addictive behaviors contain different systems, in which mesolimbic dopaminergic system is a traditional focus in the field of METH addiction mainly mediated by altering dopamine release [4,5]. More and more studies, recently, have suggested that glutamatergic systems may be involved in behavioral effects of METH [6–8].

It is well recognized that the levels of synaptic cleft glutamate is mainly maintained by excitatory amino acid transporters (EAATs) located in plasma membrane and vesicular glutamate transporters (VGLUTs) located in membrane of synaptic vesicles. EAAT2 is responsible for removing glutamate from the extracellular space by reuptaking [9], while the main function of VGLUTs is to mediate the packaging of glutamate into synaptic vesicles and can therefore control the amount of glutamate released into the synaptic cleft. Controlling the function of these transporters could regulate glutamate levels of synaptic cleft and then potentially modulate the efficiency of excitatory neurotransmission [10]. A body

Abbreviations: CSB6B, Chicago sky blue 6B; METH, methamphetamine; EAATs, excitatory amino acid transporters; VGLUTs, vesicular glutamate transporters; TFB-TBOA, (2S,3S)-3-{3-[4-(trifluoromethyl)benzoylamino]benzyloxy}aspartate; ANOVA, analysis of variance; i.c.v., intracerebroventricular; s.c., subcutaneously.

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of evidence showed that controlling glutamate reuptaking potentially modulated METH-induced addictive behaviors. Conditioned rewarding effect of METH was significantly inhibited through overexpression of EAAT2 [11] and increasing the activity of EAAT2 [12]. TFB-TBOA, an EAAT2 inhibitor, significantly facilitated the expression of METH-induced behavioral sensitization [13]. On the other hand, it has been reported that METH prolonged increases in the extracellular glutamate in the striatum of rats [14], and the long-lasting increases in striatal glutamate after METH may be result from increasing VGLUT1 synthesis and expression [15]. However, whether regulation glutamate levels in synaptic cleft by controlling expression or activity of VGLUTs will also modulate METH-induced addictive behaviors is still unknown.

Chicago sky blue 6B (CSB6B), a VGLUTs inhibitor, mediates sequestration of glutamate by synaptic vesicles. It has previously shown that CSB6B inhibited loading glutamate into synaptic vesicles in vitro [16] and increased feed intake in Ross 208 broilers cockerels after acute i.c.v. injection [17]. It is unclear whether VGLUTs were involved in METH dependence. In the present research, we investigated effects of CSB6B, a VGLUTs inhibitor, on METH-induced hyperactivity and behavioral sensitization in mice after acute or chronic intracerebroventricular administration.

2. Materials and methods

2.1. Animals

Male Kunming mice, initially weighing 18–20 g, were purchased from Beijing Animal Center, China. The animals were housed in groups of four to six in clear plastic cages with free access to water and food in a room with a climate-controlled environment on a standard 12 h light/dark cycle (lights on 7 a.m., lights off 7 p.m.). The mice were acclimated to the housing conditions and handled for 3–4 days before experiments. All experiments were performed during the daytime. All experiments were conducted according to the he National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The experimental procedures were approved by the Local Committee on Animal Care and Use.

2.2. Drugs

DL-METH (purity >99%) was provided by Institute of Crime Detection, China. CSB6B was purchased from Sigma (St. Louis, MO, USA). All drugs were dissolved in sterile normal saline to the final concentrations. The dose of METH was 1 mg/kg injected subcutaneously (s.c.) in a volume of 10 ml/kg. The CSB6B dose was selected based on published observations that the 5 μmol/animal of CSB6B by intracerebroventricular (i.c.v.) successfully increased feed intake in Ross 208 broilers cockerels by inhibiting activity of VGLUTs [17] and our preliminary experiment. Thus, we chose the 1.5 μg/μl dose in the current study, the volume of i.c.v. injection was 5 μl per animal.

2.3. Intracerebroventricular injection (i.c.v. injection)

Mice were anesthetized by pentobarbital sodium (80 mg/kg, intraperitoneally) as described earlier [18] and placed in a stereotaxic apparatus (Stoelting Co., USA). Their skulls were carefully exposed and a small hole was drilled with a 25-gauge needle above the left lateral ventricle, 3.0 mm posterior and 1.5 mm lateral to the bregma. A stainless steel guide cannula (RWD Life Science Co., china) was implant 3.0 mm ventrally in lateral cerebral ventricle from the surface of the skull. To prevent occlusion, a dummy cannula was inserted into the guide cannula. Dental cement was used to fix the guide cannula to the skull. After surgery, the animals were allowed to recover for at least 5 days.

2.4. Behavioral sensitization

For the behavioral sensitization experiment (Fig. 1A), mice were injected with METH (1 mg/kg) once daily for 7 days to induce behavioral sensitization (development period), as previously reported [19], then washout period of sensitization lasted 6 days, following sensitization expression, in which mice were challenged with METH (1 mg/kg).

2.5. Procedure

2.5.1. Experiment 1

The aim of this experiment was to test effects of CSB6B (7.5 μg) on the acute 1 mg/kg METH-induced hyperactivity in mice. Four groups were treated with one of the following drug pairs: saline (i.c.v.) + saline (s.c.), saline (i.c.v.) + METH (s.c.), CSB6B (i.c.v.) + saline (s.c.) and CSB6B (i.c.v.) + METH (s.c.) with a 2.5 h interval between the two injections. After the second injection, the mice were put into the test chambers to record their locomotor activity for 1 h.

2.5.2. Experiment 2

This experiment was designed to investigate effects of CSB6B (7.5 μg) on development of behavioral sensitization to METH (1 mg/kg) in mice (Fig. 1B). Mice were treated for 7 days with one of the following drug pairs: saline (i.c.v.) + saline (s.c.), saline (i.c.v.) + METH (s.c.), CSB6B (i.c.v.) + saline (s.c.) and CSB6B (i.c.v.) + METH (s.c.) with a 2.5 h interval between the two injections. After a 6-day washout period, all mice were challenged with METH (1 mg/kg) and put into the test chambers and then the locomotion was recorded for 1 h to observe the effects of CSB6B on the development of METH-induced behavioral sensitization.

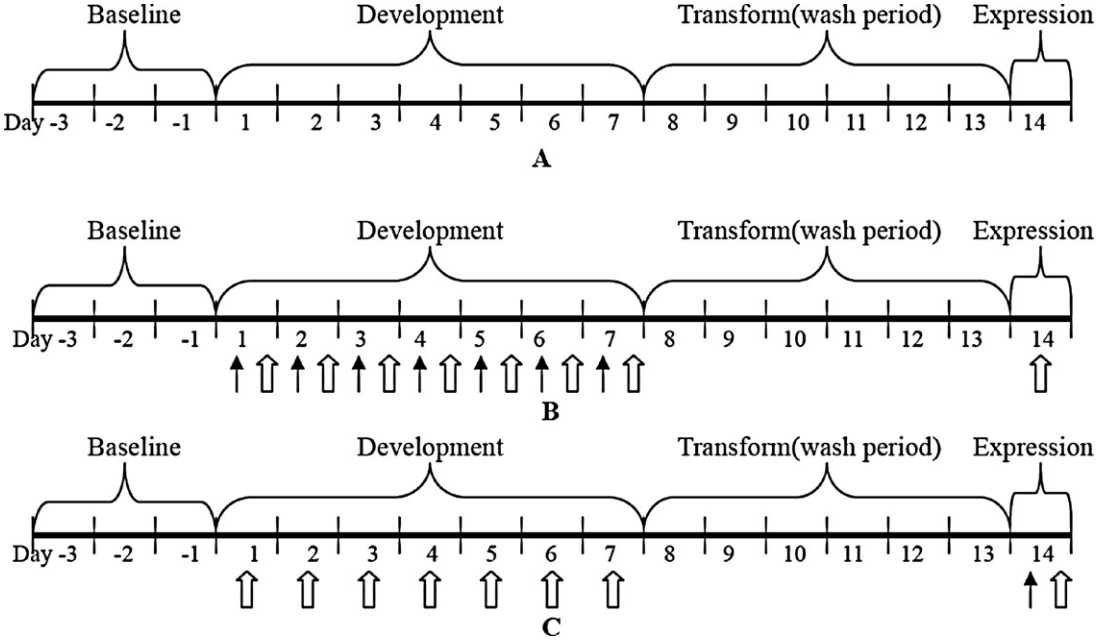

Fig. 1. Schematic representation of the experimental design. Black horizontal bars represent the periods of experimentation, where contains three different periods. Black arrows represent i.c.v. saline or CSB6B (7.5 μg) prior to s.c. saline or METH (1 mg/kg) and white arrows represent s.c. saline or METH (1 mg/kg). (A) Schematic diagram of behavioral sensitization. (B) Schematic diagram of experiment 2. (C) Schematic diagram of experiment 3.