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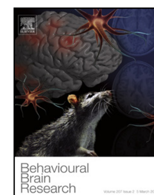

Research report

Protective effects of cholecystokinin-8 on methamphetamine-induced behavioral changes and dopaminergic neurodegeneration in mice

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HIGHLIGHTS

- Repeated administration of METH induced behavioral changes and dopaminergic neurotoxicity in mice.
- CCK-8 attenuated METH-induced behavioral sensitization and stereotypic behaviors in mice.
- CCK-8 inhibited hyperthermia and reduction of nigrostriatal TH and DAT in METH-treated mice.

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ABSTRACT

We investigated whether pretreatment with the neuropeptide cholecystokinin-8 affected methamphetamine (METH)-induced behavioral changes and dopaminergic neurodegeneration in male C57/BL6 mice. CCK-8 pretreatment alone had no effect on locomotion and stereotypic behavior and could not induce behavioral sensitization; however, it attenuated, in a dose-dependent manner, hyperlocomotion and behavioral sensitization induced by a low dose of METH (1 mg/kg). CCK-8 attenuated METH-induced stereotypic behavior at a dose of 3 mg/kg but not at 10 mg/kg. CCK-8 pretreatment attenuated METH (10 mg/kg)-induced hyperthermia, the decrease of tyrosine hydroxylase (TH) and dopamine transporter (DAT) in the striatum, and TH in the substantia nigra. CCK-8 alone had no effect on rectal temperature, TH and DAT expression in the nigrostriatal region. In conclusion, our study demonstrated that pretreatment with CCK-8 inhibited changes typically induced by repeated exposure to METH, such as hyperlocomotion, behavioral sensitization, stereotypic behavior, and dopaminergic neurotoxicity. These findings make CCK-8 a potential therapeutic agent for the treatment of multiple symptoms associated with METH abuse.

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1. Introduction

Abuse of methamphetamine (METH) is an extremely serious and growing global problem. Long-term abuse of METH may result in memory loss, aggression, psychotic symptoms and potential heart and brain damage [1–3]. Rodents administered single or repeated METH treatments serve as an animal model for METH abuse in humans, as they present abnormal behaviors including hyperlocomotion and repetitive and compulsive behavior [4]. Systemic administration of METH to rodents induces increased locomotor activity and behavioral sensitization, and these are replaced by stereotypic behavior and hyperthermia at higher doses [5–7]. Once initiated, the stereotypic behavior induced by METH can persist for several hours, depending on the dose [8]. It was hypothesized that the action of METH on the dopamine (DA) neuronal system may contribute to the augmentation of psychostimulant use [9,10]. Studies have demonstrated that METH is neurotoxic to striatal DA terminals, exemplified by a significant reduction in striatal DA and tyrosine hydroxylase (TH). In addition, dopamine transporter (DAT), an integral membrane protein that removes dopamine from the synaptic cleft and deposits it into surrounding cells, significantly decreased in both METH-treated animals and abuser [2,7,11].

However, the precise mechanisms by which METH elicits neurotoxic effects are still being elucidated. A number of studies have shown that METH-induced neurotoxicity involve the interaction of

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several neurotransmitters, neuropeptides, and neurotrophic factors. Cholecystokinin (CCK), a gut–brain peptide, exerts a wide range of biological activities in the gastrointestinal tract and central nervous system (CNS). It was initially isolated from the porcine duodenum as a 33 amino acid peptide [12] and acts via the CCK1 and CCK2 receptor subtypes. A number of biologically active molecular variants were subsequently described, and the most abundant peptide present in the brain was shown to be CCK-8: Asp-Tyr (SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 [13–15]. CCK-8 is involved in the regulation of feeding, pain perception, and learning and memory and possibly in the pathogenesis of anxiety and psychosis [16–20]. It modulates the release of several neurotransmitters, such as DA and gamma-aminobutyric acid (GABA), and possibly acts as a neurotransmitter/modulator [21,22]. A subpopulation of the dopaminergic neurons in the ventral tegmental area (VTA) projecting to the nucleus accumbens (NAc) contains CCK as a co-transmitter. Changes in the activity of the DA system have been observed in pharmacological studies conducted on CCK receptor-deficient mice. The targeted genetic suppression of CCK2 receptors has been shown to increase the sensitivity of pre- and post-synaptic D2 receptors. Loonam et al. found that CCK regulated neurochemical responses to METH in the striatum [23,24]. Additionally, there are reports on the role of CCK in DA-mediated behaviors, with different subregions of the NAc involved in different effects on DA-mediated locomotor activity [25]. Moreover, our previous results have shown that CCK-8 has anti-oxidative stress and anti-inflammatory effects [26,27]. In addition, it produced neuroprotective effects in neuronal injury models. These data show that CCK-8 exhibits a pharmaco-therapeutic potential for treating METH-induced neurotoxicity in CNS.

The present study aimed to evaluate the effects of CCK-8 on METH-induced behavioral changes, including hyperlocomotion, behavioral sensitization, and stereotypical behavior. Furthermore, the effects of CCK-8 on high doses of METH-induced hyperthermia and dopamine neurotoxicity were investigated.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice, initially weighing 18–20 g, were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd., China. The animals were housed in a climate-controlled environment. Constant temperature (21 ± 2 °C), humidity (approximately 60%) and a 12 h light/dark cycle (lights on at 7:00 am) were maintained. Food and water were available ad libitum. All experiments were conducted according to the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental procedures were approved by the Local Committee on Animal Care and Use and Protection of the Hebei Medical University.

2.2. Drugs

DL-METH was provided by Beijing Municipal Public Bureau, China. CCK-8 was purchased from Sigma, Ltd. (MA, USA). CCK-8 was re-suspended to a concentration of 1 mg/ml in 1% ammonia saline solution. This working solution of CCK-8 was diluted in saline to concentrations of 0.5, 0.05 and 0.005 μg/ml immediately before use.

2.3. Surgery and microinjections

Surgery and microinjections in the mouse brain were performed as previously reported [17]. The mice were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and placed in the stereotaxic apparatus (Benchmark TM Stereotaxic Instruments, USA). We drilled a hole on the skull surface corresponding to the lateral ventricle, targeted above the left or right lateral ventricle (ML, ±0.94 mm; AP, −0.22 mm). A stainless steel guide cannula was implanted 2.2 mm ventrally from the surface of the skull. The location of cannulae was illustrated in Fig. 1A. The animals were treated with penicillin (1000 μm/day i.p.) for 3 days and allowed to recover for at least 7 days. Each microinjection was prepared with a 10-μl syringe (Hamilton, USA) attached to PE tubing connected to the injection cannula and was administered at a rate of 0.5 μl/min in a volume of 2 μl using a syringe pump (KD Scientific, USA). The injection cannula extended 0.2 mm beyond the guide cannula and was left in place for 5 min following the microinjections to minimize backflow of the drug. After completion of behavioral testing, mice for i.c.v. were injected with Evans blue dye (10 μl) to test the location of cannulae (Fig. 1B). Mislocated mice were excluded.

2.4. Measurement of locomotor activity

The apparatus for measuring locomotor activity consisted of four clear Plexiglas boxes (40 cm × 40 cm). A video system was used to track the movement of the mice in each box, and the horizontal distance traveled was subsequently recorded and analyzed by an Animal Locomotor Video Analysis System (JLBeh Soft-tech Co. Ltd. Shanghai, China).

2.5. Behavioral sensitization

The protocol used in this study has been described by He et al. [28,29], and the schedule of the drug treatment is outlined in Fig. 3A. On days 1–3, the mice were placed in the locomotion test apparatus for 30 min daily. The objective of this habituation was to eliminate the baseline locomotor activity. The mice were injected with METH (1, 2 mg/kg, i.p.) once daily for the next 7 days to induce behavioral sensitization (development period); the locomotor activity was recorded on the 1st, 3rd, 5th and 7th days for 30 min, followed by a washout period for sensitization, which lasted 6 days. Next, sensitization was induced (day 14), where the mice were challenged with METH (1, 2 mg/kg, i.p.) and the locomotion was recorded for 30 min.

2.6. Stereotypic behavior

We tested the effects of CCK-8 on stereotypic behavior induced by a high dose of METH in mice. The stereotypic behaviors were scored as described by Sams-Dodd (Fig. 4A) [30]. The mice were placed in the test apparatus to assess the stereotypic behavior for 1 h after the final drug administration; the behavior was assessed in 10-min intervals, and the total scores of each group were calculated.

2.7. Immunocytochemistry

The determination of TH and DAT was performed according to Jung et al. [31–33]. The mice were killed, and the brains were harvested at the end of the predetermined time schedule. Cardiovascular perfusion with 0.9% saline and 4% paraformaldehyde was applied to the animals under anesthesia, and the brains were then harvested. The tissue samples were embedded in paraffin, and 5-μm-thick sagittal sections were prepared for staining. The sections were immunohistochemically stained using an antibody for TH (diluted 1:500, Abcam) and DAT (diluted 1:50, Proteintech) to determine changes in TH and DAT expression. Incubation was performed overnight at 4 °C. Some sections were lightly counterstained with hematoxylin.

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