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Galantamine attenuates the heroin seeking behaviors induced by cues after prolonged withdrawal in rats

Huifen Liu, Miaojun Lai, Xin Zhou, Huaqiang Zhu, Yu Liu, Anna Sun, Baomiao Ma, Fuqiang Zhang, Wenhua Zhou*

Laboratory of Behavioral Neuroscience, Ningbo Addiction Research and Treatment Center, School of Medicine, Ningbo University, 42 Xibei Str., Ningbo 315010, PR China

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ABSTRACT

Background and objective: Evidence shows that acetylcholinergic transmission in the ventral tegmental area (VTA) or nucleus accumbens (NAc) plays an important role in heroin-seeking induced by cues. Cholinergic modulation of VTA neurons arises from the lateral dorsal tegmental nucleus (LDT). The present studies investigated the effect of systemic or intra- LDT administration of galantamine, an inhibitor of acetylcholinesterase, on heroin-seeking induced by cues.

Methods: Rats were trained to self-administer heroin for 12 days, underwent extinction training for 12 days followed by two weeks in their home cages. Then the conditioned cues were introduced for the reinstatement of heroin-seeking.

Results: The reinstatement of heroin-seeking induced by cues was attenuated by the administration of galantamine (0, 0.3, 1 or 3 mg/kg, i.p.) in a dose-dependent manner. In contrast, galantamine only at the dose of 3 mg/kg could inhibit the reinstatement of sucrose-seeking. Galantamine at those doses failed to alter the locomotor activity in heroin-withdrawn rats. The inhibition of drug-seeking by galantamine was reversed by pretreatment with scopolamine (0.5 mg/kg) but not with mecamylamine (3 mg/kg) or scopolamine methobromide (1 mg/kg). Moreover, the microinjection of galantamine into the LDT blocked cue-induced heroin-seeking, while the microinjection of scopolamine into the LDT reversed the inhibitory effect of galantamine on drug-seeking behavior.

Conclusion: The results suggest that cholinergic transmission in the LDT may play a critical role in heroin-seeking behavior induced by cues and that galantamine may have the beneficial effect of blocking heroin-seeking behavior, which is mediated through its actions on the muscarinic receptors.

1. Introduction

Relapse to heroin use after abstinence is a major clinical problem in the treatment of heroin addiction (O'Brien, 1997). Opiate withdrawal produces an aversive state in humans, and this aversive state is hypothesized to promote heroin-seeking and heroin-taking behaviors (Hutcheson et al., 2001; Kenny et al., 2006; Zhou et al., 2009). More importantly, the 'intensity' of the conditioned reinstatement increases during the initial period of abstinence (Di Ciano and Everitt, 2002; Hellemans et al., 2006; Schmidt et al., 2005; Vanderschuren and Everitt, 2004). Extinction responses follow an inverted U-shaped curve, in which a higher response was evident after 6, 12 or 25 days of withdrawal from heroin compared to after 1 or 66 days (Shalev et al., 2001). Using a rat model of drug craving and relapse, we recently found that heroin-seeking behavior induced by re-exposure to drug-associated cues or a heroin-priming injection persisted for over 2 months after withdrawal from heroin self-administration (Zhou and Kalivas, 2008). Both spontaneous withdrawal and naltrexone-precipitated withdrawal can enhance the magnitude of heroin-seeking behavior induced by drug related cues (Kuntz et al., 2008; Zhou et al., 2009).

The cholinergic system plays an important role in the regulation of attention, memory, processing speed, and sensory gating (Everitt and Robbins, 1997). Several lines of evidence suggest that the cholinergic system may be disrupted following the administration of heroin (Hoebel et al., 2007). Our previous studies showed that the enhancement of acetylcholine transmission in the NAc inhibits the heroin-seeking behavior induced by cues, but in the VTA increases the seeking behavior (Zhou et al., 2007). One important

Abbreviations: AChE, acetylcholinesterase; NAc, nucleus accumbens; VTA, ventral tegmental area; LDT, laterodorsal tegmental nucleus; PPT, pendunculopontine tegmental nucleus.

* Corresponding author. Tel.: +86 574 87273530; fax: +86 574 87345976.
E-mail address: whzhou@vip.163.com (W. Zhou).

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source of cholinergic modulation of the VTA DAergic neurons arises from the lateral dorsal tegmental nucleus (LDT) (Omelchenko and Sesack, 2006), and a much smaller percentage of cholinergic projections come from the pedunculopontine nucleus (PPT). Lesions of the LDT attenuate the elevation of DA in the NAc induced by the intra-VTA microinjection of neostigmine (Blaha et al., 1996). Importantly, blocking muscarinic type 2 (M_2) receptors in the LDT with scopolamine or the more selective antagonist, methoctramine, abolishes suppression of cholinergic neurons and thus suppression of DA currents in the NAc (Forster and Blaha, 2000). Microinjection of scopolamine into the midbrain induced a marked increase in acetylcholine (ACh) efflux into this region in wild-type mice and significantly reduced ACh efflux in M_4 knock-out mice (Tzavara et al., 2004). It has been shown that LDT cholinergic neurons play an important role in modifying the reinforcing value of natural and drug rewards (Shabani et al., 2010). These data pose the possibility that cholinergic transmission in the LDT may be involved in heroin-seeking behavior.

Galantamine, an inhibitor of acetylcholine esterase (AChE), has been used for symptomatic treatment of Alzheimer's disease (Buchanan et al., 2008; Cummings, 2000; Schubert et al., 2006). In the current study, we examined the effects of galantamine on the reinstatement of heroin-seeking behavior induced by cues after a prolonged withdrawal. The subtypes of cholinergic receptors involved in the action of galantamine were further investigated using pretreatments with an nicotinic receptors (nAChR) antagonist, mecamylamine, or mAChR antagonists, scopolamine or scopolamine methobromide (minimal passage across the blood-brain barrier). To understand the role of acetylcholine in the LDT in heroin-seeking behavior, galantamine was directly microinjected into the LDT of rats.

2. Methods

2.1. Subjects and drugs

Male Sprague–Dawley rats weighing 250–275 g at the beginning of the experiment were individually housed in a temperature- and humidity-controlled vivarium on a reversed light-dark cycle (lights off from 6 AM to 6 PM). Food and water were freely available except when specified. All experiments were approved by the Animal Care and Use Committee of Zhejiang Province and conducted according to the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publications No. 80-23). The rats were weighed and handled daily for one week prior to surgery. Heroin (diacetylmorphine HCl) was obtained from the National Institute of Forensic Science (Beijing, China). Galantamine, scopolamine and scopolamine methobromide were obtained from Sigma–Aldrich (St. Louis, MO). Mecamylamine was obtained from Tocris (Ellisville, MO). These drugs were dissolved in 0.9% sterile physiological saline. Galantamine, scopolamine, or both were dissolved in an artificial cerebrospinal fluid (aCSF) consisting of the following compounds (in mM): 120 NaCl, 3.3 KCl, 1.2 $CaCl_2$, 1.0 $MgCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4, and 10 glucose with a pH of 7.4 and used for the microinjection.

2.2. Lever response training

The rats were deprived of food overnight and trained to press a lever on a fixed ratio (FR) 1 schedule of food reinforcement (45 mg pellets; Noyes, Lancaster, NH) in operant conditioning chambers ($30 \times 20 \times 24$ cm high) during a 16-h overnight food training session. The chambers were equipped with two retractable levers, a stimulus light above each lever, a food pellet dispenser between the levers, and a house light on the wall opposite of the levers. During the session, each press on the active lever resulted in the delivery of only a food pellet. Lever presses on the inactive lever were recorded but had no programmed consequences. Following the lever response training, food dispensers were permanently removed from the test chambers.

2.3. Surgery

The rats were anesthetized with ketamine HCl (87.5 mg/kg Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (5 mg/kg Rompum, Bayer, Shawnee Mission, KS), and indwelling jugular catheters were implanted as previously described (LaLumiere and Kalivas, 2008; Zhou and Kalivas, 2008). The catheter was flushed daily with heparinized saline (0.2 ml of 100 IU) and cefazolin antibiotic (0.2 ml of 0.1 g/ml). Following the surgery, the rats were allowed 7 days to recover prior to behavioral training. On the sixth day, the rats were placed on a food-restricted diet (20–25 g per day; Purina Rat Chow) for the remainder of the experiment.

In some experiments, bilateral guide cannulae (20-gauge, Small Parts Inc., Roanoke, VA) were implanted 1 mm dorsal to the LDT according to the following stereotaxic coordinates from the atlas of Paxinos and Watson (1997): AP: −8.5 mm, ML: ±1.2 mm, and DV: −6.2 mm. The guide cannulae were lowered into place and attached to the skull via dental acrylic. Obturators were extended 0.5 mm beyond the tip of each cannula to prevent the obstruction of debris.

2.4. Heroin self-administration and extinction

The rats were trained to self-administer heroin in operant chambers under an FR-1 schedule for 3 h per day for 12 consecutive days. Pressing on the active lever resulted in the infusion of heroin (0.1 mg per infusion for days 1–2, 0.05 mg per infusion for days 3–4, 0.025 mg per infusion for days 5–12) prepared in 0.9% sterile saline over 4 s as previously described (Fuchs and See, 2002; Zhou and Kalivas, 2008). Each training session began when the rats were connected to the drug delivery apparatus, the house light was illuminated and the two levers were inserted into the chamber. The delivery of each heroin infusion was accompanied with the light cue located above the active lever and was followed by a 20 s time out period during which responses on the active lever were counted but resulted in no heroin delivery.

After self-administrating heroin for 12 days, the rats underwent the extinction training for 2 h daily without any lights and drugs for 12 consecutive days in the operant chamber. During the extinction sessions, responses on the active lever were recorded but had no program consequence. The criterion used to determine extinction was when the rats pressed the active lever for less than 10% of the average response on the active lever during maintenance.

2.5. Cue-induced reinstatement

The rats stayed in the home chamber for an additional 2 weeks after the 12 days of extinction training. The rats were placed in the operant chambers for 3 h to test for the reinstatement of heroin-seeking behavior induced by heroin-associated cues. Heroin-associated cues included a house light that previously predicted the drug's availability and a 20 s light cue that was previously associated with a heroin infusion (Zhou and Kalivas, 2008; Zhou et al., 2007). The house light was illuminated, and the levers were inserted into the chamber. Each active lever press resulted in the presentation of a light cue that was previously associated with a heroin infusion.

2.5.1. Special experiments

Experiment 1. The effect of galantamine on heroin-seeking behavior induced by cues.

The rats self-administered heroin for 12 days, underwent extinction training for 12 days followed by two weeks of additional abstinence. The animals were randomly assigned to one of 4 groups and were injected with a vehicle ($n = 6$) or 0.3 mg/kg ($n = 6$), 1.0 mg/kg ($n = 7$), or 3.0 mg/kg ($n = 6$) of galantamine (i.p.) 10 min prior to testing reinstatement induced by cues.

Experiment 2. The effect of scopolamine or scopolamine methobromide on the reversal of cue-induced reinstatement by galantamine.

The rats ($n = 6$ per group) were tested for the effect of scopolamine orscopolamine methobromide on the inhibition of heroin-seeking behavior induced by a galantamine pretreatment. Ten min prior to the reinstatement testing session, the animals were injected with scopolamine (0.5 mg/kg, i.p.), scopolamine (0.5 mg/kg, i.p.) plus galantamine (3.0 mg/kg, i.p.), scopolamine methobromide (1.0 mg/kg, i.p.), or scopolamine methobromide (1.0 mg/kg, i.p.) plus galantamine (3.0 mg/kg, i.p.). The vehicle and galantamine (3.0 mg/kg) treated groups are same in Experiment 1.

Experiment 3. The effect of mecamylamine on the inhibitory action of galantamine on cue-induced heroin-seeking behavior.

The rats were tested for the effect of mecamylamine on the inhibition of heroin-seeking behavior induced by a saline or galantamine pretreatment (3.0 mg/kg), which are same in Experiment 1. Ten min prior to the reinstatement testing session, the animals were injected with 0.5 mg/kg ($n = 7$), or 3.0 mg/kg mecamylamine (i.p., $n = 6$)) or galantamine, mecamylamine (0.5 mg/kg) plus galantamine ($n = 6$), or mecamylamine (3.0 mg/kg) plus galantamine ($n = 6$).

2.6. Microinjection procedure and histology

The rats were microinjected before the reinstatement testing. All injections into the LDT were delivered by a microinjection pump (MD-1001, Bioanalytical System