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Ethological analysis of scopolamine treatment or pretreatment in morphine dependent rats

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Abstract

Although scopolamine is currently used to treat morphine addiction in humans, its extensive actions on behaviors have not been systematically analyzed yet, and the underlying mechanisms of its effects still remain ambiguous. The present study was carried out to clarify the possible mechanisms by evaluating the effects of scopolamine pretreatment and treatment on naloxone-precipitated withdrawal signs and some of other general behaviors in morphine dependent rats. Our results showed that scopolamine pretreatment and treatment attenuated naloxone-precipitated withdrawal signs including jumping, writhing posture, weight loss, genital grooming, teeth-chattering, ptosis, diarrhea and irritability, except for wet dog shakes, while general behaviors such as water intake, urine volume and morphine excretion in urine were increased. Our findings suggest that scopolamine has significant actions in the treatment of opiate addiction, which might result from increasing morphine excretion from urine.

Keywords: Morphine dependence; Morphine withdrawal signs; Scopolamine; Morphine excretion

1. Introduction

Chronic abuse of opiates causes a number of adaptive changes in the central nervous system (CNS) accompanied by behavioral alterations related to drug dependence [1,2]. It has already been shown that chronic morphine treatment effectively activates cAMP response element-binding protein (CREB) [3,4], Fos and its isoform ΔFosB [5,6], calcium/calmodulin-dependent protein kinase II (CaMKII) [7,8], NMDA receptors [9,10], dopaminergic neurons in the ventral tegmental area (VTA) [11], and protein kinase C (PKC) [12] in the brain, all of which play important roles in the neural plasticity underlying addiction. Also, central cholinergic neurons have long been suggested to mediate many of the signs and symptoms of opiate withdrawal [13–17]. Blocking of the above elements can inhibit or even reverse morphine tolerance and dependence [18–21]. Although such evidence has suggested a close correlation between such elements and morphine addiction, the mechanism of morphine tolerance and dependence is unclear. Abrupt discontinuation of opiate consumption after prolonged abuse is followed by a series of morbid symptoms defined as withdrawal syndrome, which is a significant factor deterring a drug-dependent patient from going through rehabilitation [22].

Pharmacological blockade of central muscarinic receptors by antagonists such as scopolamine, which easily permeates the brain blood barrier, has been widely used in treatment of drug abuse, especially in opioid addiction [23,24]. In recent years, the new pharmacologic effects of scopolamine such as regulating the autonomic nervous system [25], accelerating the excretion of morphine [26], affecting other neurotransmitters [27,28], decreasing the activity of guanylate cyclase and nitric oxide synthase [29,30], adjusting immune response [31], and influencing learning and memory [32] were discovered. Despite these extensive studies, the precise mechanisms underlying scopolamine attenuation of opiate dependence are still poorly delineated. Previous reports showed that chronic scopolamine treatment at

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Table 1
Treatment groups

Group	d1-d7 i.p.	d1-d7 pretreatment	d8-d14 treatment	Naloxone
Saline control (sal+sal+nal, $n=8$)	Saline	1 ml saline	–	2 mg/kg
Scopolamine control (sal+sco+nal, $n=8$)	Saline	Scopolamine	–	2 mg/kg
Withdrawal control (mor+sal+nal, $n=8$)	Morphine	1 ml saline	–	2 mg/kg
Scopolamine pretreatment (mor+sco+nal, $n=8$)	Morphine	Scopolamine	–	2 mg/kg
Scopolamine treatment (mor+sco+nal, $n=8$)	Morphine	–	Scopolamine	2 mg/kg
Morphine control (mor+sal+nal, $n=8$)	Morphine	–	Saline	2 mg/kg

doses around 0.5 mg/kg could attenuate the naloxone-precipitated withdrawal symptoms [32,33], and 1 mg/kg scopolamine could antagonize respiratory depression induced by 20 mg/kg morphine [34]. Therefore, we used 0.5 mg/kg scopolamine in our scopolamine treatment study, and scopolamine doses at 1/20 of morphine doses in the scopolamine pretreatment study.

Naloxone-precipitated increase in behavioral responses of morphine-dependent rats has been employed as a model for investigating the development of physical dependence [35,36]. In the previous studies, scopolamine was only given 30 min before naloxone administration [25,33], whose effect was only limited in detoxification instead of substantial treatment of opioid addiction. In this research, scopolamine was given 15 min before each morphine injection in the scopolamine pretreatment study or twice a day in the scopolamine treatment study to the morphine dependent rats for investigating the effects of scopolamine on morphine withdrawal signs. Scopolamine actions on general behaviors such as food consumption, water intake and urine volume were recorded and used to evaluate morphine withdrawal intensity [37]. Additionally high performance liquid chromatography (HPLC) was used to detect the quantity of morphine excreted from urine of morphine dependent rats.

2. Methods

2.1. Animals housing

Forty-eight male Sprague–Dawley rats weighing 220–250 g were acquired from the Medical Experimental Animal Center of Shaanxi Province, China. The animals were housed and maintained on a 12-h light/dark cycle (light on at 20:00) with constant temperature (22 °C) except during testing. Animals were housed individually in metabolic cages, with free access to food through a corridor and free access to water contained in a tube with a scale. Animals' food consumption, water intake, and urine volumes were precisely measured.

2.2. Drugs and reagents

Morphine hydrochloride was purchased from the Shenyang Pharmaceutical Factory (Shenyang, China). (−)-Scopolamine hydrochloride and naloxone hydrochloride were from Sigma (St. Louis, MO, USA). The drugs were dissolved in sterile saline. HPLC reagents were purchased from Fisher Scientific Ltd (Springfield, NJ, USA).

2.3. Experimental procedure

2.3.1. Morphine dependence and withdrawal

An adapted method described by Chou et al. [38] and Valverde and Roques [39] was used. Rats received morphine i.p. twice per day at 08:30 and 16:30 for 7 d. The daily dosage was 10, 20, 30, 40, 50, 50 or 50 mg/kg/injection, respectively. Rats in both saline control and scopolamine control groups received saline injections instead of morphine on the same schedule. In the scopolamine pretreatment test, the saline control group received saline injection and the other groups received a scopolamine injection 15 min before every saline or morphine injection. In the scopolamine treatment test, the morphine control group received saline injections twice per day at 08:30 and 16:30 for an additional 7 d, and the scopolamine treatment group received 0.5 mg/kg scopolamine injections instead of saline on the same schedule after the aforementioned 7-d morphine administration. One hour after the last injection of saline, morphine or scopolamine, all rats were given naloxone hydrochloride (2 mg/kg i.p.) [40,41] and immediately placed into observation tanks for behavior analysis. Animals were divided into six treatment groups shown as in Table 1.

2.3.2. General behavior

Food consumption, water intake, urine volume, and body weight of the rats were recorded every day at 4:00 PM during the whole process of the experiments. All these values compared with each other in the following were the means of the whole experimental days except for special explain.

2.3.3. Withdrawal behavior analysis

Withdrawal intensity was evaluated with nine withdrawal signs including jumping, wet dog shake, writhing posture,

Table 2
Ethogram of the rat behaviors during morphine withdrawal

Pattern	Category	Description
Body shake	Wet dog shakes	The rat shakes its body or moves the hand with sideway movement
Writhing posture	Writhing behavior	The rat lies on the floor, while the belly is firmly pressing the surface; abdominal contractions are usually present
Jumping	Escape	Leaping off the surface of the cage
Genital grooming	Self care	The rat licks its genitalia. It usually follows ejaculation
Teeth-chattering		The rat rapidly clicks teeth together
Ptosis		Closing of the eyelids
Diarrhea		Presence of soft or formless stool
Irritability		Attack or vocalization when touched at the end of the time period
Weight loss		The percentage of weight loss before and 1 h after each test