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Effect of rhynchophylline on central neurotransmitter levels in amphetamine-induced conditioned place preference rat brain

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ABSTRACT

The effects of rhynchophylline on expression of amphetamine reward using a conditioned place preference (CPP) paradigm and central neurotransmitter levels in rat brain was investigated. Rats were injected with amphetamine (2 mg/kg, per day for 4 consecutive days) and treated with rhynchophylline (60 mg/kg, per day for the later 3 days). Control rats were administered with rhynchophylline (60 mg/kg) instead of amphetamine to evaluate whether rhynchophylline by itself produced CPP. Glutamic acid, γ-aminobutyric acid, endorphin, acetylcholine, norepinephrine, dopamine, and 5-hydroxytryptamine contents were examined by encephalofluogram technology. Rhynchophylline reversed the expression of amphetamine-induced CPP and itself did not produce a CPP. Glutamic acid, dopamine, and norepinephrine contents in amphetamine-CPP rat brain were significantly higher; while γ-aminobutyric acid, endorphin, and acetylcholine contents were significantly lower than those of control rats. Rhynchophylline reversed those central neurotransmitter levels induced by amphetamine to control levels; rhynchophylline by itself had no effect on central neurotransmitter in control rats. These findings show that rhynchophylline reverses the expression of amphetamine-induced rewarding effect which is partly mediated by regulation of central neurotransmitter levels in the rat brain.

1. Introduction

Amphetamine analogues are popular among young people as recreational drug due to their energy and mood enhancing properties [1] and its abuse is associated with psychiatric adverse effects [2]. The various actions and effects of amphetamine have resulted in contradictive views among researchers regarding its degree of harmfulness [3]. Chronic drug exposure causes long-lasting neuroadaptive changes in the brain at the cellular and molecular levels that may contribute to compulsive drug use and relapse [4]. Many studies have focused on neuroadaptive responses in the glutamate and dopamine systems towards amphetamine [5,6]. Amphetamine interacts acutely with catecholamine transport and release mechanisms to enhance synaptic catecholamine concentrations, produce rewarding reinforcing effects, augment locomotion, and produce stereotyped behaviors [7]. Chronic amphetamine administration results in catecholamine depletion [8]. Glutamic acid and γ-aminobutyric acid (GABA) are the principal excitatory amino acid and inhibitory amino acid neurotransmitters in the central nervous system [9]. The drug-induced release of endorphin contributes to the positive reinforcing and motivating property of amphetamine [10].

Amphetamine increases monoamine transmission and initiates a cascade of cellular events which in turn modifies behavior. The conditioned place preference (CPP) paradigm allows the rewarding properties of a treatment to be inferred by assessing approach behavior to environmental cues, which is previously paired with the affective consequences of the treatment. CPP is thought to be related to and predictive of

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the positive reinforcing effects of drugs [11]. Understanding the neural substrates underlying pharmacological action on different neurotransmitter systems is a key facet of neuroscience research and critical in the development of new medicines in psychiatry and neurology. Recently, encephalofluogram technology, a noninvasive repeatable technology, has offered an in vivo whole-brain view on resulting changes in brain activity [12–14].

Several plant-derived compounds have been shown to have significantly anti-drug addiction mostly in preclinical studies and the exact mechanisms of these compounds are still unclear [15]. Rhynchophylline (Fig. 1) is an important active component of tetracyclic oxindole alkaloids separated from gambir plant (*Gouteng* in Chinese) which has been used in traditional medicine in southeastern Asia, Africa, and South America. As specified in the Chinese Pharmacopoeia, *Gouteng* may contain three *Uncaria* species: *Uncaria rhynchophylla* (Miq) Jacks, *Uncaria macrophylla* Wall, *Uncaria sinensis* (Oliv) Havil, or *Uncaria* sessilifructus Roxb. *Gouteng* in the prescription of traditional Chinese medicine is mainly used to treat ailments in the cardiovascular and central nervous systems, such as lightheadedness, convulsions, epilepsy, cerebral apoplexy, numbness, and hypertension [16]. In several laboratories, a significant sedative effect of rhynchophylline was observed [17,18]. In in vitro studies, rhynchophylline reduces glutamate-induced Ca^{2+} influx and protects against glutamate-induced neuronal death in cultured cerebellar granule cells [19,20]. Rhynchophylline has different effects on 5-hydroxytryptamine (5-HT) and dopamine release from different brain regions, but the whole effect on neurotransmitter contents is not clear [21].

This study was designed to investigate the effects of rhynchophylline on expression of amphetamine-induced CPP in rats and to define central neurotransmitters to the regulation of amphetamine-induced CPP by examining changes of amino acid, monoamine neurotransmitter, endorphin levels in rat brain.

2. Experiment

2.1. Animals

Wistar rats, weighing 200–260 g, were provided by the Experimental Animal Center of First Military Medical University, Guangzhou, China. All animals were adapted to the experimental conditions (temperature: (20 ± 2) °C, humidity: (60 ± 5) %, 12 h dark/light cycle) for 1 week. All rats had free access to tap water and chow. The ethical aspects of the research plan and experimental procedures had been approved by Science and Technological Committee and the Animal Use and Care Committee of First Military Medical University, Guangzhou, China.

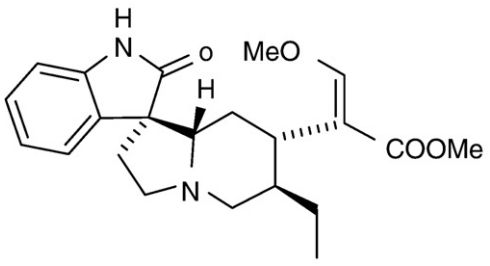

Fig. 1. Structural formula of rhynchophylline.

2.2. Drugs and reagents

Rhynchophylline (no. H1I2, purity 99.7%) was brought from Matsuura Ykugyo Co., Japan. Amphetamine sulphate (no. 1211-9301) was purchased from National Institute for the Control of Pharmaceutical and Biological Products, China. Both rhynchophylline and amphetamine sulphate were dissolved in physiological saline to final concentrations and injected in a volume of 10 ml/kg. All other chemicals used in this experiment were of reagent grade from commercial sources.

2.3. Apparatus

The CPP apparatus consists of two equal-sized compartments (30 × 30 × 30 cm), one with a white box and the other with a black box joined by a wall with a sliding door. For testing, the sliding door was raised 12 cm above the floor to allow the rat free access to both sides of the box. Encephalofluctuograph technology system was provided by TongRen Electronic Co., Beijing, China. It has been used in animal experiments for many years in China and has detailed information in a related book written by Mei Lei [14].

2.4. Conditioned place preference procedure

CPP test consisted of three phases and proceeded on 8 consecutive days. For the pre-conditioning phase (days 1–3), the rat was placed under the door which was left open to allow free access to the entire box for 15 min each day. On day 2 and day 3, the amount of time spent on each compartment was calculated and averaged to use as the pre-conditioning time of each animal. There was a significant difference between the time spent on the black compartment (not drug-paired side) (720.4 ± 46.3 s, $n = 32$) and the time spent on the white compartment (drug-paired side) (179.6 ± 46.3 s, $n = 32$) before drug conditioning ($P < 0.01$), which indicated the used CPP apparatus was of a biased design [22]. The rats that showed place preference for the white compartment in pre-conditioning phase (4 of 36 rats) were excluded from further analysis. During the conditioning phase (days 4–7), the door was shut so that two compartments were separated. The rats were divided into amphetamine-paired group and saline-paired group, and underwent two conditioning sessions each day. The first session was performed in the morning, when the rats received amphetamine (2 mg/kg, s.c.) in amphetamine-paired group or sterile physiological saline (10 ml/kg, s.c.) in saline-paired group, and were immediately confined to the white compartment for 1 h. After an interval of 6 h, the second session of the day began. All the rats received saline and were confined to the black compartment immediately for 1 h during the second session. Twenty-four hours after the last drug-paired conditioning trial, the post-conditioning phase (day 8) was carried out and was exactly the same as the pre-conditioning phase. The time that the rats spent on the drug-paired side (the white compartment) was recorded for the 15-min trial.