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Inhibiting effects of rhynchophylline on zebrafish methamphetamine dependence are associated with amelioration of neurotransmitters content and down-regulation of TH and NR2B expression

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ABSTRACT

Others and we have reported that rhynchophylline reverses amphetamine-induced conditioned place preference (CPP) effect which may be partly mediated by amelioration of central neurotransmitters and N-methyl-D-aspartate receptor 2B (NR2B) levels in the rat brains. The current study investigated the inhibiting effects of rhynchophylline on methamphetamine-induced (METH-induced) CPP in adult zebrafish and METH-induced locomotor activity in tyrosine hydroxylase-green fluorescent protein (TH-GFP) transgenic zebrafish larvae and attempted to confirm the hypothesis that these effects were mediated via regulation of neurotransmitters and dopaminergic and glutamatergic systems. After baseline preference test (on days 1–3), zebrafish were injected intraperitoneally METH (on days 4, 6 and 8) or the same volume of fish physiological saline (on days 5 and 7) and were immediately conditioned. Rhynchophylline was administered at 12 h after injection of METH. On day 9, zebrafish were tested for METH-induced CPP. Results revealed that rhynchophylline (100 mg/kg) significantly inhibited the acquisition of METH-induced CPP, reduced the content of dopamine and glutamate and down-regulated the expression of TH and NR2B in the CPP zebrafish brains. Furthermore, the influence of rhynchophylline on METH-induced locomotor activity was also observed in TH-GFP transgenic zebrafish larvae. Results showed that rhynchophylline (50 mg/L) treatment led to a significant reduction on the locomotor activity and TH expression in TH-GFP transgenic zebrafish larvae. Taken together, these data indicate that the inhibition of the formation of METH dependence by rhynchophylline in zebrafish is associated with amelioration of the neurotransmitters dopamine and glutamate content and down-regulation of TH and NR2B expression.

1. Introduction

Amphetamine-type stimulants, a group of new-type synthetic drugs mainly in psychological dependence, are abused more and more severely in recent years (Brensilver et al., 2013; Jernigan et al., 2005; Scott et al., 2007). In particular, the methylated derivative, methamphetamine (METH), is a highly addictive drug known for its potent reinforcing effects. Animal and human studies suggest that these effects are mediated through activation of the mesolimbic dopamine system, which innervates limbic and cortical areas including the ventral striatum, orbitofrontal cortex and anterior cingulate cortex to form a reward circuit (Karila et al., 2010). Although the neurobiological mechanisms of METH addiction are complex and involve the interactions of multiple brain regions and neurotransmitter systems, there is substantial evidence showing that dopaminergic and glutamatergic neuronal systems play key roles (Cadet and Jayanthi, 2013; Nordahl et al., 2003; Parsegian and See, 2014).

Dopamine, a catecholamine neurotransmitter, modulates neuronal activities in the brain (Beaulieu and Gainetdinov, 2011). METH evokes an increase of extracellular concentrations of dopamine in the striatum and blockade of dopamine neurotransmission in this region attenuates most rewarding effects of METH, such as conditioned place preference (CPP) (Wise, 1996; Yuan et al., 2010). Tyrosine hydroxylase (TH), a rate-limiting enzyme in the biosynthesis of dopamine, is commonly used as a marker for dopaminergic neurons (Arenzana et al., 2006; Pickel et al., 1975). The activity of TH reflects the change of dopamine. In addition to dopaminergic system, N-methyl-D-aspartate (NMDA)

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and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, the ionotropic glutamate receptors, are involved in mediating biological actions of drugs of abuse, particularly the psychostimulants (Cadet and Jayanthi, 2013; Suto et al., 2003). Our previous studies showed that the expression of N-methyl-D-aspartate receptor 2B (NR2B) was remarkably increased in the hippocampi of METH-induced CPP rats (Zhou et al., 2010b) and mice (Li et al., 2014). The level of glutamate receptor 2 (GluR2) was significantly up-regulated in the medial prefrontal cortex of rats following repeated administration of METH (Herrold et al., 2013).

Rhynchophylline is a predominant tetracyclic oxindole alkaloid isolated from the Chinese herbal medicine *Uncaria rhynchophylla* (Miq.) Jacks (Gouteng in Chinese), which is routinely used in traditional Chinese medicine formulas for the treatment of symptoms relevant to drug addiction and is also an important component of a Japanese neurotrophic medicine called Yokukansan (Shi et al., 2003; Zhou and Zhou, 2010). Substantial experimental evidence indicates that rhynchophylline possesses many beneficial effects such as anti-hypertensive, anti-addictive, anti-anxiety, anti-arrhythmic, anticonvulsant, sedative and neuroprotective effects in various models (Shi et al., 2003; Zhou and Zhou, 2010). Rhynchophylline has attracted considerable interest due to its potent effects on the central nervous system such as acting as a non-competitive antagonist of the NMDA receptor and a calcium channel blocker (Kang et al., 2002; Lee et al., 2003). Rhynchophylline has been demonstrated to reduce central neurotransmitters content such as dopamine, glutamate and norepinephrine in amphetamine-induced CPP rats, reverse amphetamine-induced CPP effect and do not produce a CPP effect in itself (Zhou et al., 2010a). Our recent reports have shown that rhynchophylline can significantly abolish the rewarding effect and down-regulate the expression of NR2B in the hippocampi of amphetamine-type stimulants-induced CPP rats (Zhou et al., 2010b) and mice (Li et al., 2014). In addition, rhynchophylline protects rat cortical neurons against METH-induced neurotoxicity (Mo et al., 2006), antagonizes dopamine-induced NTera 2 (NT2) neuronal damage (Shi and Kenneth, 2002) and protects against glutamate-induced neuronal death in cultured cerebellar granule cells by inhibiting Ca^{2+} influx (Shimada et al., 1999). Together, these studies clearly show that the protective effects of rhynchophylline on METH-induced damage are closely related to neurotransmitters and their receptors.

Zebrafish (*Danio rerio*) is a small freshwater teleost species which has rapidly emerged as a useful new model organism for studying the behavioral and molecular mechanisms of brain disorders due to its well-characterized genome, robust behavioral responses and physiological similarity to humans (Cachat et al., 2010; Mathur and Guo, 2010; Stewart et al., 2010). Zebrafish has a functional nervous system after 4–5 days of embryonic development. The nervous system of zebrafish is less complex than that of mammals, but is still able to control a variety of complex behaviors such as learning, addiction, aggression and locomotion (Mathur and Guo, 2010; Stewart et al., 2010). The basic structure of the central nervous system in zebrafish has all of the major domains that are found in the mammalian brain. Neurotransmitters such as dopamine, 5-hydroxytryptamine, glutamate and γ-aminobutyric acid are also found in both interneuron systems and in long neuronal pathways (McLean and Fetcho, 2004; Panula et al., 2010; Panula et al., 2006). Key proteins necessary for dopaminergic and glutamatergic signaling have been detected in the zebrafish brains that TH, NR2B and GluR2/3 are included (Arenzana et al., 2006; Filippi et al., 2010; Maximino and Herculano, 2010). There is accumulating evidence showing that administration of amphetamine, cocaine, morphine and alcohol can significantly induce placed preference in adult zebrafish, respectively (Bretaud et al., 2007; Darland and Dowling, 2001; Echevarria et al., 2011; Mathur et al., 2011; Ninkovic et al., 2006). Additionally, acute exposure to amphetamine, morphine and alcohol remarkably alters the locomotor activity in larval zebrafish (Bretaud et al., 2007; Irons et al., 2010; Ninkovic et al., 2006). Moreover, treatment of zebrafish with these drugs of abuse alters the expression of multiple central nervous system genes, some of which have been identified as key components of addiction pathways in mammals (Kily et al., 2008). Together, these data indicate that analogous circuitry that mediates reward has been identified in the zebrafish brain.

In this study, we aimed to investigate the inhibiting effects of rhynchophylline on METH-induced CPP in adult zebrafish and METH-induced locomotor activity in TH-GFP transgenic zebrafish larvae and further elucidate the possible molecular mechanisms of rhynchophylline by measuring the content of neurotransmitters and the expression of TH, NR2B and GluR2/3 in dopaminergic and glutamatergic pathways.

2. Materials and methods

2.1. Animals

3- to 6-month-old wild-type adult male zebrafish (AB strain) weighing 0.5–1 g were used in this study and were maintained on a 14-h light:10-h dark cycle at 26 °C in a multi-tank system at our Fish Facilities of the Key Laboratory of Zebrafish Modeling and Drug Screening for Human Diseases of Guangdong Higher Education Institutes, belonging to Southern Medical University, simulating their environmental condition. Adult zebrafish were fed twice a day with a mixture of flake fish food and live brine shrimps. All experimental protocols and animal handling procedures were performed in accordance with the National Institutes of Health (NIH, USA) Guide for the Care and Use of Laboratory Animals and were approved by the Experimental Animal Ethics Committee of Southern Medical University.

2.2. Drugs

Methamphetamine hydrochloride (Lot # 1212-9802) was obtained from the National Narcotics Laboratory, China. Rhynchophylline (Lot # 1108-20131018, with purity ≥98%) was bought from National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine, China. (+)-MK-801 hydrogen maleate (Lot # 022M4616V) was purchased from Sigma (St. Louis, MO, USA). Methamphetamine hydrochloride, rhynchophylline and (+)-MK-801 hydrogen maleate were dissolved in fish physiological saline to final concentrations and injected intraperitoneally by microinjection system.

2.3. Conditioned place preference (CPP) paradigm

Zebrafish were tested in a rectangular tank (16 cm long × 9 cm wide × 9 cm high) as described previously (Darland and Dowling, 2001), with minor modifications. Distinct visual cues divide the experimental tank into two halves: a dark half colored in brown and a light half colored in white with two "frightening" black spots placed at the bottom of the tank. The neutral area providing access each compartment has a transparent door. According to previous study (Mathur et al., 2011) and our pre-test, zebrafish have an innate biased preference for brown versus white environment. Therefore we identified the white compartment as the METH-paired compartment. Zebrafish were tested for baseline preference by calculating the time spent in the METH-paired compartment during a 15-min trial. The zebrafish that showed place preference for the white compartment in the preconditioning phase were excluded from further analysis. 50 qualified zebrafish in the test of baseline preference were randomly divided into 5 groups: control group, CPP model group and treatment groups which were treated with rhynchophylline at doses of 50 and 100 mg/kg and MK-801 at a dose of 0.1 mg/kg, respectively. MK-801 is a noncompetitive NMDA receptor antagonist which readily crosses the blood–brain barrier and has been shown to inhibit METH-induced CPP in mice (Kim and Jang, 1997). Therefore, we used MK-801 as a positive control drug.

CPP test consisted of 3 phases and was proceeded within 9 consecutive days. For the preconditioning phase, zebrafish were placed under the door which was left opened to allow free access to the entire tank