Low dose risperidone attenuates cue-induced but not heroin-induced reinstatement of heroin seeking in an animal model of relapse

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Abstract
The aim of the present study was to investigate the effects of pretreatment with risperidone on heroin self-administration and heroin-seeking behaviour induced by cues and heroin priming. Rats were trained to self-administer heroin under a fixed ratio 1 schedule for 2 wk and nose-poke responding was extinguished for 10 d, after which reinstatement of drug seeking was induced by conditioned cues or heroin priming. Acute risperidone administration at doses 10–100 mg/kg potently and dose-dependently inhibited reinstatement of conditioned cue-induced heroin seeking; the minimum dose of inhibition was 30 mg/kg. In contrast, risperidone at the same doses did not attenuate reinstatement induced by two priming doses of heroin (100 or 250 mg/kg s.c.). Risperidone at these doses failed to alter heroin self-administration and locomotion activity. These data demonstrate that acute treatment with low-dose risperidone inhibits conditioned cue-induced heroin seeking and risperidone may be an adjunctive therapy for the treatment of heroin addiction.

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Introduction
Relapse to drug-seeking behaviour is a primary manifestation of opiate addiction that follows prolonged abstinence. Several factors are known to contribute to craving and relapse to drug use, such as the presentation of drug-associated cues, stress or a single dose of the drug itself (Shalev et al., 2002). These trigger factors have been used in animal models of relapse, particularly in the reinstatement model following withdrawal from heroin self-administration; in this model, heroin-paired stimuli or non-contingent heroin administration robustly re-instate drug seeking (Fuchs and See, 2002; Zhou et al., 2005; Rogers et al., 2008).

Heroin use initially causes increases in dopamine neurotransmission in the nucleus accumbens (Di Chiara and Imperato, 1988). There is evidence that the release of dopamine in the nucleus accumbens correlates with heroin self-administration behaviour (Wise et al., 1995). However, other literature does not support the role of dopamine in the reward-effect of the opiates (Badiani et al., 2011). Acute or chronic injection of morphine sulphate produced a dose-dependent increase in extracellular serotonin (5-HT) in the dorsal raphe nucleus and the nucleus accumbens (Tao et al., 1998; Tao and Auerbach, 2002). The augmentation of 5-HT levels may reduce the desire for morphine during withdrawal (Harris and Aston-Jones, 2001).

Dysregulation of these neurotransmitters during withdrawal may be involved in the craving and relapse. For example, selective stimulation of dopamine D2 receptors strongly mediates cocaine- and heroin-seeking behaviour in reinstatement paradigms (Self et al., 1996; De Vries et al., 1999; Khroyan et al., 2000). The available data suggest that activation of D2-like receptors is involved in heroin reinstatement during early, but not late, withdrawal (De Vries et al., 2002) and the D2-like receptor antagonist raclopride attenuates heroin-induced relapse (Shaham and Stewart, 1996). The dopaminergic meso-accumbens pathway receives 5-HT2A receptor innervations (McMahon et al., 2001). Numerous studies have also shown that 5-HT2A receptors may be involved in cocaine prime- and cue-induced reinstatement (Fletcher et al., 2002; Burmeister et al., 2004; Filip, 2005). Moreover, the genetic studies support a contribution of D2 receptor promoter polymorphism to the heroin inhalation (Picconiet et al., 2002) and 5-HT2A receptor gene polymorphisms to...
susceptibility to heroin dependence (Saiz et al., 2008). Risperidone has a high affinity for 5-HT2 and D2 receptors in vitro (Sumiyoshi et al., 1994). The primary antipsychotic effects of risperidone are believed to involve the antagonism of D2 and 5-HT2A receptors in the mesocorticolimbic pathway (Leysen et al., 1992; Schotte et al., 1996), a circuitry that is extensively implicated in addiction and relapse (Feltenstein and See, 2008).

Risperidone has both antipsychotic and mood-stabilizing properties. In combination with mood-stabilizers, risperidone is a safe and effective therapy for the treatment of patients with manic, hypomanic and depressive symptoms of mixed episodes in the bipolar type of schizo-affective disorder (Vieta et al., 2001). It has been shown that atypical antipsychotics significantly reduce smoking and alcohol consumption in schizophrenic patients. Risperidone blocks the continuation of nicotine-type addictive behaviour, but it is ineffective on early adaptations in the initiation of nicotine addiction (Akdag et al., 2011). Due to its unique pharmacological properties, risperidone may also be effective in reducing cocaine craving and use and may increase the likelihood of completing substance-abuse treatment (Albanese and Suh, 2006). Indeed, risperidone has already been used as a candidate medication for the treatment of cocaine dependence (Broderick et al., 2003). In the literature, most of the reports have examined the role of risperidone in the modulation of cocaine dependence. There are relatively few studies on the effects of risperidone on heroin addiction.

The present study aimed to evaluate whether the 5-HT2/D2 antagonist risperidone could be a therapeutically effective agent for preventing heroin relapse. First, we examined the effects of a wide range of doses of risperidone on the primary reinforcement characteristics of heroin. Next, we assessed the effects of risperidone on conditioned cue-induced and heroin-primed reinstatement of heroin-seeking behaviour.

Method

Subjects

The subjects were male Sprague–Dawley rats (280–300 g, Experimental Animal Center of Zhejiang Province, China) that were housed individually in stainless-steel home cages in a temperature-controlled ventilated colony room with a reversed 12 h light /dark cycle (lights on 18:00 hours). Water was always available in both test and home cages. Similarly, food was available ad libitum throughout the experiments. All experiments were conducted in compliance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publications No. 80-23 revised 1996).

Drugs

Heroin (diacetylmorphine HCl) was obtained from National Institute of Forensic Science (China). The heroin dose (50 µg/kg per injection) used for the self-administration was chosen on the basis of previous studies (Zhou et al., 2009). Heroin was dissolved daily in sterile physiological saline at a concentration of 0.2 mg/ml. Risperidone (National Institute for the Control of Pharmaceutical and Biological Products, China) was dissolved in dimethy sulfoxide (DMSO), diluted in sterile saline. The final concentration of DMSO in different doses of risperidone was adjusted to 3% and injected i.p. at a volume of 1 ml/kg. Risperidone doses were chosen in the present study according to previous reports (Schotte et al., 1993; van Beijsterveldt et al., 1994).

Intravenous catheter surgery

Rats were surgically implanted with chronic indwelling i.v. catheters under sodium pentobarbital anaesthesia (50 mg/kg i.p.) as previously described (Zhou et al., 2007). The catheters were flushed daily with 0.2 ml saline–heparin solution (25 U/ml heparin) to maintain catheter patency. To prevent infection, rats were treated post-surgically with penicillin B (30 mg/kg i.m.) for 5 d. The animals were allowed to recover for at least 7 d (Zhou et al., 2007).

Heroin self-administration

The rats were trained to self-administer heroin during daily 4 h sessions under a fixed ratio 1 schedule of reinforcement as previously described (Zhou et al., 2005, 2007). Rats received a single heroin infusion (50 µg/kg) following an active nose-poke. Each infusion was paired with a 5 s illumination of a light in combination with the noise of the infusion pump, together these stimuli served as a discrete conditioned cue paired with the drug infusion. Following infusions, a time-out period was imposed for 20 s, during which responding was recorded but produced no programmed consequences. Responding in the inactive nose-poke port produced no programmed consequences. Rats were put back into their individual home cages shortly after the session. Similar to a previous report (Liu et al., 2012), the rats exhibited reliable heroin self-administration, indicated by the increase in active responding when the heroin dose was decreased, and vice versa. An acquisition criterion required that subjects’ active nose-pokes vary by ≤ 10% over the course of three consecutive maintenance days.

Extinction and reinstatement of heroin seeking

Extinction training was carried out daily in 1-h sessions for 10 d consecutively in the operant chamber. Extinction criterion was that subjects touch the active nose-poke <10% of the average responding on the active nose-poke during maintenance. During the extinction phase, responding in the active hole was recorded but had no programmed consequence. The test for cue-induced or heroin-induced reinstatement was conducted 24 h after
After the rats acquired heroin self-administration for 10 d, they were randomly assigned to five groups (n = 7 in each group) and injected with vehicle, 0.01, 0.03, 0.1 or 0.3 mg/kg risperidone (i.p.) 10 min before the testing session on day 11.

Expt 2: Effects of acute treatment with risperidone on cue-induced reinstatement of heroin self-administration

Following 2 wk heroin self-administration and 10 d extinction, the rats were injected with vehicle or risperidone (0.01, 0.03 or 0.1 mg/kg) 10 min prior to the reinstatement session. Cue-induced reinstatement was observed for 2 h after initial cue presentation.

Expt 3: Effects of acute treatment with risperidone on heroin-induced reinstatement of heroin seeking

Rats self-administered heroin for 2 wk and extinguished for 10 d as described earlier. Subsequently, the rats (n = 6 in each group) were injected with risperidone (0.01, 0.03 or 0.1 mg/kg) or vehicle 10 min prior to a heroin-induced reinstatement session in which all rats were injected with heroin (100 or 250 µg/kg s.c.) and then placed into the operant chamber.

Statistical analyses

Data were analysed separately for the training and reinstatement phases. During training, the dependent measures were infusions and nose-poke responses. During reinstatement, the dependent measures were total responses on the previously active nose-poke port and inactive nose-poke responses. One way analysis of variance (ANOVA) or repeated measures ANOVAs for training and reinstatement included risperidone dose as a between-subjects factor. Post hoc analyses were performed with the Newman–Keuls test and differences are reported for p < 0.05.

Results

Effects of risperidone on heroin self-administration

The rats were injected with risperidone after the rats acquired heroin self-administration in expt 1. A two-way repeated measures ANOVA revealed a significant main effect of risperidone treatment (F1,38 = 3.19, p = 0.027), a significant interaction of risperidone on active and inactive nose-pokes (F1,4 = 2.68, p = 0.05) and a significant difference between the active and inactive nose-pokes as within-factor (F1,38 = 184.37, p < 0.001). Multiple comparisons showed risperidone at 0.3 mg/kg significantly decreased the active responses compared with the vehicle group (p = 0.01; Fig. 1a). As shown in Fig. 1b, one-way ANOVA did not show a significant main effect of risperidone on heroin infusions during training (F1,30 = 1.06, p = 0.39).

Effects of risperidone on cue-induced heroin seeking

To test the effect of risperidone on heroin seeking induced by cues which previously associated with heroin
responses (no significant effect of risperidone treatment on inactive cues compared with the control groups. There was significantly decreased the active responses induced by heroin priming at 0.1 mg/kg one-way ANOVA revealed a significant main effect of risperidone treatment on active responses \((F_{3,28} = 6.83, p = 0.002)\) and post hoc individual group comparisons showed that risperidone at doses of 0.03 and 0.1 mg/kg significantly decreased the active responses induced by cues compared with the control groups. There was no significant effect of risperidone treatment on inactive responses \((F_{3,28} = 1.69, p = 0.20)\).

**Effects of risperidone on heroin-induced heroin seeking**

With regard to the heroin-priming reinstatement, risperidone pretreatment did not affect the heroin seeking induced by the two doses of heroin priming. As shown in Fig. 3, during the reinstatement induced by heroin at 0.1 mg/kg one-way ANOVA revealed no significant main effect of risperidone treatment on active or inactive responses induced by heroin priming at 0.1 mg/kg (active: \(F_{3,28} = 0.22, p = 0.88\); inactive: \(F_{3,28} = 2.11, p = 0.13\)). In addition, there was no significant effect of risperidone treatment on active or inactive responses induced by heroin priming at 0.25 mg/kg (active: \(F_{3,28} = 0.80, p = 0.51\); inactive: \(F_{3,28} = 0.88, p = 0.47\)).

**Effects of risperidone on locomotion activity after abstinence**

Rats were injected with risperidone after the 1-h habituation period in the chambers. As shown in Fig. 4, no significant difference was evident among all the groups during the habituation \((F_{4,25} = 0.07, p = 0.99)\). One-way ANOVA showed a significant main effect of risperidone treatment on locomotion activities \((F_{3,28} = 4.83, p = 0.005)\) and multiple comparisons showed that risperidone treatment only at doses of 0.3 mg/kg significantly decreased the locomotion activities \((p < 0.05)\).

**Discussion**

These data showed that acute pretreatment with risperidone, an atypical antipsychotic, effectively inhibited cue- but not drug-induced heroin seeking. Risperidone dose-dependently reduced cue-induced heroin seeking, with significant reductions observed at 0.03-0.1 mg/kg for most drug-seeking responses measured. Risperidone only at a dose of 0.3 mg/kg could inhibit the active responses in the heroin self-administration training. Moreover, low-dose risperidone failed to produce any significant effects on locomotion activity, inactive responding and heroin self-administration, suggesting that blockage of cue-induced reinstatement of heroin seeking by risperidone did not result from sedative or motor inhibitory effects of the drug.

Risperidone is a novel antipsychotic drug with beneficial effects on both positive and negative symptoms of...
Risperidone and heroin seeking

Peridone in the frontal cortex and the nucleus accumbens, compared with 5-HT$_2$ receptors (Sumiyoshi et al., 1994). Compared with 5-HT$_3$ receptor occupancy, a dosage 19 times higher is required to obtain the same extent of D$_2$ receptor occupancy (Schotte et al., 1996). The 50% effective dose of receptor occupancy (ED$_{50}$) for 5HT$_2$ by risperidone in the frontal cortex and the nucleus accumbens is 10–20 times less than that of the D$_2$ receptor (i.e. 0.04 and 1 mg/kg, respectively; Leysen et al., 1992). Furthermore, risperidone occupies 5-HT$_2$ receptors at very low doses (ED$_{50}$=0.067 mg/kg). Near complete occupancy (>80%) is achieved before the D$_2$ receptor becomes occupied (ED$_{50}$=0.66 mg/kg; Schotte et al., 1993). The highest risperidone affinity is for the 5-HT$_2A$ receptor, whereas the affinities for other 5-HT receptor subtypes are at least 100 times lower in vitro (Leysen et al., 1994). Thus, the dose of risperidone used in the present study might be enough to block the 5-HT$_2A$ receptor. The binding properties of this receptor may contribute to risperidone’s ability to attenuate reinstatement of heroin seeking induced by cues.

Indeed, 5-HT$_2A$ antagonists have been shown to attenuate cue-induced reinstatement of drug seeking in rats (Burmeister et al., 2004; Filip, 2005). Notably, the present study also showed that low-dose risperidone failed to affect heroin self-administration and reinstatement induced by heroin priming. Thus, risperidone diminished cue-induced heroin seeking without affecting the primary reinforcing effects of heroin. While selective stimulation of D$_2$ receptors strongly mediates cocaine- and heroin-seeking behaviour in reinstatement paradigms (De Vries et al., 1999; Khroyan et al., 2000), the activation of D$_2$-like receptors mediates the heroin reinstatement during the early stages of withdrawal (De Vries et al., 2002). In the present study, risperidone inhibited the reinstatement of heroin seeking at doses as low as 0.01 mg/kg, indicating that the D$_2$ antagonist properties of risperidone may not account for its effects on drug seeking. Although doses as high as 0.3 mg/kg risperidone may significantly blunt the reinforcing effects of heroin, these effects do not appear to be specific to drug-taking and -seeking behaviour. For example, this dose of risperidone has been shown to robustly decrease sucrose-consuming behaviour (Ricci et al., 2007). Recent data suggest that the endogenous 5-HT system affects striatal dopamine release in a state-dependent manner associated with the conditional involvement of various 5-HT receptors, including 5-HT$_{2A}$ and 5-HT$_C$ (Navailles and De Deurwaerdere, 2011). Activation of cortical 5-HT$_{2A}$ receptors increases the release of dopamine from the mesocortical system (Bortolozzi et al., 2005); this effect may be mediated by increases in glutamate release from corticotegmental projections to the ventral tegmental area (Pehek et al., 2006). The selective regulation of the mesoaccumbens circuit by 5-HT$_{2A}$ and 5-HT$_C$ receptors implies these 5-HT receptors are critical to the behavioural outcomes of systemic cocaine administration (McMahon et al., 2001; Bubar and Cunningham, 2008). Conversely, the 5-HT$_{2A/C}$ antagonist, ketanserin, attenuates cue-induced reinstatement, but fails to alter cocaine-induced reinstatement (Burmeister et al., 2004). SR 46349B, a 5-HT$_{2A}$ receptor antagonist, reduces cue-induced reinstatement (Filip, 2005). Recently, M100907, a 5-HT$_{2A}$ receptor antagonist, infused directly in the ventromedial prefrontal cortex has also been shown to attenuate cue-induced reinstatement of cocaine-seeking behaviour (Pockros et al., 2011). The evidence showed that 5-HT$_{2A}$ blockade in the nucleus accumbens significantly decreases impulsive responding, which may be a characteristic of drug addiction (Robinson and Berridge, 2008). It is worth noting that risperidone inhibited cue-induced reinstatement of heroin seeking even at doses below the ED$_{50}$ for 5-HT$_{2A}$ receptor occupancy. The data inferred that antagonism of 5-HT$_{2A}$ receptor by risperidone at a low dose may be involved in its inhibitory action on the heroin seeking induced by cues.

Clinical trials have been conducted demonstrating that risperidone may reduce craving in cocaine dependence (Smelson et al., 2004; De La Garza et al., 2005) and blunt the euphoric highs associated with cocaine use in human volunteers (Grabowski et al., 2000). The doses of risperidone that effectively blocked reinstatement of heroin seeking induced by cues were equal to, or below, clinically equivalent unit doses used for the treatment of psychosis and cocaine craving (Nyberg et al., 1993; Newton et al., 2001). Moreover, in the present study, risperidone at low doses may have possible clinical applications for the treatment of heroin addiction. Although risperidone is unlikely to find broad acceptance with the treatment-seeking population due to its potential side-effects, at low doses it may be a useful treatment approach for heroin addiction. Our results support further assessment of risperidone in clinical trials for the treatment of heroin addiction.

In conclusion, the present results demonstrated that risperidone as an atypical antipsychotic, at low doses, can be used as a treatment for heroin seeking or craving induced by conditioned cues previously associated with heroin reward. These results suggest that risperidone could be used as an adjunctive therapy for heroin addiction.

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Statement of Interest
None.

References


