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published in
International Journal of Neuropsychopharmacology
2010

DOI (link to publisher)
10.1017/S1461145710000428

document version
Publisher's PDF, also known as Version of record

International Journal of Neuropsychopharmacology (2010), **13**, 961–965. Copyright © CINP 2010
doi:10.1017/S1461145710000428

BRIEF REPORT

CINP

5-HT$_6$ antagonism attenuates cue-induced relapse to cocaine seeking without affecting cocaine reinforcement

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Abstract

Re-exposure to drug-related cues elicits drug-seeking behaviour and relapse in humans even after months of abstinence. Similarly, in laboratory rats, drug-associated stimuli reinstate cocaine seeking after prolonged withdrawal periods, thus providing a model to study mechanisms underlying cocaine relapse. 5-HT$_6$ receptors (5-HT$_6$Rs) are abundantly expressed in brain areas such as the nucleus accumbens and prefrontal cortex, which are critically involved in cocaine reinforcement and relapse. Nevertheless, the role of 5-HT$_6$Rs in relapse mechanisms has not been investigated. We report here that the 5-HT$_6$R antagonists SB-271046 and Ro-04-6790 significantly attenuate cue-induced cocaine seeking. However, effective doses of both 5-HT$_6$R antagonists did not affect cocaine self-administration. This indicates that 5-HT$_6$Rs are specifically involved in the secondary reinforcing properties of cocaine, leaving primary reinforcement and ability to perform an operant response unaffected. As such, 5-HT$_6$Rs may represent a novel target for the prevention of relapse to cocaine seeking.

Received 22 September 2009; Reviewed 29 October 2009; Revised 9 March 2010; Accepted 22 March 2010; First published online 29 April 2010

Key words: Cocaine self-administration, drug addiction, relapse, 5-HT$_6$.

Introduction

In abstinent cocaine users, the propensity to relapse is high and the effectiveness of therapeutic interventions even after successful detoxification is rather limited. Therefore, a major focus of drug addiction research is the identification of novel therapeutic targets for drug development. There is accumulating evidence that the serotonin (5-hydroxytryptamine, 5-HT) system plays a modulator role in cocaine addiction (Dhonnchadha & Cunningham, 2008; Homberg *et al*. 2008). However, the underlying mechanisms and the role of specific 5-HT receptors are not fully understood.

In the past decade, the 5-HT$_6$ receptor (5-HT$_6$R) has come into focus as a novel target for treatment of various diseases. Evidence for a role of 5-HT$_6$R in addiction-related processes is limited, but emerging. For instance, pharmacological blockade of 5-HT$_6$Rs was found to enhance amphetamine-induced locomotor activity and amphetamine self-administration, while leaving cocaine-mediated behaviour unaffected (Frantz *et al*. 2002). More recently, overexpression of 5-HT$_6$Rs in the nucleus accumbens by viral-mediated gene transfer resulted in reduced place preference conditioning whereas pharmacological blockade of 5-HT$_6$Rs enhanced place preference conditioning to cocaine. However, these manipulations did not affect cocaine-induced stereotypy (Ferguson *et al*. 2008). Together, these studies suggest that 5-HT$_6$Rs may play a role in associative learning processes triggered by cocaine, but not in the pharmacological effects of the drug *per se*. Indeed, there is growing evidence for a critical role of 5-HT$_6$Rs in memory formation (Meneses, 2001; Rogers & Hagan, 2001).

Associative learning processes play an important role in the initiation and persistence of cocaine seeking. Re-exposure to drug-associated cues can provoke drug craving and subsequent relapse to drug use even

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after prolonged abstinence periods (Childress *et al*. 1988). In the present study we set out to investigate the role of 5-HT_6Rs in cue-induced reinstatement of cocaine seeking, in a so-called 'reinstatement model' (De Vries *et al*. 2001; Epstein *et al*. 2006). Rats were pretreated with two chemically distinct 5-HT_6R antagonists and tested for relapse behaviour induced by stimuli previously associated with cocaine delivery. In addition the effect of these manipulations on cocaine self-administration was evaluated.

Material and methods

Male Wistar rats (weighing 250–300 g, Harlan, The Netherlands) were used. The animals were housed individually in a temperature- and humidity-controlled room on a reversed 12-h light/dark cycle (lights off 07:00 hours) with food and water available *ad libitum*. Experimental procedures were approved by the Animal Welfare Committee (VU University, Amsterdam, The Netherlands) and were performed in accordance with the recommendations and policies of the United States National Institutes of Health Principles of Laboratory Animal Care (Clark *et al*. 1996).

Behavioural procedures and surgery were as previously described (De Vries *et al*. 2001). In brief, intravenous (i.v.) silicon catheters were implanted in the right jugular vein under isoflurane (1.5–2.0%) anaesthesia. Lidocain and adrenalin were used for local anaesthesia during the surgery. After surgery, animal received Ketofen (5 mg/kg s.c.) and Baytril (1.25%, 0.2 ml s.c.). Animals were allowed to recover from surgery for 5–7 d before training started. All training and testing was conducted in 16 Plexiglas operant chambers surrounded by sound-attenuating ventilated cubicles (TSE Systems, Germany). The chambers were fitted with a grid floor, a dim red houselight and a wall with two nose-poke holes. A red stimulus light was located above both the nose-poke holes and a yellow stimulus light within the holes.

One nose-poke hole served as the active hole and the other as the inactive hole. Pokes made in the active hole resulted in a delivery of a 40 μl cocaine solution (0.5 mg/kg per infusion, i.v.) for 2 s. During cocaine delivery, the active nose-poke hole was illuminated for 2 s and the houselight and red stimulus light were switched off for 15 s. In this time-out period, nose-poking was without consequences. During the whole session, pokes in the inactive hole were monitored, but were without consequences.

Animals were trained on a fixed-ratio 1 schedule (FR1 schedule) for 10 daily sessions (every active response resulted in a reward, except in time-out periods; sessions were for 60 min). The maximum number of infusions was set at 50. When responding was stable (<10% variation during three consecutive sessions), the extinction period was started. In this 3-wk period (15 daily sessions of 60 min), animals were placed in the operant chambers, were not connected to the swivel, and cocaine and cocaine-related stimuli (discrete and discriminative cues) were not available.

After extinction, the animals were tested for cue-induced reinstatement (session duration 30 min). Here, the experimental conditions were similar to the conditions during training on a FR1 schedule, with the exception that active responses did not result in a cocaine infusion – only the presentation of cues.

The animals were tested twice for cue-induced reinstatement: once with one of the doses of the tested compound and once with vehicle, in random order. Between these two test days there was a 7-d period of further extinction training and wash-out. Treatment groups were balanced by nose-poke responses during the last two cocaine self-administration sessions. SB-271046 (5-chloro-*N*-(4-methoxy-3-piperazin-1-ylphenyl)-3-methyl-2-benzothiophenesulfonamide) and Ro-04-6790 [(4-amino-*N*-(2,6-bis-methylamino-pyramidin-4-yl)-benzene sulphonamide] were synthesized at Abbott. Both compounds were suspended in 0.5% hydroxypropyl methylcellulose in water and injected i.p. 30 min before the test, 0 mg/kg (vehicle), 1, 3 and 10 mg/kg (SB-271046) and 3, 10 and 30 mg/kg (Ro-04-6790).

In the cocaine self-administration tests, each compound was intraperitoneally injected 30 min before the test. The test lasted 60 min, during which time the animals could self-administer cocaine (0.5 mg/kg per infusion) on a FR1 schedule of reinforcement. The dose range of 5-HT_6R antagonist tested was similar to the range used for the reinstatement tests. The test design was a latin squares, hence each animal ($n = 9$) received each dose. Between the test days were 2 or 3 d during which cocaine self-administration was maintained.

A mixed-factor repeated-measures ANOVA, was used for analysis of the reinstatement data with drug dose as the between factor and drug treatment (vehicle or drug) as the within factor. Further *post-hoc* testing was done by pairwise comparisons using a paired *t* test. The homogeneity of variance across groups was determined using Levene's test of equality of error variances. In case of violation of homogeneity, a square-root transformation of the data was performed. A Kruskal–Wallis test (non-parametric *K*-independent-samples test), followed by a Mann–Whitney test was

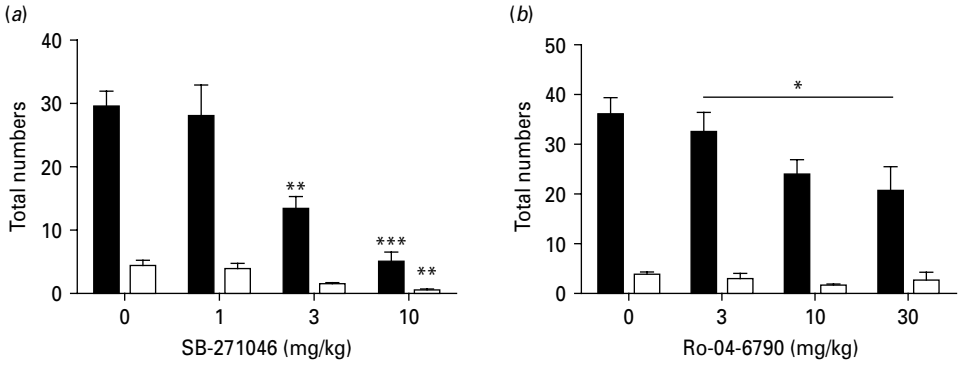

Fig. 1. (*a*) SB-271046 ($n=10$) dose-dependently reduced cue-induced reinstatement of cocaine seeking. The highest does decreased responding on the inactive nose-poke hole. (*b*) The Ro-04-6790 compound ($n=9$) also significantly reduced cue-induced reinstatement of cocaine seeking. No effect of treatment on inactive nose-poke responding was found. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to vehicle. ■, Active nose-pokes; □, inactive nose-pokes.

used when there was no homogeneity after square-root transformation.

Results

Cue-induced reinstatement of cocaine seeking

A mixed-factor repeated-measures analysis of the effects of SB-271046 on nose-pokes made in the active (previous cocaine-paired) hole revealed a significant effect of drug treatment ($F_{1,27}=38.193$, $p<0.001$), a significant drug × dose interaction ($F_{2,27}=7.757$, $p=0.002$) and a significant effect of dose ($F_{2,27}=8.425$, $p=0.001$). *Post-hoc* testing showed that SB-271046 significantly reduced nose-pokes made in the active hole at 3 ($p=0.01$) and 10 mg/kg ($p<0.001$; paired t test, Fig. 1*a*). Moreover nose-pokes made in the inactive holes were significantly affected by treatment ($\chi^2=13.911$, Mann–Whitney test, $p=0.003$). SB-271046 attenuated inactive nose-pokes made at 10 mg/kg ($p<0.01$).

Analysis of the effects of Ro-04-6790 on active nose-pokes during the relapse test revealed a significant effect of drug treatment ($F_{1,24}=11.95$, $p=0.002$; Fig. 1*b*), no drug × dose interaction and no effect of dose. Ro-04-6790 did not affect the number of inactive nose-pokes.

Analysis of the time-course of the effects of both antagonists on cue-induced reinstatement revealed that the inhibitory effects on active nose-poke responding were observed during the first 5 min of the relapse tests (data not shown).

Cocaine self-administration

SB-271046 did not affect the number of rewards received ($F_{3,24}=1.594$, $p=0.217$) or nose-pokes made in the inactive hole ($F_{3,24}=1.360$, $p=0.281$) or total number of nose-pokes made in the active hole ($F_{3,24}=2.747$, $p=0.115$; Fig. 2*a*). Ro-04-6790 also did not affect cocaine self-administration: number of rewards ($F_{3,21}=1.055$, $p=0.389$), inactive nose-pokes ($F_{3,21}=1.427$, $p=0.271$) and total number of active nose-pokes ($F_{3,21}=1.363$, $p=0.288$; Fig. 2*b*).

Discussion

This is the first demonstration that 5-HT$_6$Rs are involved in the long-term neuronal processes underlying relapse to cocaine seeking. The 5-HT$_6$R antagonists SB-271046 and Ro-04-6790 dose-dependently attenuated cue-induced reinstatement of cocaine seeking in animals with a history of cocaine self-administration. However, both of the 5-HT$_6$R antagonists did not affect responding for cocaine itself. This indicates that 5-HT$_6$R antagonists selectively interfere with the reinforcing properties of cocaine-associated stimuli, but not with the primary reinforcing effects of cocaine or with general operant behaviour. Time-course analysis revealed that both antagonists suppressed responding for the cocaine cues immediately at the start of the session, indicating that 5-HT$_6$R blockade does not facilitate extinction learning, but rather reduces the impact of cocaine-associated cues. Since cocaine-associated cues are a major trigger for relapse to cocaine seeking, blockade of the 5-HT$_6$Rs may represent a promising novel pharmacological intervention strategy for the treatment of cocaine addiction.

SB-271046 and Ro-04-6790 did not affect responding for a 0.5 mg/kg dose of cocaine at doses that attenuate cue-induced cocaine seeking, nor have these

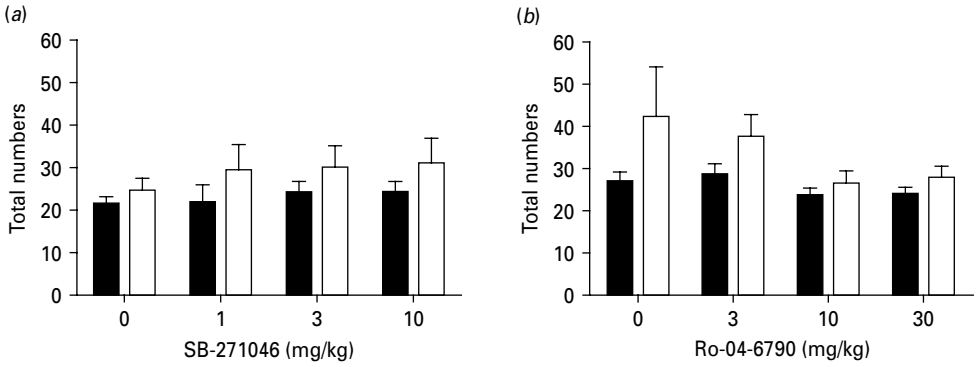

Fig. 2. (*a*) SB-271046 and (*b*) Ro-04-6790 did not affect cocaine self-administration ($n = 9$). ■, Infusions; □, total nose-pokes.

compounds been reported to induce pro-cognitive effects (e.g. King *et al*. 2004) or reduce spontaneous locomotor activity (Van Gaalen & Relo, unpublished results). This lack of effect of 5-HT_6R blockade on cocaine self-administration confirms an earlier report using SB-258510A (Frantz *et al*. 2002). Recently, the 5-HT_6R antagonist Ro-4368554 was found to facilitate conditioned place preference to cocaine, but did not affect cocaine-induced stereotypy (Ferguson *et al*. 2008). Facilitation of conditioned place preference, an associative spatial learning task, by 5-HT_6R blockade may therefore reflect a role of 5HT_6Rs in spatial learning and memory, independent of the pharmacological properties of cocaine. This view is supported by observations showing that 5-HT_6R antagonists enhance retention of a water maze task (Rogers & Hagan, 2001).

The molecular mechanisms underlying the relapse-attenuating effects of 5-HT_6R antagonists remain unclear. 5-HT_6R mRNA is highly expressed in the striatum, nucleus accumbens, olfactory tubercles, hippocampus and cortex (Monsma *et al*. 1993; Ward *et al*. 1995), brain areas that are involved in cognitive functioning and reward-related behaviour. 5-HT_6R messenger RNA is mainly localized in 5-HT projection fields rather than regions of 5-HT containing cell bodies (Ward *et al*. 1995). In the hippocampus and striatum, no effect on 5-HT_6R mRNA levels were found after a lesion with the serotonergic neurotoxin 5,7-DHT, indicating that 5-HT_6Rs are located post-synaptic to serotonergic neurons and do not function as autoreceptors (Gerard *et al*. 1996). This is in line with results from microdialysis studies in which it was demonstrated that SB-271046 does not affect 5-HT concentrations in the prefrontal cortex (Dawson *et al*. 2001; Lacroix *et al*. 2004). Dopamine and noradrenaline release in the frontal cortex have been reported to be enhanced after application of 5-HT_6R antagonists in the frontal cortex by some (Lacroix *et al*. 2004) but not by others (Dawson *et al*. 2001; Frantz *et al*. 2002). Furthermore, 5-HT_6R antagonists increase acetylcholine and glutamate release in the frontal cortex, but not in the striatum, nucleus accumbens or hippocampus (Dawson *et al*. 2001; Marcos *et al*. 2006; Riemer *et al*. 2003). Such increase in excitatory neurotransmission in the prefrontal cortex may be of particular relevance to the observed relapse-attenuating effects. Glutamatergic projections from prefrontal areas to the ventral striatum are known to play a key role in reinstatement of drug seeking induced by various stimuli, including drug-associated cues. (Kalivas & McFarland, 2003). We have shown recently that re-exposure to heroin-associated stimuli resulted in an acute decrease in AMPA receptor functioning in the ventral part of the medial prefrontal cortex (Van den Oever *et al*. 2008). The resulting loss of inhibitory control over drug seeking could be rescued by restoring AMPA receptor functioning. Similarly, but still speculative, enhancement of excitatory transmission in the prefrontal regions by 5-HT_6R antagonists may strengthen corticostriatal projections and allow a better control over drug-seeking behaviour. However, the effect of 5-HT_6R antagonism on the reinforcing effects of cocaine and amphetamine appears to be divergent. Therefore, studying reinstatement of drug seeking in animals with a history of self-administration of other classes of drugs of abuse are required to determine whether 5-HT_6R antagonism is specifically involved in the secondary reinforcing properties of cocaine.

Acknowledgements

SB-271046 and Ro-04-6790 were provided by Abbott.

Statement of Interest

M. M. van Gaalen is full time employee of Abbott.

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