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A 5-HT_{2C} receptor antagonist potentiates a low dose amphetamine-induced conditioned place preference

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Abstract

This study was designed to determine whether a 5-HT_{2C} receptor antagonist could induce a conditioned place preference indicative of reward and/or abuse potential. Here, we present the first evidence that a selective 5-HT_{2C} receptor antagonist, 6-chloro-5-ethoxy-N-(pyridin-2-yl)indoline-1-carboxamide hydrochloride (CEPC), can potentiate a low dose (0.5 mg/kg) amphetamine-induced positive conditioned place preference (CPP). CEPC did not produce any CPP given alone at doses of either 2.0 or 4.0 mg/kg, whereas low dose amphetamine alone produced only a slight, but statistically nonsignificant, place preference. These studies suggest that 5-HT_{2C} receptor antagonists can indirectly potentiate the rewarding effects of amphetamine, and perhaps other psychostimulants. If the results can be translated to man, putative 5-HT_{2C} receptor antagonist treatments for anxiety or depression may enhance or potentiate the rewarding effects of drugs of abuse such as amphetamine, which release dopamine.

Keywords

Conditioned place preference; 5-HT_{2C} receptor; amphetamine; dopamine; reward; drug abuse

Introduction

Conditioned Place Preference (CPP) is a behavioral assay that utilizes Pavlovian or classical conditioning to pair an unconditioned stimulus such as a drug with a neutral stimulus such as an environment [8]. A positive CPP is defined as an increase in preference for the drug-paired environment, and it is strongly correlated with self-administration of drugs in rats [2]. CPP has potential to predict rewarding effects and assess abuse potential in drug development.

Serotonin receptors remain a lucrative target in drug development for several disorders including schizophrenia [22], and clinical depression [17]. In particular, 5-HT_{2C} receptors are an emerging target for antidepressant [4] and anxiolytic therapies [27]. In animal models of anxiety, 5-HT_{2C} receptor antagonists have shown consistent anxiolytic [19;21] and antidepressant [9] properties. In fact, a mixed melatonin agonist/5-HT_{2C} receptor antagonist,

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agomelatine, is currently marketed for treating depression and has shown clinical effectiveness [18].

The 5-HT_{2C} receptor is a member of the Family A G-protein coupled receptors. As a principal signaling mechanism they couple to Gαq and lead to phospholipase C activation and phosphoinositide hydrolysis [25]. They are expressed in a number of brain areas, but of relevance to the present study is their localization on GABAergic cells in or near the ventral tegmental area (VTA) [7]. The VTA provides mesolimbic and mesocortical dopamine innervation to the limbic and cortical systems, respectively, and 5-HT_{2C} receptors present in the VTA have been postulated to modulate dopamine efflux [10]. Agonists and antagonists of the 5-HT_{2C} receptor also have been shown to decrease [13] and increase [11], respectively, dopamine release in the nucleus accumbens (NAcc). Drugs that increase mesolimbic dopamine levels, such as amphetamine, widely used as Adderall to treat attention deficit disorder [24], produce a robust positive CPP [5] and have a long history of abuse. Microinjections of amphetamine directly into the shell of the NAcc also produce a positive CPP [26].

The finding that 5-HT_{2C} receptor antagonists increase [11] dopamine release in the NAcc led us to hypothesize a potential role for the 5-HT_{2C} receptor in mediating the rewarding effects of amphetamine. This issue has not been thoroughly investigated, but there is evidence for potentiation of low-dose (0.5 mg/kg) amphetamine-induced locomotor activity after treatment with the 5-HT_{2C} receptor antagonist, SB242084 [14]. There are, however, no conditioned place preference studies of any selective 5-HT_{2C} receptor antagonist.

We therefore sought to test whether there were any rewarding effects mediated by a 5-HT_{2C} receptor antagonist given alone or in combination with selective dopamine releasers such as amphetamine. To carry out in vivo studies, however, we required relatively large amounts of a selective 5-HT_{2C} receptor antagonist, but those that were commercially available were prohibitively expensive. Thus, we synthesized a close analogue of SB242084 that had also been reported by SmithKline Beecham: 6-chloro-5-ethoxy-N-(pyridin-2-yl)indoline-1-carboxamide hydrochloride (CEPC), identified as compound **40** in Bromidge et al [6]. CEPC was reported to have 150-fold selectivity for 5-HT_{2C} over 5-HT_{2A} receptor affinity, and also had oral activity at 3.4 mg/kg in reversing mCPP-induced hypolocomotion.

We first asked whether CEPC alone could produce a positive CPP indicative of reward and possible abuse potential. In addition, we also sought to investigate whether CEPC could potentiate a low-dose (0.5 mg/kg) amphetamine-induced CPP, based on the reported 5-HT_{2C} receptor antagonist potentiation of rat locomotor activity after the same dose of amphetamine [14].

Material and Methods

Drugs

The 5-HT_{2C} receptor antagonist (CEPC) used for our study was synthesized in our laboratory according to methods described by Bromidge et al [6]. It had physical and chemical properties in agreement with those reported, and spectral and elemental analysis values consistent with the expected structure. CEPC was prepared for injection by soaking the desired weight of the compound in 2–3 drops of 80% lactic acid overnight and adding the remaining volume of saline under sonication. *S*-amphetamine sulfate was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in physiological saline (0.9% NaCl). All drugs were administered intraperitoneally (i.p.) in a volume of 1 mL/kg.

Animals

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used for all conditioned place preference experiments. All rats weighed between 250 and 275 grams and were approximately nine weeks of age at the start of experiments. Rats were housed individually in polycarbonate cages with free access to food and water under a 12:12 hour light:dark schedule with lights on at 10:00 and off at 22:00. Rats were allowed to acclimate upon arrival for at least one week and were handled each day for the following week. All experimental procedures were conducted between 12:00 and 20:00. All animal use procedures conformed to the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and were approved by the Purdue Animal Care and Use Committee (PACUC).

CPP apparatus and procedure

The apparatus used for all conditioned place preference experiments was a shuttle box chamber from Lafayette Instruments (Lafayette, IN) with two compartments of equal sizes ($23 \times 31 \times 21$ cm) separated by a guillotine door. One compartment was painted black with a grid floor and the other compartment had unpainted metal walls with a solid metal floor. Time spent in each compartment was measured by a weight sensitive timer for each compartment, which started when the rat placed all four paws in the chamber, and stopped when all four paws were out of the chamber.

For pre-conditioning days 1–4, rats were weighed, placed in transport cages, and when the session began were allowed free access to both of the compartments with the guillotine door open. Placement of rats in either compartment was counterbalanced by session day and by subject number. Rats were allowed to explore freely for 20 minutes and were then taken out and placed back in transport cages. Both sides of the compartment were cleaned with 10% ethanol after every individual session to remove any olfactory cues. On the final day of pre-conditioning (day 4), time spent in each compartment was measured to assess compartment preference (pre-conditioning test). The compartment that the rats spent less time in was designated the non-preferred side, and the compartment where the rat spent more time was designated the preferred side. Rats were randomly assigned to an experimental group receiving a pretreatment and test drug combination (Vehicle/Saline, 2.0 mg/kg CEPC/Saline, 4.0 mg/kg CEPC/Saline, Vehicle/0.5 mg/kg *S*-amphetamine, 4.0 mg/kg CEPC/0.5 mg/kg *S*-amphetamine, and Vehicle/2.0 mg/kg *S*-amphetamine).

For conditioning days 5–12, procedures were similar to days 1–4 except that the guillotine door was closed and rats were allowed access only to one compartment. On days 5, 7, 9, and 11, rats were administered the pretreatment drug and then 20 minutes later administered a test drug. After 30 minutes, rats were placed in the non-preferred compartment for 20 minutes. For days 6, 8, 10, and 12, procedures were similar to days 5, 7, 9, and 11 except that the pretreatment and test drugs were both saline and placement was in the preferred compartment. All pretreatment and test drugs were administered in transport cages and cleaning procedures after every session were the same as preconditioning days. For post-conditioning test day 13, rats were allowed free access to both compartments for 20 minutes and time spent in each compartment was measured to assess conditioned place preference.

Data Analysis

For pre-conditioning test day 4 and post-conditioning test day 13, time spent in each compartment was converted to a percentage of total time spent in the test. Change in percentage of time spent in the non-preferred (drug-paired) compartment was measured by subtracting the percent time spent in the non-preferred side before drug pairing on preconditioning test day 4 from percent time spent in the non-preferred compartment on

post-conditioning test day 13 after drug pairing. A positive change in percent time spent in the drug-paired side would indicate a positive CPP; a negative change in percent time spent in the drug-paired side would indicate a negative CPP; and a no change in percent time spent in the drug-paired side would indicate an absence of CPP. Change in percentage of time spent in the non-preferred (drug-paired) compartment was graphed and statistical analysis was performed using Graphpad Prism 4 Software (San Diego, California).

Results

Figure 1 shows percent change in time spent in the drug paired compartment (post-conditioning test minus pre-conditioning test) for all experimental groups. One-way ANOVA reveals a significant difference among all conditioning groups ($F_{5,30}=9.612$, $p<0.0001$). The vehicle/saline control group showed no significant changes in preference, whereas vehicle/2.0 mg/kg amphetamine showed a robust positive conditioned place preference ($p<0.01$, compared to vehicle/saline, Dunnett's Multiple Comparison Test). Neither group, 2.0 mg/kg CEPC/saline or 4.0 mg/kg CEPC/saline, showed any significant difference compared to vehicle/saline. The vehicle/0.5 mg/kg *S*-amphetamine group showed an increase in preference, but this increase was not statistically significant compared to vehicle/saline ($p>0.05$, Dunnett's Multiple Comparison Test). The 4.0 mg/kg CEPC/0.5 mg/kg *S*-amphetamine group did, however, show a statistically significant increase in preference compared to saline ($p<0.01$, Dunnett's Multiple Comparison Test). A multiple comparison using a Bonferroni test also indicated that the 4.0 mg/kg CEPC/0.5 mg/kg *S*-amphetamine group was significantly different from vehicle/0.5 mg/kg *S*-amphetamine ($p<0.05$), but not from vehicle/2.0 mg/kg *S*-amphetamine.

Discussion

These experiments reveal two major findings about the CPP effects of 5-HT_{2C} receptor antagonists. First, there was no effect on place conditioning with either dose (2 or 4 mg/kg) of the 5-HT_{2C} receptor antagonist, CEPC. The second major finding is that when a 4.0 mg/kg dose of CEPC is given before a low dose of *S*-amphetamine (0.5 mg/kg), positive CPP is observed, indicative of reward similar to a higher 2.0 mg/kg dose of amphetamine. This latter finding demonstrates that the 5-HT_{2C} receptor antagonist, CEPC, potentiated the establishment of CPP with low dose amphetamine. These results provide further evidence that the 5-HT_{2C} receptor is able to modulate the rewarding properties of *S*-amphetamine in rats, as measured by the conditioned place preference assay.

These findings are consistent with locomotor activity studies using another 5-HT_{2C} receptor antagonist, SB242084 [14]. In these studies, SB242084 potentiated locomotor activity of the same low 0.5 mg/kg dose of amphetamine. Furthermore, this same study also reported potentiation by SB242084 of other drugs that increase mesolimbic dopamine levels, including cocaine, methylphenidate, and nicotine.

The lack of a rewarding effect with either dose of CEPC may be surprising in view of the fact that most 5-HT_{2C} receptor antagonists, including SB242084, lead to an increase in mesolimbic dopamine release, as measured by *in vivo* microdialysis [12]. According to Hutson and colleagues, however, an increase in mesolimbic dopamine was not observed with SB242084 alone, but occurred only when dopamine release was increased by PCP [15]. Moreover, several differences in mesolimbic dopamine modulation by 5-HT_{2C} receptor antagonists have been attributed to inverse agonism versus silent antagonism and also to constitutive activity of the 5-HT_{2C} receptor, as well as tissue distribution [1;20]. Regardless, the rewarding effects of SB242084 or any other selective 5-HT_{2C} receptor antagonist have never been tested using CPP.

Although we did not observe a statistically significant positive CPP with 0.5 mg/kg *S*-amphetamine alone, meta-analysis studies have shown the size of the effect of CPP with amphetamine to be dependent on various factors, including breed, number of pairings, and is even diminished when amphetamine is paired with the non-preferred side [3], the method utilized in our study. The dose of amphetamine (0.5 mg/kg) was chosen based on the effects observed by Fletcher et al. [18] in locomotor activity studies.

To our knowledge, this is the first study to report on the possible rewarding effects of a 5-HT_{2C} receptor antagonist using CPP. There is, however, evidence for blocking a THC-induced positive CPP by disrupting 5-HT_{2C} receptor PTEN coupling with a small interfering peptide administered directly into the VTA [16]. PTEN coupling to 5-HT_{2C} receptor is thought to dephosphorylate and inhibit the 5-HT_{2C} receptor agonist-induced reduction in mesolimbic dopamine [23]. Interestingly, Ji and colleagues [16] also found that SB242084 reversed the 5-HT_{2C} receptor PTEN interfering peptide's ability to block THC-induced positive CPP. Although these authors' results demonstrate the importance of 5-HT_{2C} receptor PTEN coupling in regulating mesolimbic dopamine function, they did not test SB242084 alone or in combination with THC without the interfering peptide. If they had tested SB242084 in combination with THC as a control, our results indicate that a potentiation of the CPP might have occurred.

More investigation is needed, however, into the possible rewarding effects of 5-HT_{2C} receptor antagonists either alone or in combination with other drugs. Several 5-HT_{2C} receptor antagonists, such as SB242084 and agomelatine, have never been tested using CPP either alone, or in combination with common psychostimulant drugs of abuse such as cocaine or methamphetamine. Further studies are needed to investigate whether therapeutic doses of 5-HT_{2C} receptor antagonists in combination with therapeutic doses of prescription dopamine releasers such as Adderall might lead to enhanced abuse potential.

Conclusions

In conclusion, we have demonstrated that a selective 5-HT_{2C} receptor antagonist does not produce rewarding effects by itself, but can potentiate the positive CPP produced by a low dose of amphetamine. These results indicate that selective 5-HT_{2C} receptor antagonists developed for depression and anxiety could lead to abuse if given in combination with medications that have psychostimulant-like properties. Furthermore, this study illustrates the need for extensive study of 5-HT_{2C} receptor drugs using conditioned place preference or other tests of reward, with concomitant study of dopamine release and dopamine releasing drugs.

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References

1. Aloyo VJ, Berg KA, Spampinato U, Clarke WP, Harvey JA. Current status of inverse agonism at serotonin2A (5-HT_{2A}) and 5-HT_{2C} receptors. *Pharmacol Ther.* 2009; 121:160–173. [PubMed: 19109993]
2. Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl).* 2000; 153:31–43. [PubMed: 11255927]
3. Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neurosci Biobehav Rev.* 1995; 19:39–51. [PubMed: 7770196]

4. Berg KA, Harvey JA, Spampinato U, Clarke WP. Physiological and therapeutic relevance of constitutive activity of 5-HT 2A and 5-HT 2C receptors for the treatment of depression. *Prog Brain Res.* 2008; 172:287–305. [PubMed: 18772038]
5. Bowling SL, Bardo MT. Locomotor and rewarding effects of amphetamine in enriched, social, and isolate reared rats. *Pharmacol Biochem Behav.* 1994; 48:459–464. [PubMed: 8090815]
6. Bromidge SM, Dabbs S, Davies DT, Duckworth DM, Forbes IT, Ham P, Jones GE, King FD, Saunders DV, Starr S, Thewlis KM, Wyman PA, Blaney FE, Naylor CB, Bailey F, Blackburn TP, Holland V, Kennett GA, Riley GJ, Wood MD. Novel and selective 5-HT_{2C/2B} receptor antagonists as potential anxiolytic agents: synthesis, quantitative structure-activity relationships, and molecular modeling of substituted 1-(3-pyridylcarbamoyl)indolines. *J Med Chem.* 1998; 41:1598–1612. [PubMed: 9572885]
7. Bubar MJ, Cunningham KA. Distribution of serotonin 5-HT_{2C} receptors in the ventral tegmental area. *Neuroscience.* 2007; 146:286–297. [PubMed: 17367945]
8. Cunningham CL, Gremel CM, Groblewski PA. Drug-induced conditioned place preference and aversion in mice. *Nat Protoc.* 2006; 1:1662–1670. [PubMed: 17487149]
9. Dekeyne A, Mannoury la CC, Gobert A, Brocco M, Lejeune F, Serres F, Sharp T, Daszuta A, Soumier A, Papp M, Rivet JM, Flik G, Cremers TI, Muller O, Lavielle G, Millan MJ. S32006, a novel 5-HT_{2C} receptor antagonist displaying broad-based antidepressant and anxiolytic properties in rodent models. *Psychopharmacology (Berl).* 2008; 199:549–568. [PubMed: 18523738]
10. Di M, Cacchio VM, Di GC, Esposito E. Role of serotonin(2C) receptors in the control of brain dopaminergic function. *Pharmacol Biochem Behav.* 2002; 71:727–734. [PubMed: 11888564]
11. Di M, Di VGG, Di MM, Esposito E. Selective blockade of serotonin_{2C/2B} receptors enhances dopamine release in the rat nucleus accumbens. *Neuropharmacology.* 1998; 37:265–272. [PubMed: 9680252]
12. Di M, Di VGG, Di MM, Esposito E. SB 242084, a selective serotonin_{2C} receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology.* 1999; 38:1195–1205. [PubMed: 10462132]
13. Di M, Di VGG, Di MM, Esposito E. Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin(2C) receptors. *Brain Res.* 2000; 865:85–90. [PubMed: 10814735]
14. Fletcher PJ, Sinyard J, Higgins GA. The effects of the 5-HT(2C) receptor antagonist SB242084 on locomotor activity induced by selective, or mixed, indirect serotonergic and dopaminergic agonists. *Psychopharmacology (Berl).* 2006; 187:515–525. [PubMed: 16832658]
15. Hutson PH, Barton CL, Jay M, Blurton P, Burkamp F, Clarkson R, Bristow LJ. Activation of mesolimbic dopamine function by phencyclidine is enhanced by 5-HT(2C/2B) receptor antagonists: neurochemical and behavioural studies. *Neuropharmacology.* 2000; 39:2318–2328. [PubMed: 10974315]
16. Ji SP, Zhang Y, Van CJ, Jiang W, Liao M, Li L, Wan Q, Backstrom JR, Zhang X. Disruption of PTEN coupling with 5-HT_{2C} receptors suppresses behavioral responses induced by drugs of abuse. *Nat Med.* 2006; 12:324–329. [PubMed: 16474401]
17. Kennedy SH, Rizvi SJ. Emerging drugs for major depressive disorder. *Expert Opin Emerg Drugs.* 2009; 14:439–453. [PubMed: 19637989]
18. Kennedy SH, Rizvi SJ. Agomelatine in the treatment of major depressive disorder: potential for clinical effectiveness. *CNS Drugs.* 2010; 24:479–499. [PubMed: 20192279]
19. Kennett GA, Pittaway K, Blackburn TP. Evidence that 5-HT_{2c} receptor antagonists are anxiolytic in the rat Geller-Seifter model of anxiety. *Psychopharmacology (Berl).* 1994; 114:90–96. [PubMed: 7846211]
20. Leggio GM, Cathala A, Neny M, Rouge-Pont F, Drago F, Piazza PV, Spampinato U. In vivo evidence that constitutive activity of serotonin_{2C} receptors in the medial prefrontal cortex participates in the control of dopamine release in the rat nucleus accumbens: differential effects of inverse agonist versus antagonist. *J Neurochem.* 2009; 111:614–623. [PubMed: 19702657]
21. Martin JR, Ballard TM, Higgins GA. Influence of the 5-HT_{2C} receptor antagonist, SB-242084, in tests of anxiety. *Pharmacol Biochem Behav.* 2002; 71:615–625. [PubMed: 11888553]

22. Meltzer HY, Li Z, Kaneda Y, Ichikawa J. Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2003; 27:1159–1172. [PubMed: 14642974]
23. Muller CP, Carey RJ. Intracellular 5-HT 2C-receptor dephosphorylation: a new target for treating drug addiction. *Trends Pharmacol Sci*. 2006; 27:455–458. [PubMed: 16876260]
24. Sallee FR, Smirnoff AV, Adderall XR: long acting stimulant for single daily dosing. *Expert Rev Neurother*. 2004; 4:927–934. [PubMed: 15853519]
25. Sanders-Bush E, Conn PJ. Effector systems coupled to serotonin receptors in brain: serotonin stimulated phosphoinositide hydrolysis. *Psychopharmacol Bull*. 1986; 22:829–836. [PubMed: 3025911]
26. Schilwein S, Agmo A, Huston JP, Schwarting RK. Intraaccumbens injections of substance P, morphine and amphetamine: effects on conditioned place preference and behavioral activity. *Brain Res*. 1998; 790:185–194. [PubMed: 9593886]
27. Wood MD. Therapeutic potential of 5-HT_{2C} receptor antagonists in the treatment of anxiety disorders. *Curr Drug Targets CNS Neurol Disord*. 2003; 2:383–387. [PubMed: 14683466]

Highlights

- A 5-HT_{2C} antagonist alone did not produce positive conditioned place preference (CPP).
- A selective 5-HT_{2C} antagonist potentiated a low dose amphetamine-induced CPP.
- 5-HT_{2C} antagonists may lack abuse potential, but may potentiate effects of psychostimulants.

Figure 1.

The effect of the 5-HT_{2C} receptor antagonist, CEPC, at two doses (mg/kg) and in combination with *S*-amphetamine (0.5 mg/kg) on conditioned place preference. Data represent means and error bars represent SEM of the difference in the time spent in the drug paired side between the post- and pre-conditioning tests. Each value represents the mean of six rats. Numbers next to conditions indicate dose (mg/kg, i.p.) * $p < 0.01$ compared to vehicle/saline group, # $p < 0.05$ compared to vehicle/0.5 mg/kg *S*-amphetamine.