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Selective serotonin 5-HT$_{2C}$ receptor activation suppresses the reinforcing efficacy of cocaine and sucrose but differentially affects the incentive-salience value of cocaine- vs. sucrose-associated cues

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ABSTRACT

Serotonin (5-HT) controls affective and motivational aspects of palatable food and drug reward and the 5-HT$_{2C}$ receptor (5-HT$_{2C}$R) has emerged as a key regulator in this regard. We have evaluated the efficacy of a selective 5-HT$_{2C}$R agonist, WAY 163909, in cocaine and sucrose self-administration and reinstatement assays employing parallel experimental designs in free-fed rats. WAY 163909 dose-dependently reduced the reinforcing efficacy of cocaine (ID_{50} = 1.19 mg/kg) and sucrose (ID_{50} = 0.7 mg/kg) as well as reinstatement (ID_{50} = 0.5 mg/kg) elicited by exposure to cocaine-associated contextual cues, but not sucrose-associated contextual cues. The ID_{50} of WAY 163909 predicted to decrease the reinforcing efficacy of cocaine or sucrose as well as reinstatement upon exposure to cocaine-associated cues was ~5–12-fold lower than that predicted to suppress horizontal ambulation (ID_{50} = 5.89 mg/kg) and ~2–5-fold lower than that predicted to suppress vertical activity (ID_{50} = 2.3 mg/kg). Thus, selective stimulation of the 5-HT$_{2C}$R decreases the reinforcing efficacy of cocaine and sucrose in freely-fed rats, but differentially alters the incentive-salience value of cocaine- vs. sucrose-associated cues at doses that do not impair locomotor activity. Future research is needed to tease apart the precise contribution of 5-HT$_{2C}$R neurocircuitry in reward and motivation and the learning and memory processes that carry the encoding for associations between environmental cues and consumption of rewarding stimuli. A more complete preclinical evaluation of these questions will ultimately allow educated proof-of-concept trials to test the efficacy of selective 5-HT$_{2C}$R agonists as adjunctive therapy in chronic health maladies including obesity, eating disorders and drug addiction.

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1. Introduction

Survival is ensured by neural circuits engaged by basic needs (*e.g.*, food, water) and states (*e.g.*, hunger, thirst) that motivate the organism to sustain important biological functions. Brain motivational systems are sensitive to endogenous and exogenous stimuli that become linked to natural and drug rewards, and neuroplasticity in these circuits engender affective states that powerfully drive such behaviors as bingeing on palatable food (*e.g.*, fat, sucrose) or abused drugs (*e.g.*, cocaine). A complex appetitive process links the motivation to consume (*e.g.*, hunger) with the palatability and incentive salience of the rewarding stimulus (drug or food) and its associated conditioned cue properties as well as with the satiety signals that terminate further intake (Halford and Harrold, 2008). Studies over the last 20 years have identified cellular and behavioral mechanisms of these appetitive processes to include dopamine, glutamate, and their intracellular signaling webs in the limbic–corticostriatal–hypothalamic circuit (Kelley, 2004). Serotonin (5-HT) is additionally important in the control over the affective and motivational aspects of palatable food and

Abbreviations: 5-HT, serotonin; 5-HT$_{2x}$R, 5-HT$_{2x}$ receptor; CHO, Chinese Hamster Ovary; FR, fixed ratio; i.p., intraperitoneal; Veh, vehicle; WAY, WAY 163909.

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drug reward, which has been described to occur at the level of satiety (Hewitt et al., 2002; Lyness et al., 1980; Blundell et al., 1980) as well as palatability or reinforcing efficacy (Wogar et al., 1991). In particular, there is a growing literature based upon genetic and pharmacological manipulations that the serotonin 5-HT_{2C} receptor (5-HT_{2C}R) signaling regulates such neurobehavioral processes which may underlie important chronic health maladies including obesity, eating disorders and drug addiction (Halford et al., 2010; Bubar and Cunningham, 2008; Steiger, 2004).

The selective activation of signaling through the 5-HT_{2C}R has been associated with reduced feeding and decreased body weight in animals (Fletcher et al., 2009; Clifton et al., 2000) and most recently in humans (Smith et al., 2009, 2010). The constitutive knockout of the 5-HT_{2C}R in mice results in hyperphagia (Tecott et al., 1995) the phenotype also includes adult-onset obesity and depressed metabolic rates (Tecott et al., 1995). Selective 5-HT_{2C}R antagonists have been shown to increase baseline food intake (Bonhaus et al., 1997; Thomsen et al., 2008) (but see, Hewitt et al., 2002) in the same dose range shown to block the effects of a 5-HT_{2C}R selective agonist to decrease feeding (Thomsen et al., 2008; Grottick et al., 2000). Analogous to these findings with palatable food reward, rates for responding for cocaine in a self-administration task were suppressed by pretreatment with a preferential 5-HT_{2C}R agonist and enhanced by a selective 5-HT_{2C}R antagonist (Fletcher et al., 2008; Grottick et al., 2000), suggesting an important role for the 5-HT_{2C}R in control of the overt rewarding efficacy of cocaine. A preferential 5-HT_{2C}R agonist also suppressed intake of nicotine (Grottick et al., 2001) and alcohol (Tomkins et al., 2002). The mechanisms are complex but a role for the 5-HT_{2C}R to enhance satiety and/or suppress incentive-motivational aspects of appetitive food or drug reinforcers are both supported (Fletcher et al., 2010; Burbassi and Cervo, 2008; Rocha et al., 2002; Vickers et al., 1999; Hewitt et al., 2002).

There is much less known about the role of serotonin in general, and the 5-HT_{2C}R in specific, to regulate drug- or food-seeking behavior upon exposure to environmental stimuli previously associated with drug or palatable food, respectively. Loss of 5-HT neurons after intraventricular infusion of a 5-HT neurotoxin decreased cocaine-seeking but enhanced sucrose-seeking during extinction in freely-fed rats previously trained to self-administer cocaine or sucrose, respectively (Tran-Nguyen et al., 2001). The selective 5-HT reuptake inhibitor fluoxetine was shown to suppress palatable food intake most potently in freely-fed female rats pre-exposed to food cues (Cifani et al., 2009). The preferential 5-HT_{2C}R agonists MK 212 (Neisewander and Acosta, 2007) and Ro 60-0175 (Fletcher et al., 2008) have been shown to suppress cue-evoked reinstatement in cocaine self-administration. However, the interpretation of findings across studies and the development of an overarching appreciation of how 5-HT_{2C}R neurocircuitry controls drug or palatable food intake or "relapse" in the face of reward-related cues are hampered by two issues. The first challenge is that selective agonists with singular high affinity and efficacy for the 5-HT_{2C}R have only recently become commercially available. The second challenge is that few studies have established the sensitivity of palatable reward vs. reward-related cues to a specific manipulation of 5-HT_{2C}R function employing comparable assay methodologies in a given species [for a recent review of these methodologies, see (Nair et al., 2009)].

The 5-HT_{2C} receptor (5-HT_{2C}R) shares high homology with the two other members (5-HT_{2A}R, 5-HT_{2B}R) of the 5-HT_2R family of G-protein-coupled receptors. Until recently, only "preferential" 5-HT_{2C}R ligands which frequently display affinity (agonists, antagonist) and/or efficacy (agonists) at the 5-HT_{2A}R and 5-HT_{2B}R have been available and some experimental outcomes with non-selective 5-HT_{2C}R ligands have led to ambiguous conclusions concerning the biological roles for this receptor. Furthermore, as 5-HT_{2A}R or 5-HT_{2B}R agonists would be expected to evoke hallucinations (Nichols, 2004) or cardiac valvulopathy (Fitzgerald et al., 2000; Roth, 2007), respectively, therapeutically-useful 5-HT_{2C}R agonists must not have demonstrable efficacy at 5-HT_{2A}R or 5-HT_{2B}R *in vivo*. Such selectivity has been difficult to achieve, providing challenges to the careful preparation of preclinical analyses in support of ultimate proof-of-concept studies of selective 5-HT_{2C}R agonists in humans for treatment of obesity, eating disorders or addiction.

In a compound series recently developed at Wyeth Research, vabicaserin (SCA-136) was identified as a selective 5-HT_{2C}R full agonist (K_i = 3 nM; efficacy 100% relative to 5-HT), a 5-HT_{2B}R antagonist (IC_{50} = 29 nM) and a very weak 5-HT_{2A}R antagonist (IC_{50} = 1650 nM) (Rosenzweig-Lipson et al., 2007a; Tong et al., 2010a; Tong et al., 2010b). Vabicaserin has been in clinical trials to evaluate antipsychotic potential (www.clinicaltrials.gov). WAY 163909 is chemically-similar to vabicaserin with high affinity (K_i = 10.5 nM) and full efficacy (90% relative to 5-HT) at the 5-HT_{2C}R. WAY 163909 exhibits a low affinity (K_i = 212 nM) at the 5-HT_{2A}R, and no efficacy and is a weak partial agonist at the 5-HT_{2B}R at concentrations 23-fold greater (Dunlop et al., 2005). In the present study, we have evaluated the efficacy of WAY 163909 to suppress the reinforcing effects of the abused psychostimulant cocaine and the palatable food sucrose as well as cue-evoked reinstatement in parallel experimental designs in freely-fed rats. To date, the only other preferential 5-HT_{2C}R agonists to be profiled in a somewhat similar fashion are MK 212 and Ro 60-0175; Table 1 provides a comparison of affinity and efficacy at the three 5-HT_2R for MK 212, Ro 60-0175 and WAY 163909.

The present study investigated the hypothesis that WAY 163909 would suppress cocaine or sucrose self-administration or cue-evoked reinstatement in freely-fed male rats under conditions that minimized potential confounds imposed by food (or water) restriction [see (Grottick et al., 2000; Fletcher et al., 2002; Nic Dhonnchadha et al., 2009), for discussion] and operant pretraining for an appetitive reinforcer (Grottick et al., 2000; Fletcher et al., 2002; Nic Dhonnchadha et al., 2009). The doses of cocaine for reliable self-administration and reinstatement are well-established

Table 1
Affinity and efficacy of MK 212, Ro 60-0175 and WAY 163909 for 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R

Agonist	5-HT_2 Receptor Family[a]					
	h5-HT_{2A}R		h5-HT_{2B}R		h5-HT_{2C}R	
	Affinity K_i (nM)	Efficacy E_{max}	Affinity K_i (nM)	Efficacy E_{max}	Affinity K_i (nM)	Efficacy E_{max}
MK 212	1023	<50%	617	75%	98	100%
Ro 60-0175	36.3	69%	5.4	79%	6.0	84%
WAY 163909	212	0%	2101	40%	10.5	90%

[a] Published studies employed radioligand binding assays to establish the affinity (K_i) and efficacy (E_{max}) of MK 212 (Knight et al., 2004; Cussac et al., 2002; Porter et al., 1999) and Ro 60-0175 for the 5-HT_2R subtypes in h5-HT_{2A}R- or h5-HT_{2C}R-transfected Human Embryonic Kidney (HEK)-293 (Knight et al., 2004) and h5-HT_{2A}R-, h5-HT_{2B}R-,or h5-HT_{2C}R-transfected Chinese Hamster Ovary (CHO)-K1 clonal cell lines (Porter et al., 1999; Cussac et al., 2002) while the affinity of WAY 163909 for each h5-HT_2R subtype was established in CHO-K1 cells (Dunlop et al., 2005). The efficacy of MK 212 (Porter et al., 1999), Ro 60-0175 (Porter et al., 1999) and WAY 163909 (Dunlop et al., 2005) was established in agonist-stimulated mobilization of intracellular calcium in CHO-K1 cells with a fluorometric imaging plate reader; the E_{max} is expressed as a percentage of the maximal response to 5-HT. MK 212 did not exhibit efficacy (IC_{50} > 1 μM) for the 5-HT_{2A}R (Knight et al., 2004). Ro 60-0175 did not exhibit efficacy (IC_{50} > 1 μM) for a multitude of proteins with the highest affinity for β_2 adrenoceptor (β_2-noradrenergic receptor; IC_{50} = 251; Martin et al., 1998). WAY 163909 exhibited modest affinity for the dopamine D_4R (K_i = 245 nM), 5-HT_7R (K_i = 343 nM) and $\alpha_1$adrenoceptor (K_i = 665 nM; Dunlop et al., 2005).