Sigma Receptor Agonists: Receptor Binding and Effects on Mesolimbic Dopamine Neurotransmission Assessed by Microdialysis

Linda Garcés-Ramírez, Jennifer L. Green, Takato Hiranita, Theresa A. Kopajtic, Maddalena Mereu, Alexandra M. Thomas, Christophe Mesangeau, Sanju Narayanan, Christopher R. McCurdy, Jonathan L. Katz, and Gianluigi Tanda

Background: Subtypes of sigma (σ) receptors, σ_1 and σ_2, can be pharmacologically distinguished, and each may be involved in substance-abuse disorders. σ-Receptor antagonists block cocaine place conditioning and σ-receptor agonists are self-administered in rats that previously self-administered cocaine. Self-administration of abused drugs has been related to increased dopamine (DA) neurotransmission, however, σ-receptor agonist effects on mesolimbic DA are not fully characterized.

Methods: Receptor-binding studies assessed affinities of σ-receptor ligands for σ-receptor subtypes and the DA transporter; effects on DA transmission in the rat nucleus accumbens shell were assessed using in vivo microdialysis.

Results: Cocaine (.1–1.0 mg/kg intravenous [IV]), the nonselective $\sigma_{1/2}$-receptor agonist DTG (1.0–5.6 mg/kg IV), and the selective σ_1-receptor agonist PRE-084 (.32–10 mg/kg IV) dose-dependently increased DA to ~275%, ~150%, and ~160% maxima, respectively. DTG-induced stimulation of DA was antagonized by the nonselective $\sigma_{1/2}$-receptor antagonist BD 1008 (10 mg/kg intraperitoneal [IP]) and the preferential σ_2-receptor antagonist SN 79 (1–3 mg/kg IP), but not by the preferential σ_1-receptor antagonist, BD 1063 (10–30 mg/kg IP). Neither PRE-084 nor cocaine was antagonized by BD 1063 or BD 1008.

Conclusions: σ-Receptor agonists stimulated DA in a brain area critical for reinforcing effects of cocaine. DTG effects on DA appear to be mediated by σ_2-receptors rather than σ_1-receptors. However, DA stimulation by cocaine or PRE-084 does not likely involve σ-receptors. The relatively low potency on DA transmission of the selective σ_1-receptor agonist, PRE-084, and its previously reported potent reinforcing effects, suggest a dopamine-independent reinforcing pathway that may contribute to substance-abuse disorders.

Key Words: Abuse liability, addiction, cocaine, dopamine microdialysis, nucleus accumbens, reinforcing effects, sigma receptors

Sigma (σ) receptors, initially proposed as opioid (1) and later phencyclidine receptors (2), were demonstrated to represent a unique binding site in the mammalian brain and peripheral organs (3–5) and are expressed throughout the central nervous system. The σ-receptor system has been implicated in a variety of physiological functions and disease states (6).

Pharmacological and structural studies distinguished two receptor subtypes: sigma 1 (σ_1) and sigma 2 (σ_2) (5,7). The σ_1-receptor was cloned and characterized as a 29 kDa single polypeptide having no homology with any other known mammalian proteins (8). In contrast, the σ_2-receptor is an 18 to 21 kDa protein that has not yet been cloned (9). The σ_1-receptors possess two transmembrane domains and have been localized subcellularly at the endoplasmic reticulum. It has been suggested that, after stimulation by σ ligands, σ_1-receptors translocate to the plasma membrane (10).

Several classes of compounds, including neurosteroids, neuroleptics, dextrobenzomorphans, and psychostimulants, such as methamphetamine and cocaine, have been shown to bind to σ-receptors (11,12). The psychomotor stimulant and reinforcing effects of cocaine are primarily mediated through stimulation of dopamine (DA) neurotransmission by inhibiting DA reuptake. However, its moderate affinity for σ-receptors (13–15) suggests that cocaine-induced effects could involve actions at σ_1-receptor and/or σ_2-receptor subtypes (16). Consequently, studies have examined the effects of σ-receptor ligands on various effects of cocaine related to its abuse (see reviews [11,17,18]). For example, σ-receptor antagonists blocked the locomotor-stimulant effects of cocaine (19–22) and the development of cocaine-induced locomotor sensitization (22,23). Further, σ-receptor antagonists attenuated cocaine-induced place conditioning (24), though these compounds failed to substantially alter cocaine self-administration (25,26), indicating that σ-receptors are not directly involved in the reinforcing effects of cocaine. However, the preferential σ_1-receptor antagonist N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino)ethylamine (BD 1047) attenuated the reinstatement of cocaine-reinforced behavior induced by cocaine-related stimuli, suggesting that σ-receptors might be involved in brain mechanisms and behavioral activities that trigger cocaine use. Finally, several σ-receptor antagonists can block or attenuate acute cocaine-induced toxicities, such as convulsions and lethality (13). Together, these studies suggest that σ_1-receptors participate in the mechanisms of action of cocaine and that σ-receptor antagonists might be potentially useful as medications for treating cocaine abuse, dependence, and overdose (24,27).

From the Psychobiology Section (LG-R, JLG, TH, TAK, MM, AMT, JLK, GT), Medication Discovery Research Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services; and Japanese Society for the Promotion of Science (TH), Research Fellowship for Japanese Biomedical and Behavioral Researchers at National Institutes of Health, Baltimore, Maryland; Escuela Nacional de Ciencias Biológicas (LG-R), Instituto Politécnico Nacional, Mexico City, México; and Department of Medicinal Chemistry (CM, SN, CRM), University of Mississippi, Oxford, Mississippi.

Address correspondence to Gianluigi Tanda, Ph.D., National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Intramural Research Program, Psychobiology Section, Medications Discovery Research Branch, Building C, Room 321, 5500 Nathan Shock Drive, Baltimore, MD 21224; E-mail: gtanda@intra.nida.nih.gov.

Received Mar 24, 2010; revised Jul 26, 2010; accepted Jul 28, 2010.

Recently, Hiranita *et al.* (26) showed reinforcing effects of σ-receptor agonists in rats trained to self-administer cocaine. Reinforcing effects of most drugs of abuse appear to be mediated by stimulation of DA transmission, and it has been repeatedly shown that the acute effects of drugs abused by humans at doses able to maintain self-administration in rodents preferentially or selectively increase DA transmission in the nucleus accumbens (NAc) shell compared with the NAc core or dorsal striatum (28–30). Increases in DA transmission induced by nonselective σ-receptor agonists have been reported (31,32), though contrasting results and sometimes biphasic effects have been reported after systemic or local intrastriatal administration (33–36). Thus, the goal of the present study was to test the hypothesis that intravenous (IV) administration of σ-receptor agonists would increase DA transmission in the NAc shell. To this end, rats were implanted with microdialysis probes in the NAc shell and effects of various IV doses of 1,3-di(2tolyl)guanidine (DTG), a nonselective σ-receptor agonist, and 2-(4-morpholinoethyl)-1-phenylcyclohexane-1-carboxylate (PRE-084), a selective σ_1-receptor agonist, on extracellular DA levels were compared with the effects of IV cocaine. In addition, the effects of the σ-receptor agonists as well as cocaine were studied in combination with σ-receptor antagonists to confirm the pharmacological specificity of the effect. The σ-receptor ligands were also studied in binding experiments to test their affinities for σ_1-receptor and σ_2-receptor subtypes, as well as the DA transporter (DAT), the main pharmacological target of cocaine.

Methods and Materials

σ_1 and σ_2 Receptor Binding

Frozen whole guinea pig brains (minus cerebellum) were used and processed as already published (37) (see also Supplement 1 for complete details). The guinea pig brains were preferred over rat brains due to their use as a standard for σ-receptor binding because of the relatively higher density of those receptors in that tissue compared with the rat (38). Ligand binding experiments were conducted for σ_1-receptor studies with 3 nmol/L [^{3}H](+)-pentazocine (specific activity 28 Ci/mmol) and 8.0 mg tissue. Nonspecific binding was determined using 10 μmol/L haloperidol. For σ_2-receptor studies, each tube contained 3 nmol/L [^{3}H]1,3-di(2tolyl)guanidine ([^{3}H]DTG) (specific activity 48 Ci/mmol), 200 nmol/L (+)-pentazocine, and 8.0 mg tissue. Nonspecific binding was determined using 100 μmol/L haloperidol.

DAT Binding

Brains from male Sprague-Dawley rats weighing 200 g to 225 g (Taconic Laboratories, Germantown, New York) were used, and experiments were carried out as published previously (39). Binding was assessed with .5 nmol/L [^{3}H](-)-2β-Carbomethoxy-3β-(4-fluorophenyl)tropane, [^{3}H]WIN 35,428 (specific activity 84 Ci/mmol) and 1.0 mg striatal tissue. Nonspecific binding was determined using .1 mmol/L (-)-cocaine hydrochloride.

In Vivo Microdialysis Studies

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Massachusetts) weighing 275 g to 350 g served as subjects, and all methods were as described previously (30,40–42) (see details in Supplement 1). Rats were doubly housed in a temperature- and humidity-controlled room maintained on a 12-hour light/dark cycle (lights on: 07:00–19:00 hours) and had free access to food and water. Experiments were conducted during the light phase.

Surgical procedures were conducted under a mixture of ketamine and xylazine (60.0 and 12.0 mg/kg intraperitoneal [IP], respectively) anesthesia. Rats were first implanted with a silastic catheter into the external jugular vein, with the catheter exiting the skin at the back between the shoulders. Rats were then placed in a stereotaxic apparatus and implanted with concentric dialysis probes aimed at the NAc shell (uncorrected coordinates from the rat brain atlas of Paxinos and Watson [43]: anterior = +2.0 mm from bregma, lateral = ±1.0 mm from bregma, vertical = −7.9 mm from dura). The histological confirmations of probe placement are included in Supplement 1.

Experiments were performed on freely moving rats, about 22 to 24 hours after probe implant. Dialysate was sampled every 10 minutes and immediately analyzed. After stable DA values (less than 10% variability) were obtained for at least three consecutive samples (after about 1–2 hours), rats were treated with one dose of one of the test drugs (cocaine, DTG, PRE-084, or saline) or with an antagonist (N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl)ethylamine dihydrobromide [BD 1008], N-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine dihydrochloride [BD 1063], or 6-acetyl-3-(4-(4-(4-fluorophenyl)piperazin-1-yl)butyl) benzo[d]oxazol-2(3H)-one di-hydrochloride [SN 79]) followed 30 minutes (or 10 minutes for SN 79) later by saline or one of the test drugs. Different groups of naive rats were used for each different treatment. In another series of experiments in different groups of naive rats, after obtaining stable DA levels, microdialysis probes were perfused with calcium-free Ringer's solution (147.0 mmol/L NaCl, and 4.0 mmol/L KCl) for 60 minutes before IV injections of DTG or PRE-084 (44,45).

DA was detected in dialysate samples by high-performance liquid chromatography coupled with a coulometric detector (5200a Coulochem II, or Coulochem III, ESA, Chelmsford, Massachusetts).

Table 1. Affinities of Various σ-Receptor Ligands and Cocaine on Binding to σ_1-Receptor and σ_2-Receptor Subtypes and to the DAT as Labeled with [^{3}H](+)-Pentazocine, [^{3}H]DTG, and [^{3}H]WIN 35428, Respectively

Compound	σ_1 K_i Value (nmol/L)	σ_2 K_i Value (nmol/L)	σ_2/σ_1	DAT K_i Value (nmol/L)
DTG[a]	57.4 (49.3–66.7)	21.9 (14.8–32.4) 3520 (257–48,200)	.382	93,500 (80,000–109,000)
PRE-084	53.2 (44.8–63.2)	32,100 (23,100–44,700)	603	19,600 (17,600–21,900)
BD 1008	2.13 (1.77–2.56)	16.6 (13.0–21.1) 20,500 (9640–43,500)	7.79	2510 (2250–2790)
BD 1047	3.13 (2.68–3.65)	47.5 (36.7–61.4) 55,300 (25,000–122,000)	15.2	3220 (2820–3670)
BD 1063	8.81 (7.15–10.9)	625 (447–877) 53,700 (16,500–174,000)	70.9	8020 (7100–9060)
Cocaine	5190 (3800–7060)	19,300 (16,000–23,300)	3.72	76.6 (72.6–80.5)

σ1, sigma 1; σ2, sigma 2.
[a]Values for σ_2-receptors are K_d values obtained by homologous competition experiments.