Preference for Distinct Functional Conformations of the Dopamine Transporter Alters the Relationship between Subjective Effects of Cocaine and Stimulation of Mesolimbic Dopamine

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Background: Subjective effects of cocaine are mediated primarily by dopamine (DA) transporter (DAT) blockade. The present study assessed the hypothesis that different DAT conformational equilibria regulate differences in cocaine-like subjective effects and extracellular DA induced by diverse DA-uptake inhibitors (DUIs).

Methods: The relationship between cocaine-like subjective effects and stimulation of mesolimbic DA levels by standard DUIs (cocaine, methylphenidate, WIN35,428) and atypical DUIs (benztropine analogs: AHN1-055, AHN2-005, JHW007) was investigated using cocaine discrimination and DA microdialysis procedures in rats.

Results: All drugs stimulated DA levels with different maxima and time courses. Standard DUIs, which preferentially bind outward-facing DAT conformations, fully substituted for cocaine, consistently producing cocaine-like subjective effects at DA levels of 100–125% over basal values, regardless of dose or pretreatment time. The atypical DUIs, with DAT binding minimally affected by DAT conformation, produced inconsistent cocaine-like subjective effects. Full effects were obtained, if at all, only at a few doses and pretreatment times and at DA levels 600–700% greater than basal values. Importantly, the linear, time-independent, relationship between cocaine-like subjective effects and DA stimulation obtained with standard DUIs was not obtained with the atypical DUIs.

Conclusions: These results suggest a time-related desensitization process underlying the reduced cocaine subjective effects of atypical DUIs that may be differentially induced by the binding modalities identified using molecular approaches. Since the DAT is the target of several drugs for treating neuropsychiatric disorders, such as attention-deficit/hyperactivity disorder, these results help to identify safe and effective medications with minimal cocaine-like subjective effects that contribute to abuse liability.

Key Words: ADHD, benztropine analogs, cocaine discrimination, dopamine microdialysis, drug abuse and addiction, nucleus accumbens shell

Most illicit drugs produce effects described as euphoric, and these effects are assumed to underlie substance abuse disorders (1). The study of these subjective effects in the laboratory is often accomplished with drug discrimination procedures, and several previous studies have indicated that the discriminative stimulus effects of drugs are closely related to their subjective effects in humans (2,3). The subjective, reinforcing, and discriminative stimulus effects of psychomotor stimulants in humans and laboratory animals are thought to involve increased dopamine (DA) transmission in the mesolimbic system (4–8). In human subjects, a relationship between the subjective effects of cocaine and DA transporter (DAT) occupancy in striatal areas has been demonstrated (9). In animals, several studies have demonstrated a relationship between the reinforcing and discriminative stimulus effects of stimulants and their binding to the DAT (7,8,10–12), and these effects are thought to involve increased DA transmission in the nucleus accumbens (4–8).

Certain structural classes of DA uptake inhibitors (DUIs), including benztropine (BZT) analogs (13), are atypical with regard to their DAT binding and are less efficacious than cocaine in producing many of its characteristic effects (14–17). Standard DUIs, such as cocaine, bind more potently to the DAT when it is in a conformation open to the extracellular space compared with a conformation open to the cytosol. In contrast, atypical DUIs are more tolerant to DAT conformational status; potency of these drugs is decreased less compared with cocaine with the DAT in an inward, cytosol-facing conformation (13). Further, it has been suggested that differences in the inward/outward conformational equilibrium of the DAT might underlie the slower DAT association and dissociation rates for atypical DUIs (13), which could contribute to their atypical effects (17,18).

The present study explored the possibility that a preference for binding the outward-facing versus inward-facing DAT conformation could influence the potential of DUIs to produce cocaine-like subjective effects. We assessed the cocaine-like discriminative

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stimulus effects of three cocaine-like DUIs: cocaine, its analog WIN35,428, and methylphenidate (a Food and Drug Administration approved medication for treatment of attention-deficit/hyperactivity disorder [ADHD]) (19). Each of these compounds has been shown to preferentially bind the outward-facing conformation of the DAT (13,20,21). Additionally, three atypical DUIs (17), analogs of BZT (22,23), AHN1-055, AHN2-005 (a potential ADHD medication) (24,25), and JHW007, were assessed. These compounds are relatively insensitive to a change in DAT conformation (13,20). We compared these drugs across a wide range of doses and times after injection so that their effects could be compared under conditions in which the drugs similarly stimulated DA levels in the nucleus accumbens shell (NAS), assessed by brain microdialysis (26–29), as previous studies have implicated this area as critically involved in the discriminative stimulus effects of stimulant drugs (4,5,8).

Methods and Materials

Subjects

Experimentally naïve male Sprague-Dawley rats, 275 g to 350 g, were habituated for at least 1 week before experiments and maintained in an environmentally controlled vivarium. Experiments were conducted during the light phase of a 12-hour cycle (see Supplement 1 for detailed procedures).

In Vivo Brain Microdialysis

Probes had an active dialyzing surface of 1.8 mm to 2.0 mm and were implanted during surgical procedures [uncorrected coordinates (30): anterior = +2.0 mm and lateral = ±1.0 mm from bregma; vertical = −7.9 mm from dura (see Figure S1 in Supplement 1 for probe placements)] under a mixture of ketamine and xylazine anesthesia, 60.0 and 12.0 mg/kg intraperitoneally (IP), respectively, as described previously (31–33).

Experiments were performed on freely moving rats approximately 22 to 24 hours after probe implant. Dialysates were sampled every 10 minutes and immediately analyzed. After reaching stable DA values (three consecutive samples, <10% variability), rats were treated with drugs. Cerebrospinal fluid (CSF) (10 μL) was sampled every 10 minutes for the first 2 hours and every 20 minutes thereafter for 4 hours, after which CSF was sampled every 30 minutes. Because some BZT analogs had long-lasting effects, CSF was sampled as necessary up to 27 hours after drug or saline injection.

Dopamine was detected in dialysate samples by high-performance liquid chromatography coupled with a coulometric detector (5200a Coulochem II or III; ESA, Chelmsford, Massachusetts). The average basal DA values in dialysates in the present experiments were 51.5 ± 3.1 fmol (±SEM) in a 10 μL sample, $n = 161$. No significant differences were found in basal DA concentrations from the different experimental groups (analysis of variance [ANOVA], $F_{29,131} = 1.066$, $p = .39$).

Drug-Discrimination Studies

Rats were trained during daily sessions in operant conditioning chambers to press one lever after cocaine (10 mg/kg, IP) and the other after saline (IP) injection, both administered 5 minutes before the session. The 20th consecutive response produced a food pellet (fixed ratio [FR]), and the right versus left assignment of cocaine- and saline-associated levers was counterbalanced among subjects.

Sessions started at various times after injection with a 5-minute time-out during which responses had no consequences. The time-out was followed by illumination of chamber lights until the completion of the FR requirement and delivery of a food pellet. A 20-second time-out followed each food pellet, and sessions ended after 20 food presentations or 15 minutes, whichever comes first. Cocaine or saline training sessions were scheduled in a mixed sequence and continued until subjects met the criteria of four consecutive sessions with >85% cocaine-appropriate or saline-appropriate responses during the entire session and the first FR. After meeting these criteria, testing began with different doses of cocaine, standard DUIs (methylphenidate, WIN35,428), or atypical DUIs (BZT analogs: AHN1-055, AHN2-005, JHW007) each administered at various times before sessions. Test sessions were identical to training sessions with the exception that completion of the FR requirement on either key was reinforced.

Drugs

The drugs tested were (-)-cocaine HCl (1–30 mg/kg), methylphenidate (.3–17 mg/kg), WIN35,428 (.03–3 mg/kg), AHN1-055 (.3–10 mg/kg), AHN2-005 (1–30 mg/kg), and JHW007 [synthesized as per Agoston *et al.* (22) and Newman *et al.* (23), 1–17 mg/kg]. Drugs were dissolved in .9% sodium chloride or sterile water with heat and sonication, as necessary, and were injected IP (1.0 mL/kg of body weight).

Data Analyses

Microdialysis results were expressed as a percentage of basal DA values. Statistical analysis was carried out using one-way or two-way ANOVA (factors: time, dose) for repeated measures over time with significant results subjected to post hoc Tukey's test. For drug discrimination, percentages of cocaine lever responses were calculated by dividing the total responses on the cocaine-associated lever by total number of responses on both levers. Full substitution was defined as >85% and partial substitution was defined as between 85% and 15% cocaine-appropriate responding. Dose-effect curves for percent cocaine lever responding were analyzed using standard linear regression techniques, from which effective dose 50 (ED50) values with 95% confidence limits were calculated (34).

To examine the relation between levels of extracellular DA and cocaine-like discriminative stimulus effects, linear regression analyses were performed on the percent cocaine-lever responding as a function of the percent change in extracellular DA. The percentage of basal DA levels were calculated from levels in 10-minute bins during the time period most closely corresponding to the duration of the drug discrimination sessions (15 minutes plus the 5-minute presession time-out) for each subject. Data from 0% to the lowest dose producing ≥85% cocaine-lever responding were regressed on percent change in DA levels and the slopes of the best fit lines were compared across pretreatment times for each drug and between cocaine and each of the drugs using the extra sum of squares F-test. Further details are presented in Supplement 1.

Results

Microdialysis Studies

Effects of the Standard DUIs. Cocaine (1.0–30 mg/kg, IP) dose-dependently and significantly stimulated DA levels in dialysates from the NAS (Figure 1A). Two-way ANOVA indicated significant ($p < .001$) main effects of dose ($F_{5,31} = 38.0$), time ($F_{18,558} = 38.6$), and their interaction ($F_{90,558} = 11.0$). At the