Behavioral and Neurochemical Effects of the Dopamine Transporter Ligand 4-Chlorobenztpine Alone and in Combination with Cocaine In Vivo

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ABSTRACT

The current studies evaluated the novel diphenylmethoxytropane analog 4-chlorobenztpine (4-Cl-BZT), cocaine, and combinations of the two drugs for their abilities to stimulate locomotor activity, produce cocaine-like discriminative stimulus effects, and elevate extracellular dopamine (DA) in the nucleus accumbens (NAc) as measured by in vivo microdialysis. Peripherally administered cocaine was approximately twice as efficacious as 4-Cl-BZT as a locomotor stimulant and was behaviorally active at a lower dose than was 4-Cl-BZT. Cocaine also was more efficacious than 4-Cl-BZT in producing discriminative-stimulus effects in rats trained to discriminate i.p. injections of 10 mg/kg cocaine from saline. The time course of behavioral activation differed markedly between the two drugs, with much shorter onset and duration of locomotor stimulant effects for cocaine relative to 4-Cl-BZT. Similarly, i.p. cocaine (10 and 40 mg/kg) induced a pronounced, rapid, and short-lived increase in DA in the NAc, whereas i.p. 4-Cl-BZT was effective only at the higher dose and produced a more gradual, modest, and sustained (>2 h) elevation in accumbens DA. In contrast to i.p. administration, local infusion of 4-Cl-BZT (1–100 μM) into the NAc through the microdialysis probe elevated extracellular DA to a much greater extent than did local cocaine (nearly 2000% of baseline maximally for 4-Cl-BZT versus 400% of baseline for cocaine) and displayed a much longer duration of action than cocaine. However, when microinjected bilaterally into the NAc at 30 or 300 nmol/side, cocaine remained a more efficacious locomotor stimulant than 4-Cl-BZT. Finally, pretreatment with i.p. 4-Cl-BZT dose dependently enhanced the locomotor stimulant, discriminative stimulus effects, and NAc DA response to a subsequent low-dose i.p. cocaine challenge. The diphenylmethoxytropane analog also facilitated the emergence of stereotyped behavior and convulsions induced by high-dose cocaine. The current results demonstrate that DA transporter ligands that do not share the neurochemical and behavioral profiles of cocaine nevertheless may enhance the effects of cocaine in vivo.

Cocaine abuse remains a significant public health problem in the United States. At present there are no effective medications for the treatment of cocaine dependence. However, growing understanding of the neurobiological mechanisms of cocaine’s behavioral and subjective effects may facilitate the development of a successful pharmacotherapy for cocaine addiction (Johnson and Vocci, 1993; Rothman and Glowa, 1995). Considerable evidence has implicated mesolimbic dopamine (DA) neurotransmission in the psychomotor stimulant, reinforcing, and subjective effects of cocaine (Wise, 1984; Johanson and Fischman, 1989; Woolverton and Johnson, 1992). By binding to a site on the DA transporter to inhibit the neuronal reuptake of DA (Kennedy and Hanbauer, 1983; Reith et al., 1986; Madras et al., 1989; Carroll et al., 1992), cocaine elevates synaptic DA levels in both terminal field regions (nucleus accumbens (NAc) and prefrontal cortex) and cell body regions (ventral tegmental area) of mesolimbic DA neurons (Di Chiara and Imperato, 1988; Bradberry and Roth, 1989; Hurd and Ungerstedt, 1989; Mogaddam and Bunney, 1989). This action, particularly in the NAc, is thought to underlie acute cocaine-induced locomotor stimulation (Roberts et al., 1975; Kelly and Iversen, 1976; Di Chiara and Imperato, 1988; Giros et al., 1996) and cocaine self-administration in animals (Roberts et al., 1977; Wise, 1984; Ritz et al., 1987). In addition, recent studies have

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ABBREVIATIONS: 4-Cl-BZT, 4-chlorobenztpine (4’-Chloro-3α-(diphenylmethoxy)tropane); aCSF, artificial cerebrospinal fluid; DA, dopamine; NAc, nucleus accumbens.
associated DA transporter occupancy (Volkow et al., 1997) and dopaminergic activity (Breiter et al., 1997) with the euphorigenic and other subjective effects of cocaine in humans. Because cocaine’s action at the DA transporter may be central to its abuse liability, modulation of cocaine binding to the transporter represents one potentially important strategy in the development of a medication for cocaine dependence (Rothman, 1990; Witkin, 1994; Rothman and Glowa, 1995).

To this end, Rothman and colleagues have proposed the use of high-affinity DA uptake inhibitors as cocaine antagonists or substitution agents in human cocaine abusers (Rothman, 1990; Rothman et al., 1991; Baumann et al., 1994; Rothman and Glowa, 1995). Although the DA uptake inhibitors methylphenidate (Gawin et al., 1985) and mazindol (Preston et al., 1993; Stine et al., 1995) are of no reliable therapeutic benefit in cocaine addicts, recent preclinical studies suggest that the selective DA transporter inhibitor GBR 12909 can modulate the effects of cocaine on mesolimbic DA transmission in the rat in vivo (Rothman et al., 1991; Baumann et al., 1994; but see Gifford et al., 1993) and GBR 12909 or its derivatives can attenuate or delay cocaine self-administration in rats and primates (Glowa et al., 1996; Tella et al., 1996). Like most compounds that inhibit DA uptake with high affinity in vitro, GBR 12909 exhibits a cocaine-like behavioral profile in animals when administered alone (Heikkila and Manzino, 1984; Bergman et al., 1989; Spealman et al., 1989). Thus, it is perhaps not surprising that the closely related analog GBR 12935 can potentiate other effects of cocaine, including the locomotor stimulant, discriminative stimulus, and convulsive effects in rodents (Acri et al., 1996).

In contrast to cocaine, GBR 12909, and most other drugs that inhibit DA uptake with high affinity in vitro, recent studies of benzotropine and structurally related phenyltropane analogs have identified at least one compound that binds to the DA transporter with high affinity in vitro but lacks cocaine-like behavioral effects in vivo (Newman et al., 1994, 1995; Acri et al., 1996; Kline et al., 1997). More potent at the DA transporter in vitro than cocaine (Newman et al., 1994) or its parent compound benzotropine (van der Zee and Hespe, 1978; van der Zee et al., 1980), the 4’-substituted analog 4'-chloro-3a-(diphenylmethoxy)tropane (4-Cl-BZT) is less efficacious as a locomotor stimulant than cocaine and does not substitute for cocaine as a discriminative stimulus across a range of doses (Newman et al., 1994; Kline et al., 1997). It is currently unknown whether the lack of cocaine-like behavioral efficacy for this compound results from a reduced ability to elevate mesolimbic DA in vivo via its interaction with the DA transporter (pharmacodynamic factors), from diminished or delayed uptake into brain (pharmacokinetic or dispositional factors), or from a potential functional antagonism exerted by additional site(s) of action.

In an attempt to better characterize the actions of 4-Cl-BZT in vivo, the present study was designed to address the following issues. First, after full dose-response characterization of the acute locomotor-stimulant effects of 4-Cl-BZT and cocaine, the locomotor response and the NAc DA response to selected doses of 4-Cl-BZT were evaluated simultaneously in freely moving rats and compared with responses to the same doses of cocaine. Because DA uptake inhibitors are known to vary widely in their rates of entry into the brain (Stathis et al., 1995), a variable that can have a profound influence on the behavioral and subjective effects of abused drugs (Sellers et al., 1989), the time course of both behavioral activation and neurochemical effects for each drug was carefully characterized and compared. Second, to address potential dispositional factors in the differences between 4-Cl-BZT and cocaine, the locomotor and NAc DA responses to systemic administration of each drug were compared with those after local administration of either drug directly into the NAc. Finally, the present study evaluated whether systemic 4-Cl-BZT administration could antagonize the neurochemical effects, the locomotor-stimulant effects, or the discriminative stimulus effects of a subsequent systemic cocaine challenge.

**Materials and Methods**

**Animals and Drugs**

Animals used in microdialysis, microinjection, and locomotor dose-response studies were housed at the San Francisco Veterans Affairs Medical Center animal care facility. Male Sprague-Dawley rats (Simonsen, Gilroy CA) weighing between 200 and 350 g were housed in pairs on a 12-h light/12-h dark cycle (lights on at 6:00 AM) and received food and water ad libitum. Rats used for microinjection and microdialysis experiments were housed individually after surgery. For cocaine discrimination studies, male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 310 to 385 g were housed individually at the National Institute on Drug Abuse Intramural Research facility with free access to water under a 12-h light/12-h dark cycle (lights on at 7:00 AM). Rats were fed daily 15 g of Purina chow 30 min after testing. Cocaine hydrochloride (Sigma, St. Louis, MO) was dissolved in isotonic saline for i.p. injections and in artificial cerebrospinal fluid (aCSF: 125 mM NaCl, 0.5 mM NaH2PO4, 2.5 mM NaHCO3, 2.5 mM KCl, 1.2 mM CaCl2, 1 mM MgCl2, pH 7.4) for microinjections and microdialysis studies. 4-Chlorobenzotropine (van der Zee et al., 1980; Newman et al., 1994) was dissolved with extensive sonication in saline for i.p. injections and in aCSF for microinjection/microdialysis studies.

**Locomotor Activity**

Test chambers (Opto-Varimex Minor, Columbus Instruments, Columbus, OH) measured 50 × 50 cm and were equipped with 15 photocell beams in each direction located 4 cm off the cage floor. Interruptions of photocell beams were cumulatively computer-registered in 10-min intervals (15-min in microdialysis groups). In all test groups, rats were allowed to habituate to the test chamber for 1 h while being monitored for baseline locomotor activity. At the end of this period, rats received drug treatments (see Experimental Design section below) and were monitored further for locomotor activity and stereotypy for at least 2 h. Frequency and intensity of stereotyped behavior was scored by a trained observer (often, but not in all cases, blind to the treatment conditions) for 10 s every 10 min according to the following scale (Steketee and Kalivas, 1991): 1: asleep or still; 2: inactive, grooming, mild licking; 3: locomotion, rearing, or sniffing; 4: any combination of two of locomotion, rearing, or sniffing; 5: continuous sniffing for 10 s without locomotion or rearing; 6: continuous sniffing for 10 s with locomotion or rearing; 7: patterned sniffing for 5 s; 8: patterned sniffing for 10 s; 9: continuous gnawing; 10: bizarre diskinetic movements or seizures. Scored behavior is presented as the percentage of animals exhibiting stereotypy as defined for a given experiment (scores of 5 or higher during any 10-min interval for single drug treatments, and scores of 8 or higher during any 10-min interval for drug combination studies employing a high challenge dose of cocaine). The threshold defining stereotypy was raised in drug combination studies to allow comparison of pretreatment effects; such comparisons were impossible using the lower-score criteria because all animals exceeded scores of 5 regardless of pretreatment in these experiments.
Cocaine Discrimination

Rats were tested in operant-conditioning chambers (modified Med Associates model ENV 007; St. Albans, VT) housed within light- and sound-attenuating enclosures with white noise present throughout testing. Ambient illumination was provided by lamps mounted at the top of the front panel. Two response keys (levers) were set 17 cm apart, with three stimulus lights above each. A force of 0.4 N through 1 mm was required to register a response, and each response produced an audible click from a relay mounted behind the front panel of the chamber. Reinforced responses produced one 45-mg pellet (BioServe, Frenchtown, NJ) delivered from a dispenser mounted behind the front panel into a food tray located centrally between the response keys. On-line experimental control and data collection were by MS-DOS computers operating Med Associates software (Med Associates).

Rats initially were trained to press both keys under a fixed-ratio schedule of food reinforcement. Responding on each key was trained separately in a mixed order, with the active key on a given training session indicated by illumination of the lamps directly above it. Rats subsequently were trained to discriminate i.p. injections of cocaine (10 mg/kg) from i.p. injections of saline. After cocaine injections, responses on only one key were reinforced; after saline injections, responses on the alternate key were reinforced. The assignment of cocaine- and saline-appropriate keys was counterbalanced across rats. Immediately after injection, rats were placed inside the experimental chambers and a 5-min time-out period was initiated, during which all stimulus lamps were extinguished and responding produced feedback clicks but had no other scheduled consequences. All lamps then were illuminated and responses on the appropriate key were reinforced. The fixed-ratio (FR) value was increased to 10 or 20 (depending on the experiment; see figure legends) over several training sessions. Responses on the inappropriate key reset the FR-response requirement on the appropriate key. Each food presentation was followed by a 20-s time-out period during which all lamps were off, and responding had no scheduled consequences other than the feedback clicks. Sessions ended after 20 food presentations or 15 min, whichever occurred first.

As the FR value reached 20, training sessions for which cocaine (C) and saline (S) injections were administered were ordered in a CSS-CSS...sequence, with test sessions conducted after consecutive SC or CS training sessions. On test sessions, different doses of cocaine, 4-Cl-BZT, or their combination were substituted for cocaine or saline. A test session was conducted if the subject achieved criteria on both of the immediately preceding saline and cocaine training sessions. The criteria were at least 80% cocaine- or saline-appropriate responding overall and during the first FR of the session. Test sessions were identical with training sessions, with the exception that 20 consecutive responses on either key were reinforced.

Microdialysis

All animals were experimentally naive, and microdialysis probes (CMA/12; CMA/Microdialysis, Acton MA) were unused before surgical procedures and testing. Rats were anesthetized with ketamine/xylazine and placed in a stereotaxic instrument (Kopf, Tujunga, CA). A new 2-mm microdialysis probe was implanted into the left NAc with xylazine and placed in a stereotaxic instrument (Kopf, Tujunga, CA). Microdialysis sessions were identical with training sessions, with the exception that responding overall and during the first FR of the session. Test sessions were conducted if the subject achieved criteria on both of the immediately preceding saline and cocaine training sessions. The criteria were at least 80% cocaine- or saline-appropriate responding overall and during the first FR of the session. Test sessions were identical with training sessions, with the exception that 20 consecutive responses on either key were reinforced.

Experimental Design

Five experiments were designed and conducted as follows.

**Experiment 1.** The effects of i.p. 4-Cl-BZT, cocaine, or the combination of the two drugs on locomotor activity were evaluated as follows. For studies of each drug alone, rats were habituated to the test chambers for 1 h and then were injected with saline (1 ml/kg), cocaine (1, 10, 20, 40, or 50 mg/kg i.p.), or 4-Cl-BZT (1, 10, 20, 40, or 60 mg/kg i.p.) and monitored for locomotor activity for 2 h. For combination groups, after 1 h habituation rats were injected with saline or 4-Cl-BZT (20 or 40 mg/kg i.p.) and returned to the test chamber. Thirty minutes later, all rats were challenged with 40 behavioral data and corresponding perfusate samples began. Behavioral data and perfusate samples were collected simultaneously in 15-min intervals throughout the experiment as described below. Comparisons of behavioral and microdialysis time courses took into consideration a 15- to 20-min time lag in DA response (or in drug delivery for studies of drugs perfused locally through the microdialysis probe) because of dead space in the collection tubing. Perfusates were stored at −20°C until assayed (within 1 week of collection). All rats were euthanized for histological analysis of the probe and tract locations upon completion of the experiment.

HPLC

Perfusate sample aliquots (20 μl) were analyzed for DA content by reverse-phase HPLC connected to an electrochemical detector (LC-4B; Bioanalytical Systems, West Lafayette, IN) equipped with a dual glassy carbon electrode set at 0.65 V. The mobile phase (0.06 M Na2HPO4, 0.09 M EDTA, and 1.3 mM 1-octanesulfonic acid in an 18% methanol solution adjusted to pH 2.95 with phosphoric acid) was pumped (model 510; Waters, Milford, MA) at 0.6 ml/min through a reversed-phase, ion-pairing column (100 × 3.2 mm) prepacked with Phase II octadecylsilane 5-μm particulate at 0.6 ml/min (model 6213, Bioanalytical Systems). Retention times and concentrations of DA, 3,4-dihydroxyphenylacetic acid, and 5-hydroxyindoleacetic acid in dialysates were determined by comparisons with individual standards and standard mixtures of known concentration run daily before and after microdialysis samples. Linearity of standard curves was observed over a wide range of concentrations (0.01–200 pmol). Chromatographic data were recorded using a dual pen chart recorder (BD 41; Kipp and Zonen, Boheimia, NY) with signal amplitudes set at 0.5 and 5 V. The limit of detection for DA was approximately 10 fmol.

Intracranial Microinjections

Rats were anesthetized with ketamine/xylazine and placed in a stereotaxic instrument. Bilateral guide cannulas (22-gauge; Plastics One, Roanoke, VA) were implanted into the NAc (A/P, 1.0 mm; M/L: 1.3 mm; D/V, 8.3 mm from bregma according to the atlas of Paxinos and Watson (1986)). The effects of i.p. 4-Cl-BZT, cocaine, or the combination of the two drugs on locomotor activity were evaluated as follows. For studies of each drug alone, rats were habituated to the test chambers for 1 h and then were injected with saline (1 ml/kg), cocaine (1, 10, 20, 40, or 50 mg/kg i.p.), or 4-Cl-BZT (1, 10, 20, 40, or 60 mg/kg i.p.) and monitored for locomotor activity for 2 h. For combination groups, after 1 h habituation rats were injected with saline or 4-Cl-BZT (20 or 40 mg/kg i.p.) and returned to the test chamber. Thirty minutes later, all rats were challenged with 40
mg/kg cocaine and were monitored for locomotion, stereotypy, and convulsions for 2 h.

**Experiment 2.** The effects of i.p. 4-Cl-BZT, cocaine, and the combination of the two drugs on NAc DA were evaluated simultaneously with locomotor activity in freely moving rats. Baseline dialysate samples were collected every 15 min while rats were habituated to the test chambers for 1 h. After i.p. injection of saline, 4-Cl-BZT (10 or 40 mg/kg), or cocaine (40 mg/kg) dialysates and behavioral measures were collected every 15 min for 2 h. All rats then were challenged with 10 mg/kg cocaine, and dialysates and behavioral measures were collected for another 2 h.

**Experiment 3.** The effects of unilateral intra-accumbens 4-Cl-BZT or cocaine perfusion through the microdialysis probe on extracellular DA were evaluated simultaneously with locomotor activity in freely moving rats. Baseline perfusate samples were collected every 15 min while rats were habituated to the test chambers for 1 h. After habituation, cocaine or 4-Cl-BZT (1, 10, or 100 µM in aCSF) was perfused locally through the microdialysis probe for 1 h while samples were collected every 15 min. Artificial CSF alone then again was perfused and samples were collected every 15 min for 3 to 5 h.

**Experiment 4.** The effects of bilateral microinjections of 4-Cl-BZT or cocaine into the NAc on locomotor activity were compared with respect to total locomotion and time course of activity. Doses were chosen based on previous studies of intra-accumbens cocaine (Delfs et al., 1990; Jones et al., 1994). On the test day rats were habituated to the test chamber for 1 h, obturators were removed, and 26-gauge injection cannulas were inserted into the guide canulas. Microinjections of aCSF (0.5 µl/side), cocaine (30 or 300 nmol/0.5 µl/side), or 4-Cl-BZT (30 or 300 nmol/0.5 µl/side) were made over 60 s using a bilateral polyethylene connector (Plastics One) and two 1-µl Hamilton syringes. An additional 60 s was allowed for drug diffusion, injection cannulas were removed, and obturators were replaced before monitoring locomotor activity for 2 h.

**Experiment 5.** The effects of 4-Cl-BZT, cocaine, or the combination of the two drugs on rats discriminating cocaine injections were evaluated as follows. Daily sessions were conducted and performances were evaluated to assess whether subjects met the testing criteria. Rats were administered the drug i.p. and placed in the darkened experimental chamber. Responses during an initial 5-min time-out had no scheduled consequences. After the time-out, completion of the FR requirement on either lever produced a food pellet followed by the 20-s time-out period. Test sessions were conducted with saline (1 ml/kg i.p.), cocaine (1.0, 3.0, 10.0 mg/kg i.p.), 4-Cl-BZT (1.0, 2.5, 5.0, 10.0, 25.0 mg/kg i.p.), or cocaine (0.1, 0.3, 0.56, 1.0, 3.0, 10.0 mg/kg i.p.) preceded by 4-Cl-BZT (3.0 and 10.0 mg/kg i.p.). The distribution of responses on the two levers was monitored until 20 food pellets were presented or for 15 min, whichever occurred first.

**Statistical Analysis**

Effects of drug and dose on cumulative locomotor responses were analyzed by one-way and two-way between-subject ANOVA followed by Newman-Keuls and Dunnett’s post hoc tests. Time course locomotor and neurochemical data were analyzed by mixed factorial (between subject = drug or dose, within subject = time) ANOVA. Secondary analysis of time course interactions compared the two drugs for onset and duration of action. Onset of action was analyzed by one-way ANOVA comparisons of time to peak effects, and duration of action was quantified for behaviorally active doses of each drug as mean time required for dissipation to 50% of peak effects. Stereotypy data were analyzed by Kruskal-Wallis and Mann-Whitney U tests and confirmed by one-way ANOVA. Basal DA data were analyzed as raw values (fmol/20 µl). Because of interindividual differences in basal DA efflux within treatment groups, microdialysis data were converted to percentages of control values, defined as the mean extracellular DA content measured in the four samples before drug administration. Statistical comparisons for drug effects on neurochemical measures were made using percent control values and subsequently were confirmed using raw data (fmol/20 µl).

For cocaine discriminative stimulus studies, the overall response rate and the percentage of responses occurring on the cocaine-appropriate lever were calculated for each subject and mean values were determined for each measure at each drug dose. Data from any rat that failed to produce at least 20 responses were not included in the calculation of mean cocaine-appropriate responding at that dose. If fewer than three rats met the response rate requirement, no mean value was calculated for percentage of cocaine-appropriate responding at that dose. Standard ANOVA and linear regression techniques were used to calculate mean effective dose (ED_{50}) values and their 95% confidence limits (Snedecor and Cochran, 1967). A significant difference in ED_{50} values is indicated when the 95% confidence limits do not overlap. To assess relative potency of cocaine in 4-Cl-BZT-treated subjects, the dose-effect data also were analyzed by standard parallel-line bioassay techniques as described by Finney (1964). This analysis involves a one-way ANOVA that determines whether the slopes of the two dose-response curves are different from parallel and fits a common slope to the two dose-response curves. It then compares the ratio of doses for a given effect (in this case a 50% substitution for the training dose of cocaine) to provide a value for relative potency. This value represents the dose of cocaine in mg/kg for 4-Cl-BZT-treated rats equal to 1 mg/kg cocaine in subjects not pretreated. A significant relative potency difference is indicated when the 95% confidence limits for that ratio do not include 1.0.

**Results**

**Locomotor Activity: Intraperitoneal Dose Response.** Although both cocaine [F(5,50) = 19.24, p < .0001] and 4-Cl-BZT [F(6,33) = 4.19, p < .005] dose-dependently stimulated locomotor activity when administered i.p., cocaine exhibited a lower threshold dose for behavioral activity [drug × dose interaction F(3,43) = 4.74, p < .007] and was clearly more efficacious as a locomotor stimulant than 4-Cl-BZT across the broad range of doses tested [drug effect F(1,53) = 7.29, p < .01; Fig. 1A]. At 80 mg/kg, twice the maximally active dose of cocaine, 4-Cl-BZT produced marked locomotor stimulation in only one of six rats; the remaining five animals in this dose group exhibited significantly less locomotion than the mean response to 40 mg/kg of cocaine. Analysis of scored behavior revealed no significant differences between cocaine and 4-Cl-BZT in the induction of stereotypy [Mann-Whitney U test, p > .05; F(1,56) = .40, p = .53; Fig. 1B]. The two drugs displayed markedly different kinetic profiles with respect to their locomotor stimulant effects when injected i.p. [drug × time interaction: F(34,1139) = 7.30, p < .0001]. Analysis of this interaction revealed a significantly longer time to peak stimulant effect for 4-Cl-BZT [58 ± 7 min versus 26 ± 3 min for cocaine across all active doses; F(1,45) = 16.81, p < .0002; Fig. 1C]. This was true at all doses [no dose effect, F(5,59) = 1.58, p = 0.19 or drug × dose interaction, F(1,39) = 20, p = .066]. In addition, the duration of action of 4-Cl-BZT was longer than that of cocaine. Whereas the locomotor stimulant effect of cocaine rapidly dissipated to 51% of peak effect by 60 min postinjection, 4-Cl-BZT continued to stimulate locomotion to 83% of its peak effect up to 120 min postinjection, when testing ended (Fig. 1C).

**NAc DA: Intraperitoneal Administration.** The above behavioral results of i.p. 4-Cl-BZT and cocaine administration were paralleled by the effects of i.p. administration of the two drugs on extracellular DA in the NAc. Whereas basal DA did not differ among i.p. treatment groups [F(3,14) = .13, p = 0.94; see Fig. 2 legend for absolute values], a significant effect of drug challenge on NAc DA over 2 h was present [F(3,144)
Secondary analysis revealed that 4-Cl-BZT significantly elevated NAc DA relative to saline at 40 mg/kg i.p. [F(1,72) = 25.14, p < .0007; Fig. 2A (□) after the first injection] but not at 10 mg/kg [F(1,72) = 25, p = 0.63; Fig. 2A (△) after the first injection]. Within-subject comparison revealed that 10 mg/kg 4-Cl-BZT also had no significant effect on DA relative to baseline [F(8,32) = 1.13, p = 0.37; Fig. 2A, compare △ before and after the first injection]. Although cocaine was not administered at 10 mg/kg as a first injection in the i.p. microdialysis experiment, this dose of cocaine did elevate NAc DA significantly above baseline (190% control) in rats that had been treated 2 h previously with i.p. saline [F(8,32) = 7.72, p < .0001; Fig. 2A (○) after the second injection]. At 40 mg/kg, cocaine produced a pronounced and rapid elevation of NAc DA relative to saline [F(1,80) = 24.83, p < .0006; Fig. 2A, compare ◇ and ○ after the first injection]. Averaged over the full 2-h period after injection, the NAc DA response to 40 mg/kg cocaine was not significantly different from that of 40 mg/kg 4-Cl-BZT (Newman-Keuls, p > .05). However, the time course of action of cocaine was significantly different from...
that of 4-Cl-BZT [drug \times time interaction $F(24,144) = 8.12$, $p < .0001$, and the peak DA overflow after 4-Cl-BZT (245%) did not reach that of cocaine (370%, Fig. 2A, compare ◇ and ■ after the first injection).

As depicted in Fig. 2B, comparison of behavioral responses in microdialysis rats revealed no differences in baseline locomotor activity among i.p. treatment groups [$F(3,57) = 1.32$, $p < .30$; compare points before the first injection], but a significant effect of drug challenge over 2 h was present [$F(3,21) = 68.84$, $p < .0001$; points after the first injection]. Significant locomotor activity was induced by 4-Cl-BZT only at the 40-mg/kg dose (Dunnett's, $p < .05$; Fig. 2B, compare ■ and ◇ after the first injection). Post hoc comparison of locomotor data over the 2-h period revealed a significantly higher locomotor response to cocaine relative to 4-Cl-BZT at 40 mg/kg (Newman-Keuls, $p < .05$; compare ◇ and ■). As was the case for NAc DA in the same rats, the temporal profile of the locomotor stimulant effects of cocaine and 4-Cl-BZT differed significantly [drug \times time interaction $F(24,168) = 7.21$, $p < .0001$].

**NAc DA: Local Administration.** No differences in basal DA levels among the NAc drug treatment groups were detected [$F(2,35) = .68$, $p = .51$]. Perfused locally through the microdialysis probe, both 4-Cl-BZT [$F(3,304) = 3.95$, $p < .03$] and cocaine [$F(3,266) = 9.32$, $p < .005$] dose dependently elevated DA levels in the left NAc (Fig. 3, A and B). Secondary analysis revealed that local 4-Cl-BZT stimulated NAc DA overflow at 10 μM [$F(1,152) = 284.77$, $p < .0001$] and at 100 μM [$F(1,171) = 5.34$, $p < .05$], but not at 1 μM [$F(1,171) = 1.21$, $p = .30$]. Similarly, locally perfused cocaine elevated DA at 10 μM [$F(1,171) = 10.88$, $p < .01$] and at 100 μM [$F(1,190) = 20.49$, $p < .002$], but not at 1 μM [$F(1,110) = 1.19$, $p = .31$]. Post hoc comparisons at each active dose revealed significantly greater elevation of accumbens DA by 4-Cl-BZT than by cocaine at the 100-μM dose but not at the 10-μM dose (Newman-Keuls, $p < .05$). As was the case with peripheral administration, the onset and duration of local cocaine's effects on NAc DA were significantly shorter than those of locally perfused 4-Cl-BZT [drug \times time interaction: $F(19,437) = 3.79$, $p < .0001$]. Whereas cocaine-induced DA overflow peaked by the third sample after beginning infusion and remained elevated for only 1 h after removal of cocaine, local 4-Cl-BZT was maximally active 2 h from the start of infusion and NAc DA levels remained elevated above baseline for more than 3 h after cessation of 4-Cl-BZT infusion (Fig. 3, A and B). Neither cocaine nor 4-Cl-BZT induced locomotor activity at any dose when perfused locally through the microdialysis probe [$F(2,360) = .953$, $p = .40$; Fig. 3, C and D).

**Bilateral NAc Microinjections.** No significant differences in basal locomotion were found among drug treatment groups [$F(2,28) = 2.054$, $p = .15$; Fig. 4A, preinjection data points]. A significant effect of drug was present [$F(2,28) = 3.79$, $p < .05$], with microinjections of either 4-Cl-BZT [$F(2,15) = 5.23$, $p < .02$] or cocaine [$F(2,14) = 4.11$, $p < .05$] eliciting locomotor stimulation (Fig. 4A). Post hoc analysis revealed no differences between doses of either cocaine or 4-Cl-BZT (Newman-Keuls, $p > .05$). Secondary comparisons of 4-Cl-BZT and cocaine revealed no differences between drugs in cumulative locomotor stimulation at either the 30-nmol/side [$F(1,10) = 1.43$, $p = .26$] or the 300-nmol/side [$F(1,12) = .323$, $p = .58$] doses. However, as was the case with peripheral administration, intra-accumbens cocaine and 4-Cl-BZT exhibited significantly different time courses of action at both doses [drug \times time interaction: $F(22,308) = 9.55$, $p < .0001$]. Secondary analysis revealed that the locomotor response to either dose of cocaine was markedly higher than to either dose of 4-Cl-BZT in the first 30 min after microinjection (Dunnett's, $p < .05$). No significant differences among all treatment groups were uncovered at any other time point (Fig. 4B).
Drugs Combination Studies. In rats simultaneously assessed for locomotor activity and extracellular DA, 4-Cl-BZT pretreatment potentiated the NAc DA and locomotor responses to a low dose (10 mg/kg i.p.) of cocaine administered 2 h later (Fig. 2, points after the second injection). Post hoc analysis of the significant effect of pretreatment on the DA response to 10 mg/kg cocaine \([F(3,120) = 8.17, \ p < .002]\) revealed that pretreatment 2 h earlier with 40 mg/kg 4-Cl-BZT resulted in significantly higher NAc DA levels upon cocaine injection relative to pretreatment with saline (Fig. 2A, compare ■ with □ after the second injection), 10 mg/kg 4-Cl-BZT (▲), or 40 mg/kg cocaine (◇) (Newman-Keuls, \(p < .05\)). Pretreatment with 10 mg/kg 4-Cl-BZT or cocaine did not alter the NAc DA response to 10 mg/kg cocaine relative to saline pretreatment (Newman-Keuls, \(p > .05\)).

It should be noted that the results of pretreatment comparisons depend, in part, on the manner in which the data are expressed. Notably, the levels of extracellular DA at the time of the cocaine challenge were already elevated in rats pretreated with 40 mg/kg 4-Cl-BZT (Dunnett’s, \(p < .05\); Fig. 2A ▲ between the two injections; Table 1, column A). Thus, if the cocaine-induced DA response is expressed as a percentage of DA levels immediately preceding 10 mg/kg cocaine challenge, then the subsequent elevation in NAc DA was marginally \([F(1,10) = 4.24, \ p < .067]\) less robust in rats pretreated with 40 mg/kg 4-Cl-BZT than in saline-pretreated rats (Table 1, column D). However, in absolute terms the rise in DA overflow in 4-Cl-BZT-pretreated rats after cocaine challenge was slightly, if not significantly \([F(1,10) = 2.42, \ p = 0.15]\), greater than that in the saline-pretreated group (Table 1, column C) and appeared to be additive with preexisting DA levels (Table 1, compare column C for 40 mg/kg 4-Cl-BZT and saline).

The locomotor response to 10 mg/kg cocaine challenge in rats that underwent microdialysis also was dose dependently potentiated and prolonged by pretreatment with 4-Cl-BZT (Fig. 2B). This effect was clearly greater than strict additivity between the actions of the two drugs alone. Post hoc analysis of an overall pretreatment effect \([F(3,18) = 26.07, \ p < .0001]\) revealed that pretreatment 2 h before with 40 mg/kg 4-Cl-BZT ( ■), but not with 10 mg/kg 4-Cl-BZT (▲) or cocaine ( ◇),

**Table 1**

Extracellular DA and locomotor responses to 10 mg/kg i.p. cocaine challenge in subjects pretreated with 4-Cl-BZT or cocaine

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>A Final Preinjection</th>
<th>B Peak Postinjection</th>
<th>C Absolute Change</th>
<th>D Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Extracellular DA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>85.7 ± 8.2</td>
<td>151.7 ± 17.9</td>
<td>66.0 ± 15.3</td>
<td>79.8 ± 18.1</td>
</tr>
<tr>
<td>10 mg/kg 4-Cl-BZT</td>
<td>94.6 ± 24.5</td>
<td>154.6 ± 51.1</td>
<td>60.0 ± 8.7</td>
<td>77.2 ± 16.2</td>
</tr>
<tr>
<td>40 mg/kg 4-Cl-BZT</td>
<td>284.5 ± 81.7</td>
<td>398.1 ± 104.4</td>
<td>113.6 ± 25.9</td>
<td>43.7 ± 5.3</td>
</tr>
<tr>
<td>Cocaine</td>
<td>95.6 ± 11.9</td>
<td>139.1 ± 6.7</td>
<td>43.5 ± 10.4</td>
<td>48.8 ± 14.8</td>
</tr>
<tr>
<td><strong>Locomotor activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>224 ± 33</td>
<td>1384 ± 298</td>
<td>1160 ± 298</td>
<td>647.1 ± 257.4</td>
</tr>
<tr>
<td>10 mg/kg 4-Cl-BZT</td>
<td>368 ± 86</td>
<td>2920 ± 1389</td>
<td>2552 ± 1376</td>
<td>741.1 ± 337.9</td>
</tr>
<tr>
<td>40 mg/kg 4-Cl-BZT</td>
<td>2809 ± 534</td>
<td>7273 ± 977</td>
<td>4464 ± 1183</td>
<td>218.6 ± 70</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1927 ± 566</td>
<td>1725 ± 291</td>
<td>−202 ± 819</td>
<td>10.1 ± 38.2</td>
</tr>
</tbody>
</table>

For all values, calculations were made for each individual animal and then averaged within each treatment group. The (−) sign associated with locomotor change in cocaine-pretreated rats indicates an average net lower locomotion after 10 mg/kg cocaine challenge than immediately before.

![Fig. 4](image-url)
magnified peak cocaine-induced locomotion roughly 5-fold over saline pretreatment (Dunnett’s, p < .05; Fig. 2B, compare points after the second injection; Table 1, column B). As was the case for NAc DA in the same rats, the results of pretreatment comparisons of locomotor activity depend in some cases on the manner in which the data are expressed. At the time of the cocaine challenge injection, ongoing locomotor activity was already elevated in rats pretreated with cocaine or 40 mg/kg 4-Cl-BZT relative to saline [overall F(3,18) = 15.34, p < .0001; post hoc by drug: Dunnett’s, p < .05]. The percent change from preinjection locomotion did not differ among pretreatment groups [F(3,18) = 1.48, p = 0.25] but tended to be lower in rats pretreated with 40 mg/kg cocaine or 40 mg/kg 4-Cl-BZT (Table 1, column D). In absolute terms (Table 1, column C), the change in locomotion after cocaine was significantly higher in rats pretreated with 40 mg/kg 4-Cl-BZT relative to saline [Student’s t test, t(1,10) = 7.00, p < .03], although differences of all pretreatment groups did not reach statistical significance by one-way ANOVA [F(3,18) = 2.54, p = 0.08]. In contrast, the locomotor response to 10 mg/kg cocaine challenge was blunted in rats pretreated with 40 mg/kg cocaine (Table 1, column C). However, analysis of a pretreatment × time interaction [F(24,144) = 2.54, p < .0004] revealed that both 40 mg/kg 4-Cl-BZT [F(8,80) = 5.80, p < .0001] and cocaine [F(8,56) = 3.39, p < .005] prolonged the locomotion induced by 10 mg/kg cocaine (Fig. 2B).

The effects of 4-Cl-BZT pretreatment on the behavioral and toxic properties of cocaine from saline under a fixed-ratio 10-response schedule of reinforcement. Abscisae: drug dose in mg/kg (log scale). Ordinate (upper): percentage of responses emitted on the lever on which rats were trained to cocaine from saline. Ordinate (lower): rates at which responses were emitted as a percentage of response rate after saline administration. Each point represents the effect in four to six rats. Ordinate (upper): percentage of responses emitted on the lever on which rats were trained to respond after injections of cocaine. Each point represents the effect in four to six rats. Ordinate (lower): rates at which responses were emitted as a percentage of response rate after saline administration. Each point represents the effect in four to six rats. Points above S represent control data after saline injections. Points above C represent control data after cocaine injections.

TABLE 2
Effects of 4-Cl-BZT pretreatment on behavioral and toxic properties of cocaine

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Total Locomotion</th>
<th>Stereotypy</th>
<th>Convulsions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>30,883 ± 2,662</td>
<td>0.0</td>
<td>0/6</td>
</tr>
<tr>
<td>20 mg/kg 4-Cl-BZT</td>
<td>34,589 ± 2,223</td>
<td>67.7*</td>
<td>2/6</td>
</tr>
<tr>
<td>40 mg/kg 4-Cl-BZT</td>
<td>28,425 ± 1,509</td>
<td>100.0*</td>
<td>3/8*</td>
</tr>
</tbody>
</table>

* One lethality after intense tonic-clonic seizure.

** Significantly different from saline pretreatment, p < 0.05.

Cocaine Discrimination. As has been shown previously, subjects trained to discriminate cocaine (10.0 mg/kg) readily acquired the discriminative performance with greater than 80% of responses on the cocaine-appropriate lever after an injection of the training dose of cocaine and less than 20% of the responses on the cocaine-appropriate lever after saline injection (Fig. 5, unconnected points above C and S, respectively). Substitutions of varying doses of cocaine produced a dose-related increase in the percentage of responses on the cocaine-appropriate lever up to full substitution for cocaine at doses of 5.6 and 10.0 mg/kg (Fig. 6, •). In contrast to the dose-related increase in cocaine-appropriate responding with cocaine, none of the doses of 4-Cl-BZT produced a maximum level of drug-appropriate responding that exceeded saline levels (Fig. 5, upper graph, connected points). This lack of substitution for cocaine occurred despite the study of a range of doses of 4-Cl-BZT, from those having no effect to those that substantially decreased response rates. Similar results have been reported previously for this compound (Newman et al., 1994). When studied in combination with cocaine, 4-Cl-BZT shifted the cocaine dose-effect curve to the left (Fig. 6, △). The shift in the cocaine dose-effect curve was dose-related, with no lethality being induced by 40 mg/kg cocaine in naive rats or in any other pretreatment group.

No lethality was induced by 40 mg/kg cocaine in naive rats or in any other pretreatment group.

Fig. 5. Effects of 4-Cl-BZT in rats trained to discriminate injections of cocaine from saline under a fixed-ratio 10-response schedule of reinforcement. Abscisae: drug dose in mg/kg (log scale). Ordinate (upper): percentage of responses emitted on the lever on which rats were trained to respond after injections of cocaine. Each point represents the effect in four to six rats. Ordinate (lower): rates at which responses were emitted as a percentage of response rate after saline administration. Each point represents the effect in six rats. Points above S represent control data after saline injections. Points above C represent control data after cocaine injections.
The higher dose of 4-Cl-BZT shifting the curve further to the left. The change in potency of cocaine with 4-Cl-BZT pretreatments is quantified further with dose-related changes in the ED50 value for cocaine (Table 3). As can be seen in the table, doses of 3.0 and 10.0 mg/kg 4-Cl-BZT significantly decreased the ED50 value from 3.5 mg/kg to 1.7 and 0.15 mg/kg, respectively. These changes in ED50 values represent 1.7- and 16.2-fold increases in the potency of cocaine (Table 3).

**Discussion**

The current results confirm and extend previous reports that 4-Cl-BZT lacks a cocaine-like behavioral profile despite its high affinity for the DA transporter and its potency in inhibiting DA reuptake in vitro (Newman et al., 1994, 1995; Kline et al., 1997). As in previous studies demonstrating weak locomotor stimulant efficacy at early time points (0–30 min) in mice (Newman et al., 1994; Kline et al., 1997), 4-Cl-BZT was found in the current study to induce only modest locomotor activation in rats across a wide range of doses for up to 2 h postinjection (Fig. 1A). Although this behavioral activation appeared to differ from that of cocaine quantitatively rather than qualitatively, the temporal profiles of the two drugs’ locomotor stimulant effects were clearly distinct. Unlike the rapid onset and dissipation of pronounced locomotor stimulation by cocaine, the response to active doses of 4-Cl-BZT remained virtually unchanged throughout the 2-h test period (Fig. 1B). The lower locomotor stimulant efficacy of 4-Cl-BZT was not the result of greater induction of stereotypy relative to cocaine (Fig. 1C); like its parent compound benztrpine (Scheel-Kruger, 1972), 4-Cl-BZT produced less stereotypy than equal doses of cocaine across all time points of the 2-h test session (data not shown). Thus, other mechanisms must account for the low behavioral efficacy of systemic 4-Cl-BZT. Previously, it has been unknown whether the ability of 4-Cl-BZT to inhibit DA uptake in vitro is conserved in vivo, whether the interaction of 4-Cl-BZT with the DA transporter is analogous to that of a partial agonist (as defined by its effects on locomotor activity), or whether penetration of 4-Cl-BZT into the brain may be poor or delayed relative to cocaine, resulting in insufficient brain levels for neurochemical and behavioral stimulant effects. Each of these mechanisms/factors is addressed by the present results.

**Systemic versus Local Administration.** As depicted in Fig. 2, peripherally administered 4-Cl-BZT clearly is able to inhibit DA uptake in vivo, as demonstrated by its dose-dependent stimulation of NAc DA overflow in freely moving rats. In fact, the locomotor stimulation produced by 4-Cl-BZT is highly concordant with its stimulation of DA overflow in the NAc (Fig. 2); only the higher (40 mg/kg i.p.) dose that elevates NAc DA elicits locomotor activation, and both the neurochemical and behavioral effect are similarly blunted relative to those of cocaine at the same dose. Moreover, allowing for the 15- to 20-min time lag in DA response due to dead space in the collection tubing, the time course of locomotor stimulation correlates well with the respective time.

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**TABLE 3**

Potency of cocaine for production of discriminative stimulus effects when administered alone and in combination with 4-Cl-BZT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ED50 Value</th>
<th>Relative Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Cl-BZT alone</td>
<td>3.47</td>
<td></td>
</tr>
<tr>
<td>Cocaine alone</td>
<td>3.47</td>
<td></td>
</tr>
<tr>
<td>Cocaine + 3.0 mg/kg 4-Cl-BZT</td>
<td>1.70</td>
<td>1.66*</td>
</tr>
<tr>
<td>Cocaine + 10.0 mg/kg 4-Cl-BZT</td>
<td>0.15*</td>
<td>16.20*</td>
</tr>
</tbody>
</table>

* A significant relative potency difference is indicated when 95% confidence limits for that ratio do not include 1.0.
course for stimulation of NAc DA overflow for each drug, although peak locomotor stimulation by 4-Cl-BZT appeared to slightly precede peak elevation of NAc DA (Fig. 2). As is

the case for locomotor stimulation (Figs. 1B and 2B), the rapid and short-lived peak in NAc DA overflow after cocaine injection contrasts with the slow and persistent elevation of extracellular DA by 40 mg/kg 4-Cl-BZT (Fig. 2A). Although 4-Cl-BZT thus clearly penetrates into the brain after systemic administration, the degree and rate at which this occurs relative to cocaine, the distribution of the drug within the brain, and the relative influence of these factors on the behavioral effects of the drugs remain unknown.

If dispositional factors underlie the efficacy differences in the NAc DA and locomotor stimulant effects of 4-Cl-BZT and cocaine, then local administration of 4-Cl-BZT directly into the NAc would be expected to stimulate NAc DA overflow and locomotor activity to a greater extent than systemic administration and to a similar extent as local cocaine. Alternatively, if 4-Cl-BZT is less effective than cocaine as an indirect agonist at the DA transporter in vivo, then the NAc DA response to 4-Cl-BZT should be reduced relative to that of cocaine regardless of the route of administration. In fact, 4-Cl-BZT exhibited significantly higher efficacy than cocaine in elevating NAc DA when either drug was perfused locally through the microdialysis probe (Fig 3, A and B). This result resembles previous microdialysis data from studies comparing cocaine with the unsubstituted parent compound benztropine. Although benztropine exerts a modest stimulation of DA overflow when administered peripherally (Church et al., 1987), extracellular DA elevation by benztropine is significantly higher relative to cocaine when perfused locally into the striatum (Nomikos et al., 1990). Thus, local administration studies demonstrate that benztropine and 4-Cl-BZT are not “partial indirect agonists” as defined by their effects on DA overflow in vivo, despite such an appearance in studies using peripheral administration. It should be noted that even when administered directly into the NAc, 4-Cl-BZT exerts its peak effects on extracellular DA significantly later than cocaine (Fig. 3), suggesting that 4-Cl-BZT has a slower rate of occupancy at the DA transporter than cocaine regardless of its uptake into the brain from the periphery. Likewise, the duration of action of 4-Cl-BZT on NAc DA is significantly longer than that of cocaine whether administered locally or peripherally, suggesting slower dissociation from the DA transporter or delayed metabolism relative to cocaine.

Although the degree to which the two drugs cross the probe membrane was not determined and compared in the present study, it is unlikely that the higher efficacy of 4-Cl-BZT in elevating NAc DA is the result of augmented delivery of 4-Cl-BZT into the brain relative to cocaine. Closely related compounds of similar molecular weights are unlikely to differ widely in probe membrane permeability, as shown in previous microdialysis studies reporting similar in vitro recoveries for cocaine and benztropine (Nomikos et al., 1990). The higher efficacy of local 4-Cl-BZT in elevating NAc DA is significantly longer than that of cocaine whether administered locally or peripherally, suggesting slower dissociation from the DA transporter or delayed metabolism relative to cocaine.

Although the degree to which the two drugs cross the probe membrane was not determined and compared in the present study, it is unlikely that the higher efficacy of 4-Cl-BZT in elevating NAc DA is the result of augmented delivery of 4-Cl-BZT into the brain relative to cocaine. Closely related compounds of similar molecular weights are unlikely to differ widely in probe membrane permeability, as shown in previous microdialysis studies reporting similar in vitro recoveries for cocaine and benztropine (Nomikos et al., 1990). The higher efficacy of local 4-Cl-BZT in elevating NAc DA is significantly longer than that of cocaine whether administered locally or peripherally, suggesting slower dissociation from the DA transporter or delayed metabolism relative to cocaine.

![Fig. 7. A, tract locations of microdialysis probes (vertical bars) and microinjection cannulas (●) in the NAc of selected rats used in local administration studies. Probe tracts are from rats that received aCSF or 100 μM 4-Cl-BZT and are representative of those of accurate placement in other treatment groups. Microinjection tracts are from rats that received aCSF or 300 nmol/side 4-Cl-BZT and are representative of those of accurate placement in other treatment groups. Animals with tract placements outside the NAc were excluded from the behavioral analysis. Although tract placements are represented schematically as occurring in a single anterior-posterior plane, tracts that met medial-lateral and dorsal-ventral criteria were accepted as accurate from approximately 1.7 to 0.7 mm from bregma according to the atlas of Paxinos and Watson (1986). Photomicrographs (×40) of tract locations and tissue damage associated with a 2-mm microdialysis probe delivering 100 μM 4-Cl-BZT (B) or microinjection cannulas delivering aCSF (C) or 4-Cl-BZT (D) at 300 nmol/side. In each case, tissue was harvested 18–24 h after behavioral testing.](ASPEJ-jpet2017.jpg)
(Newman et al., 1994). However, it should be noted that the potential for differences between 4-Cl-BZT and cocaine in probe membrane permeability, calcium-independent DA overflow, and rate of clearance/metabolism complicates direct comparison of potencies of these drugs at the DA transporter in vivo. Thus, the apparent equipotency of local 4-Cl-BZT and cocaine to elevate NAc DA should be interpreted as approximate and provisional.

Considering that the relative efficacies of 4-Cl-BZT and cocaine in elevation of accumbens DA depend on the site of administration, the same might be expected of their relative efficacies as locomotor stimulants. As shown in Fig. 3, neither drug was able to induce locomotor activity when perfused unilaterally through the microdialysis probe at 1 to 100 μM despite obvious effects on DA overflow. It should be noted that at a perfusion rate of 2 μl/min, local administration of the highest (100 μM) concentration used in the current study results in a total delivery of 12 nmol of drug over a 1-h period, assuming 100% diffusion efficiency of either drug across the probe membrane. In fact, because in vitro probe recovery of cocaine and related compounds is typically 7–10% (Nomikos et al., 1990), it may be estimated that the maximal dose of either drug delivered into the NAc through the microdialysis probe was approximately 1 nmol over 60 min. Previous studies have shown that the dose range of intraNAc cocaine that elicits locomotor activity is approximately 30 to 300 nmol/side when microinjected bilaterally (Delfs et al., 1990; Jones et al., 1994), orders of magnitude higher than those administered through the probe in the current study. For this reason, bilateral microinjections were used to assess the effects of local administration of 4-Cl-BZT on locomotor activity. Surprisingly, local administration of 4-Cl-BZT did not stimulate locomotor activity to a greater extent than systemic 4-Cl-BZT, nor did intraNAc 4-Cl-BZT induce locomotion to a greater degree than intraNAc cocaine, as would be predicted by the local perfusion microdialysis results (Fig. 4). In fact, the respective behavioral profiles of the two drugs administered into the NAc closely resembled those after systemic administration, both temporally and cumulatively (Figs. 1 and 4).

One potential explanation of these results is that 4-Cl-BZT may lack cocaine's effects at other macromolecular sites that promote cocaine-induced locomotor stimulation. In addition to its inhibition of DA uptake, cocaine is known to inhibit the neuronal reuptake of both norepinephrine and serotonin (Ritz et al., 1987), both of which may influence its locomotor stimulant effects (Snoddy and Tessel, 1985; Reith, 1990). In contrast, 4-Cl-BZT binds with much lower affinity to norepinephrine and serotonin transporters than to DA transporters in vitro (Newman et al., 1995). Alternatively, 4-Cl-BZT may exert actions at other sites that antagonize the locomotor stimulation associated with elevated NAc DA levels. For example, 4-Cl-BZT is approximately 7-fold more potent in binding to M₁ muscarinic receptors than in binding to the DA transporter in vitro (Katz et al., 1999). As evidence that such an antimuscarinic effect could antagonize the locomotor stimulant effects of DA uptake inhibition, Acri et al. (1996) have shown that coadministration of a high dose of atropine blocks the locomotor stimulant effects of various doses of cocaine in rats (Acri et al., 1996). However, these investigators also reported that at moderate doses, atropine had locomotor stimulant effects of its own and potentiated the locomotor response to 10 mg/kg cocaine (Acri et al., 1996). Although it is possible that the doses of 4-Cl-BZT used in the current study exert pronounced antimuscarinic effects comparable to the high dose of atropine in the Acri et al. (1996) study, such an explanation would suggest that 4-Cl-BZT could block the stimulant effects of cocaine when the two drugs are administered in combination. This prediction is not supported by drug combination results from the current study (discussed below). In fact, the literature suggests a general potentiation of stimulants by antimuscarinics (Carlton, 1961; Wilson and Schuster, 1975). In addition, Katz et al. (1999) have shown that the antimuscarinics atropine and scopolamine accentuated the locomotor stimulant and discriminative stimulus effects of cocaine. Together, these findings suggest that the antimuscarinic actions of 4-Cl-BZT are not capable of attenuating its own cocaine-like effects.

**Drug Combination Studies.** Although the effects of pretreatment with 4-Cl-BZT on responses to low dose (10 mg/kg) and high dose (40 mg/kg) cocaine were studied under different conditions, dose-dependent additivity/potentiation of cocaine's effects by 4-Cl-BZT were observed in both cases. In microdialysis experiments, the elevation of NAc DA produced by 10 mg/kg cocaine was additive with that induced by administration of 40 mg/kg 4-Cl-BZT 2 h before and was prolonged relative to saline-pretreated controls (Fig. 2A). Furthermore, 4-Cl-BZT significantly shifted the cocaine dose-effect curve for discriminative-stimulus effects to the left, despite its own lack of efficacy as a cocaine-like stimulus. Thus, 4-Cl-BZT does not antagonize the actions of cocaine at the DA transporter in the NAc in vivo, whereas such an antagonism has been suggested for the selective DA uptake inhibitor GBR 12909 (Rothman et al., 1991; Baumann et al., 1994). Interestingly, the closely related analog GBR 12935 has been shown to enhance the locomotor stimulant effects of cocaine, even at doses of GBR 12935 that do not stimulate locomotion when given alone (Acri et al., 1996). In the current study, cocaine-induced locomotion in the rats undergoing microdialysis was clearly potentiated by 4-Cl-BZT pretreatment 2 h before, although this potentiation was observed only at a dose of 4-Cl-BZT that had stimulant effects of its own (Fig. 2B).

Understanding at the biochemical level why GBR 12909, but not 4-Cl-BZT, is able to antagonize cocaine-induced elevation of NAc DA could have important implications for development of a transporter-based cocaine antagonist or substitution agent. Of particular interest in this regard is whether all inhibitors of DA uptake bind at the same or at different sites on the DA transporter and how these sites interact with the DA recognition site on the transporter protein. If the cocaine-binding domain is separate from the DA recognition domain on the transporter, it may be possible to identify a compound that antagonizes cocaine binding without inhibiting DA uptake (Rothman et al., 1991; Baumann et al., 1994; Rothman and Glowa, 1995). The theoretical possibility of such a “DA-sparing” antagonist has been suggested by site-directed mutagenesis (Kitayama et al., 1992) as well as radioligand-binding studies (Simoni et al., 1993; Dersch et al., 1994). Thus, it is conceivable that GBR 12909, and not 4-Cl-BZT, exhibits such properties to some extent in vivo. However, the degree to which the binding sites for various DA transporter ligands overlap each other remains controversial (Berger et al., 1990; Carroll et al., 1992;
Reith et al., 1992; Dersch et al., 1994), because cocaine and structurally related analogs typically bind to two sites associated with the DA transporter (Madras et al., 1989; Boja et al., 1992; Carroll et al., 1992), and, depending on assay conditions, some structurally unrelated ligands such as GBR 12935 and mazindol bind to a single site (reviewed in Carroll et al., 1992; but see Akunne et al., 1994).

The behavioral significance of cocaine's binding to both high- and low-affinity sites is not well understood, but may explain why some DA uptake inhibitors lack the potent locomotor stimulant and subjective effects of cocaine (Katz et al., 1997). Several reports have dissociated DA transporter occupancy from locomotor stimulant effects across different classes of DA uptake inhibitors (Rothman et al., 1992; Vaugois et al., 1993), especially in the case of compounds structurally unrelated to cocaine (Izenwasser et al., 1994). In humans, DA transporter occupancy is predictive of the subjective effects of cocaine (Volkower et al., 1997) but not the structurally unrelated DA uptake inhibitor methylphenidate (Volkow et al., 1996). Interestingly, several lines of evidence from structure-activity studies suggest that 4-Cl-BZT interacts with the DA transporter differently than does cocaine (reviewed in Katz et al., 1997). First, 4-Cl-BZT has been found to bind to the DA transporter and to inhibit DA uptake monophasically under the same assay conditions in which cocaine binds biphascially in vitro (Newman et al., 1994). Second, 4-Cl-BZT is able to bind with high affinity to the DA transporter despite its lack of a substitution at the 2 position of the tropane ring (Newman et al., 1994, 1995), a requirement for high-affinity binding in cocaine analogs (Carroll et al., 1992). In addition, para-substitutions on the phenyl rings alter DA transporter affinity to a much greater extent in substituted benztropine analogs (Newman et al., 1994, 1995; Katz et al., 1997) than in cocaine analogs (Carroll et al., 1992). Whether these differences in binding contribute to the behavioral differences between cocaine and 4-Cl-BZT when administered alone and whether the presence of 4-Cl-BZT leads to higher free concentrations of cocaine available to interact with the high-affinity cocaine binding site when the drugs are combined remain unclear.

Finally, pretreatment with nonconvulsant doses of 4-Cl-BZT 30 min before cocaine challenge tended to augment the incidence of stereotypy and seizures induced by a 40-mg/kg dose of cocaine (Table 2). Because other selective DA uptake inhibitors such as mazindol (Jaffe et al., 1989) and GBR 12935 (Acri et al., 1996) potentiate cocaine toxicity in rodents, it may be speculated that this potentiation is due to higher levels of free cocaine in the brain as a result of displacement of cocaine from its binding sites in the brain, in plasma, and in other organs by 4-Cl-BZT. Although other actions of 4-Cl-BZT could also be involved, it is noteworthy that atropine does not significantly enhance the convulsant effects of cocaine in mice (Acri et al., 1996), suggesting that the antimuscarinic properties of 4-Cl-BZT are unlikely to account for its effects on cocaine-induced seizures in the current study. Regardless of the mechanism by which DA uptake inhibitors potentiate cocaine toxicity, the current results underscore the need for caution in evaluating these compounds as potential therapeutic agents for cocaine dependence, especially in actively abusing populations.

In summary, the present in vivo microdialysis study demonstrates pronounced differences in behavioral and neurochemical effects of the widely abused psychostimulant cocaine and the related tropane analogue 4-Cl-BZT despite the high affinity of each for the DA transporter in vitro. Whereas the effects of 4-Cl-BZT on extracellular DA in the NAc were dependent on the site of administration, this compound lacks a cocaine-like locomotor stimulant profile whether administered i.p. or microinjected directly into the NAc. In both its locomotor stimulant and NAc DA effects, 4-Cl-BZT exhibited later onset and longer duration of action than cocaine regardless of route of administration, suggesting slower binding and dissociation kinetics at the DA transporter than cocaine in vivo. Of particular interest, pretreatment with i.p. 4-Cl-BZT exerted additive effects on NAc DA overflow, potentiated the locomotor stimulant effects of low-dose cocaine, and exacerbated the toxicity of high-dose cocaine despite its low stimulant efficacy when administered alone. In the context of previous work by Rothman and colleagues (Rothman, 1990; Rothman et al., 1991; Baumann et al., 1994; Rothman and Glowa, 1995), these results indicate that cocaine's interaction with mesolimbic DA neurons is altered differently by divergent classes of DA uptake inhibitors. The large series of benztropine analogs synthesized for structure-activity studies of the DA transporter (Newman et al., 1994, 1995; Kline et al., 1997) may be instrumental in understanding the mechanisms of such differences, providing future directions for development of a cocaine abuse treatment.

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References


Dopamine transporter site-directed mutations differentially alter substrate trans-


carrier.


