Maintained Cocaine Self-Administration Is Determined by Quantal Responses: Implications for the Measurement of Antagonist Potency

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ABSTRACT

The change in frequency of cocaine self-administration as a function of the unit dose is widely assumed to represent a graded pharmacodynamic response. Alternatively, a pharmacological theory states that during maintained self-administration, a quantal response occurs at a minimum maintained cocaine concentration (satiety threshold). Rats self-administered cocaine at unit doses spanning an 8-fold range from 0.75 to 6 $\mu\text{mol/kg}$. Despite an approximately 7-fold difference in the interinjection intervals, there were no differences in the plasma cocaine concentration at the time of lever press across this range of unit doses, consistent with the satiety threshold representing an equiactive cocaine concentration. Because self-administration always occurs when cocaine concentrations decline back to the satiety threshold, this behavior represents a process of automatic back titration of equiactive agonist concentrations. Therefore, the lower

frequency of self-administration at higher unit doses is caused by an increase in the duration of the cocaine-induced satiety response, and the graded dose-frequency relationship is due to cocaine pharmacokinetics. After the interinjection intervals at a particular unit dose were stable, rats were injected with the competitive D_1 -like dopamine receptor antagonist *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH23390; 15 nmol/kg intravenously) and the session continued. At all cocaine unit doses, SCH23390 accelerated self-administration with a concomitant increase in the calculated satiety threshold, and these equiactive cocaine concentration ratios were independent of the cocaine unit dose. Therefore, the measurement of antagonist potency requires only a single unit dose of cocaine, selected on the basis of convenience, and using multiple cocaine unit doses is redundant.

Introduction

A pharmacokinetic/pharmacodynamic theory of the cocaine self-administration paradigm states that in rats having acquired the behavior, cocaine-induced lever pressing occurs only when cocaine concentrations are above the priming threshold concentration but below the satiety threshold concentration (Norman and Tsibulsky, 2006). Furthermore, the theory states that when cocaine concentrations are above the satiety threshold, the time between successive administrations (T) varies as a function of the unit dose (Du) according to the equation $T = \ln(1 + Du/Dst)/k$, where Dst (satiety threshold) is the minimum concentration of cocaine at which a quantal pharmacodynamic response (lever press) occurs and k is the cocaine first-order elimination rate constant (Tsibulsky and Norman, 1999; Norman and Tsibulsky, 2006). This is assumed to be directly proportional to a minimum concentration of dopamine at which a lever press is triggered (Wise et al., 1995), and therefore, to a minimum fractional occupancy of a defined population of dopamine receptors (Norman et al., 2011a). It is implicit in this

mathematical model that the satiety threshold is constant during the maintenance phase of a self-administration session and this was confirmed by measuring the plasma cocaine concentration at the time of a lever press (Norman et al., 2011a). Therefore, the satiety threshold over time represents an equiactive agonist concentration. It is also implicit in the mathematical model that the satiety threshold is independent of the cocaine unit dose. This hypothesis was tested herein by measuring the plasma cocaine concentrations at the time of lever press during sessions in which different unit doses were self-administered.

The time between successive self-administrations of cocaine is decreased by competitive antagonists of D_1 -like (Koob et al., 1987) and D_2 -like (Yokel and Wise, 1975) dopamine receptors. According to the above-mentioned pharmacokinetic/pharmacodynamic theory of cocaine self-administration, this should be caused by an antagonist-induced increase in the cocaine satiety threshold. Whether the magnitude of the cocaine-induced response is dependent on, or independent of, the cocaine unit dose has implications for measuring the magnitude of the effect of competitive antagonists of the receptors mediating the cocaine-induced response. Competitive antagonists increase the equiactive agonist concentration and the magnitude of this shift (agonist concentration ratio) is

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directly proportional to the antagonist concentration (Schild, 1957). Although cocaine is an indirect agonist of dopamine receptors, the cocaine satiety threshold represents an equiactive cocaine concentration that is increased in the presence of dopamine receptor antagonists (Norman et al., 2011a) and the magnitude of the cocaine concentration ratio is directly proportional to the antagonist dose over a certain range of doses (Norman et al., 2011b). However, all of these studies used only a single unit dose of cocaine. Importantly, if the response is quantal and occurs at a particular agonist concentration that is independent of the cocaine unit dose, then the magnitude of the antagonist-induced increase in the satiety threshold should also be independent of the cocaine unit dose. This second hypothesis was also tested in the present studies and we report herein that despite the differences in the interinjection intervals across an 8-fold range of cocaine unit doses, the magnitude of the maximal effect of a dose of the competitive D_1 dopamine receptor antagonist, R -(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH23390), on the calculated cocaine satiety threshold is the same.

Materials and Methods

Cocaine Self-Administration Training. Male Sprague-Dawley rats (Harlan Laboratories, Indianapolis IN) with body weights of 350–500 g over the duration of these studies were housed individually on a 12-hour light/dark cycle (lights on at 6:00 AM) and food and water were available *ad libitum*. All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of Cincinnati. Surgical implantation and maintenance of intravenous catheters and self-administration training procedures were completed as previously described (Tsibulsky and Norman, 2005) using a cocaine unit dose of 1.5 $\mu\text{mol/kg}$ (0.5 mg/kg cocaine HCl). After self-administration was stably acquired during sessions conducted 5 days per week, the unit dose was changed in each session over a range of 0.3–12 $\mu\text{mol/kg}$ (which corresponds to 0.1–4.0 mg/kg cocaine HCl). During all sessions used in these studies, every lever press resulted in a cocaine injection (fixed ratio-1 schedule). The duration of access to cocaine during each daily session was for approximately 120–150 minutes followed by a 20- to 40-minute extinction phase after termination of access to cocaine.

Collection of Blood Samples during Cocaine Self-Administration. To test the hypothesis that the satiety threshold was independent of the cocaine unit dose during the maintenance phase of self-administration sessions, the plasma cocaine concentration at the time of lever presses was measured during sessions in which rats self-administered one of a range of unit doses. The procedure was similar to that used in a previous study (Norman et al., 2011a). In brief, rats that reliably self-administered cocaine via a jugular catheter were implanted with a second catheter in the left femoral vein for blood sampling. During sessions in which blood samples were collected at the time of a lever press, rats self-administered cocaine at a unit dose of 1.5, 3, or 6 $\mu\text{mol/kg}$. After 60–90 minutes, when the interinjection intervals were stable, rats were observed and the lever was disconnected after a self-injection of cocaine was complete. The rats were then observed until they pressed the lever, at which time they were quickly removed from the chamber and a blood sample (approximately 100–400 μl) was rapidly collected. The first 50 μl of the sample was discarded to avoid dilution of the blood with the heparinized saline within the catheter. The catheter was flushed with heparinized saline (50 μl), the rats were returned to the chamber, the lever was reconnected, and self-administration resumed. These

samples contained the minimum plasma cocaine concentrations during the maintenance phase of the session and were assumed to correspond to the cocaine satiety threshold.

Analysis of Plasma Cocaine Concentrations. The procedures used to collect blood, separate plasma and store it, and then to chemically extract cocaine and measure cocaine concentrations using gas chromatography/mass spectroscopy were the same as previously described (Norman et al., 2007, 2011a).

The Effect of SCH23390 on Self-Administration of Different Cocaine Unit Doses. The sessions in which the magnitude of effect of SCH23390 was measured were conducted on Monday, Wednesday, and Friday. Sessions were not run on Tuesdays and Thursdays. At the start of each session, the first four unit doses were 1.5 $\mu\text{mol/kg}$. Once the loading phase was complete and after stable self-administration was established, the unit dose was changed to one of either 0.75, 1.5, 3, or 6 $\mu\text{mol/kg}$. After approximately 90–120 minutes of stable maintained self-administration, SCH23390 [15 nmol/kg (5 $\mu\text{g/kg}$ i.v. SCH23390 HCl)] was rapidly administered and the session continued. Because SCH23390 was expected to increase the rate of cocaine self-administration, the 12 $\mu\text{mol/kg}$ unit dose was excluded from this phase of the study over concerns that the resulting peak cocaine concentrations may be lethal. At unit doses lower than 0.75 $\mu\text{mol/kg}$, the injection of SCH23390 caused a cessation of cocaine self-administration in a high proportion of sessions. The rate of self-administration was presented graphically as a cumulative event record.

Real-Time Calculation of Cocaine Levels in the Body. The cocaine level in the body was calculated by monitoring the amount that was administered and then using predetermined pharmacokinetic values to estimate the resulting levels in individual animals every second during each session. The volume of distribution of cocaine, approximately 1.3–1.9 l/kg (Norman et al., 2011a), was assumed to be constant for each rat and the cocaine level in the body was calculated according to a simplified linear equation for the zero-order input/first-order elimination kinetics for a two-compartment model as previously described in detail (Tsibulsky and Norman, 2005).

Agonist Concentration Ratios. On the basis of receptor occupancy theory, the magnitude of competitive antagonist-induced increases in the equiactive agonist concentration (concentration ratio) should be directly proportional to the antagonist concentration (Schild, 1947, 1957; Colquhoun, 2007). The mean of the values for the calculated level of cocaine at the time of each lever press during the maintenance phase, prior to the injection of antagonist, represented the baseline satiety threshold. As described previously (Norman et al., 2011b), the level of cocaine at the time of each lever press after the injection of antagonist was divided by the baseline value for that session and the resulting value represented the cocaine concentration ratio. The maximal magnitude of SCH23390-induced increase in the cocaine concentration ratio was calculated by the mean of the four to six maximal values for the 0.75, 1.5, and 3 unit doses and the two to four maximal values at the 6 $\mu\text{mol/kg}$ unit dose.

Materials. Cocaine HCl was obtained from Research Triangle Institute (Research Triangle Park, NC) under the National Institute on Drug Abuse drug supply program. Cocaine HCl (40 $\mu\text{mol/ml}$) was dissolved in normal saline solution containing one unit per milliliter of heparin and then passed through a sterile 0.2- μm acetate filter immediately prior to use in the self-administration studies. Heparin sodium was obtained from American Pharmaceutical Partners, Inc. (Schaumburg, IL). Streptokinase and R -(+)-SCH23390 HCl were purchased from Sigma-Aldrich (St. Louis, MO). SCH23390 was prepared daily in sterile normal saline from stock solutions (each 10 $\mu\text{mol/ml}$ in absolute ethanol and stored at -20°C). Methohexital sodium (Brevital) was manufactured by King Pharmaceuticals (Bristol, TN). Cocaine and benzoyllecgonine used as an external standard (1 mg/ml) and internal standard (cocaine- D_3 and benzoyllecgonine- D_3 each 0.1 mg/ml in methanol or acetonitrile) were purchased from Radian International LLC (Austin, TX). Rat plasma with heparin was purchased from Harlan Bioproducts for Science

(Indianapolis, IN). All other chemicals were purchased from Sigma-Aldrich or Pierce Chemicals (Rockford, IL) at the highest available purity and were used without any further purification.

Results

The Cocaine Satiety Threshold Is Independent of the Cocaine Unit Dose. As shown in Fig. 1, the plasma cocaine concentration at the time of a lever press (satiety threshold) during the maintenance phase of a session was not significantly different [$P = 0.89$, one-way analysis of variance (ANOVA) with repeated measures] at cocaine unit doses of 1.5, 3, and 6 $\mu\text{mol/kg}$. This was also indicated by the linear regression line with a slope not significantly different from zero. The mean plasma cocaine concentration corresponding to the satiety threshold was approximately 3.6 μM (1.1 mg/l) across this range of unit doses.

The Unit Dose-Dependent Rate of Cocaine Self-Administration. As shown in the representative sessions in Fig. 2A, the interinjection intervals were stable at cocaine unit doses of 0.75 and 6 $\mu\text{mol/kg}$ prior to the injection of SCH23390 and were proportional to the unit dose. The group mean $\pm$ S.E.M. interinjection intervals for these rats were 1.8 ± 0.2 minutes ($n = 6$ rats) and 12.0 ± 0.7 minutes ($n = 7$ rats) at unit doses of 0.75 and 6.0 $\mu\text{mol/kg}$, respectively. Consequently, the frequency of lever presses was approximately 6 to 7 times greater at the 0.75 $\mu\text{mol/kg}$ unit dose compared with the 6.0 $\mu\text{mol/kg}$ unit dose. After the injection of SCH23390, the rate of self-administration increased at both unit doses.

The Calculated Cocaine Satiety Threshold Was Independent of the Unit Dose. Despite the approximately 7-fold difference in mean interinjection intervals at these different unit doses, the calculated levels of cocaine at the time of each injection (shown in Fig. 2A) prior to the injection of SCH23390 were similar (Fig. 2B). The values for the group mean $\pm$ S.E.M. baseline satiety thresholds were 4.6 ± 0.4

Fig. 1. The cocaine satiety threshold is independent of the cocaine unit dose. The symbols represent the mean $\pm$ S.E.M. ($n = 5$ rats) plasma cocaine concentration at the time of a lever press during the maintenance phase of self-administration sessions. The linear regression line has a slope of 0.05, which is not significantly different from zero, indicating that there is no significant difference ($P = 0.89$, one-way ANOVA with repeated measures) in the magnitude of the satiety threshold across this range of unit doses.

Fig. 2. The effect of SCH23390 on the self-administration of different cocaine unit doses. (A) In these cumulative event records from representative sessions, each vertical increment represents a self-administration and the horizontal distance represents the interinjection interval. Rats self-administered cocaine at unit doses of 0.75 or 6 $\mu\text{mol/kg}$. There were a total of 258 and 48 self-administrations per session at unit doses of 0.75 and 6.0 $\mu\text{mol/kg}$, respectively. (B) The symbols represent the calculated cocaine level at the time of each lever press during the representative sessions shown in (A). The time of SCH23390 injection is indicated in each panel.

($n = 6$ rats), 4.2 ± 0.4 ($n = 7$ rats), 4.2 ± 0.4 ($n = 7$ rats), and 3.6 ± 0.2 $\mu\text{mol/kg}$ ($n = 7$ rats) at unit doses of 0.75, 1.5, 3, and 6 $\mu\text{mol/kg}$, respectively. There were no significant differences between these values ($P = 0.31$, one-way ANOVA).

The Magnitude of the Effect of SCH23390 Was Independent of the Cocaine Unit Dose. At both unit doses of cocaine, the SCH23390-induced increase in the calculated cocaine concentration in the body at the time of each lever press reached a peak magnitude of approximately 3-fold greater than the mean baseline value (Fig. 2B). This peak occurred at approximately 24–30 minutes and subsequently, the calculated cocaine concentration at the time of each press decreased in an apparent first-order manner. Despite the 7-fold greater number of presses at the 0.75 $\mu\text{mol/kg}$ unit dose, there was no difference in the time course of the SCH23390-induced increase in the cocaine satiety threshold.

Consistent with the representative sessions shown in Fig. 2B, there was no significant difference ($P = 0.9$, one-way

ANOVA) in the peak magnitude of the SCH23390-induced increase in the satiety threshold across the range of unit doses used (Fig. 3).

Discussion

During maintained cocaine self-administration, the plasma cocaine concentration corresponding to the satiety threshold is constant over several hours (Norman et al., 2011a). It is now demonstrated that the satiety threshold is also constant across a range of cocaine unit doses. This validates a major assumption of the satiety threshold model of maintained cocaine self-administration (Tsibulsky and Norman, 1999). Therefore, self-administration behavior represents a quantal pharmacodynamic response that occurs at the same cocaine concentration, which is independent of the agonist unit dose. This finding is consistent with previous studies in rats that self-administered amphetamine, in which the plasma concentration of amphetamine at the time of an attempt to self-administer was constant during a session and constant across a range of unit doses (Yokel and Pickens, 1974). Because the concentration of cocaine at the time of each self-administration is the same across unit doses, the corresponding increase in the interinjection intervals (Pickens and Thompson, 1968) cannot represent an increase in the magnitude of the cocaine-induced pharmacodynamic response. A more plausible explanation for the increase in interinjection intervals as a function of the cocaine unit dose is that it takes longer for the higher concentrations of cocaine produced by the higher unit doses to decline back to the constant concentration at which the quantal pharmacodynamic response occurs (Tsibulsky and Norman, 1999; Norman et al., 2011a). Therefore, the increase in the interinjection interval as a function of the unit dose represents an increase in the duration of the cocaine-induced satiety response (Tsibulsky and Norman, 2012).

Fig. 3. The peak magnitude of effect of SCH23390 as a function of cocaine unit dose. Symbols represent the mean $\pm$ S.E.M. maximum cocaine concentration ratio from seven rats at the 1.5, 3, and 6 $\mu\text{mol/kg}$ unit doses or six rats at 0.75 $\mu\text{mol/kg}$. There was no significant difference in mean values across unit doses ($P = 0.9$, one-way ANOVA) and linear regression analysis with a slope approaching zero (slope = 0.03).

The whole process of maintained self-administration can be viewed as the interinjection interval being the result of the automatic back titration of cocaine concentrations to the satiety threshold. The cycle starts when the cocaine concentration exceeds the satiety threshold. The time until the next self-administration occurs is simply the time required to metabolize the last dose of cocaine so that the concentration falls back to the satiety threshold. The new cycle is initiated automatically because of the feedback mechanism intrinsic to the arrangement of the experiment. Importantly, according to this explanation, the lower unit doses merely increase the frequency at which the cocaine satiety threshold is detected.

The cocaine satiety threshold represents an equiactive agonist concentration and competitive antagonists increase equiactive agonist concentrations (Schild, 1957). Indeed, competitive dopamine receptor antagonists increase the satiety threshold during maintained cocaine self-administration in rats (Norman et al., 2011a). In the present study, the maximal magnitude of the increase in the satiety threshold produced by a single dose of SCH23390 was independent of the cocaine unit dose. The same magnitude of effect of the antagonist at different unit doses is consistent with the unit dose changing the frequency with which the equiactive concentration of cocaine is measured. The lower the unit dose, the higher the temporal resolution of the antagonist effect, as illustrated by the greater than 7-fold increase in the number of measurements of the satiety threshold at 0.75 versus 6 $\mu\text{mol/kg}$ cocaine unit doses. Importantly, multiple cocaine unit doses are redundant when measuring the pharmacodynamic potency and pharmacokinetics of antagonists using cocaine self-administration behavior (Norman et al., 2011b). The appropriate agonist unit dose can be selected by the investigator based entirely on practical considerations. For example, the time required to inject the antagonist during the session should not interfere with the next lever press. At low unit doses, there is an increased probability that the time to inject an antagonist and return the animal to the chamber will exceed the time between lever presses. On the other hand, the frequency of measurement of the cocaine satiety threshold is lower at higher unit doses, which increases the probability of missing the time of the peak antagonist effect and thus underestimating the antagonist potency.

Because the cocaine satiety threshold concentration is calculated based on an assumed elimination rate constant of cocaine, an important caveat is that the antagonist does not change this parameter, and then the mathematical model holds. The dose of SCH23390 used in the present study has previously been shown to not change the elimination rate constant of cocaine in rats (Norman et al., 2011a). Furthermore, in the present study, the lack of effect of the unit dose of cocaine on the time course of onset and disappearance of the SCH23390-induced increase in the cocaine satiety threshold indicates that cocaine does not alter the elimination rate constant for SCH23390 in rats.

In conclusion, the pharmacodynamic response is quantal in nature and the cocaine concentration at which the quantal response occurs is constant across a wide range of unit doses. The independence of the magnitude of antagonist-induced effect as a function of cocaine unit dose is consistent with the pharmacokinetic/pharmacodynamic explanation of the cocaine self-administration paradigm. The practical implication

is that measurements of antagonist potency are appropriately accomplished using only a single cocaine unit dose, which can be selected on the basis of the required temporal resolution, and the use of multiple cocaine unit doses is redundant.

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Authorship Contributions

Participated in research design: A.B. Norman, Tsibulsky.

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Wrote or contributed to the writing of the manuscript: A.B. Norman, Tabet, M.K. Norman, Tsibulsky.

References

- Colquhoun D (2007) Why the Schild method is better than Schild realised. *Trends Pharmacol Sci* **28**:608–614.
- Koob GF, Le HT, and Creese I (1987) The D₁ dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. *Neurosci Lett* **79**:315–320.
- Norman AB and Tsibulsky VL (2006) The compulsion zone: a pharmacological theory of acquired cocaine self-administration. *Brain Res* **1116**:143–152.
- Norman AB, Tabet MR, Norman MK, Buesing WR, Pesce AJ, and Ball WJ (2007) A chimeric human/murine anticocaine monoclonal antibody inhibits the distribution of cocaine to the brain in mice. *J Pharmacol Exp Ther* **320**:145–153.
- Norman AB, Norman MK, Tabet MR, Tsibulsky VL, and Pesce AJ (2011a) Competitive dopamine receptor antagonists increase the equiactive cocaine concentration during self-administration. *Synapse* **65**:404–411.
- Norman AB, Tabet MR, Norman MK, and Tsibulsky VL (2011b) Using the self-administration of apomorphine and cocaine to measure the pharmacodynamic potencies and pharmacokinetics of competitive dopamine receptor antagonists. *J Neurosci Methods* **194**:252–258.
- Pickens R and Thompson T (1968) Cocaine-reinforced behavior in rats: effects of reinforcement magnitude and fixed-ratio size. *J Pharmacol Exp Ther* **161**:122–129.
- Schild HO (1947) pA, a new scale for the measurement of drug antagonism. *Br Pharmacol Chemother* **2**:189–206.
- Schild HO (1957) Drug antagonism and pAx. *Pharmacol Rev* **9**:242–246.
- Tsibulsky VL and Norman AB (1999) Satiety threshold: a quantitative model of maintained cocaine self-administration. *Brain Res* **839**:85–93.
- Tsibulsky VL and Norman AB (2005) Real time computation of in vivo drug levels during drug self-administration experiments. *Brain Res Brain Res Protoc* **15**:38–45.
- Tsibulsky VL and Norman AB (2012) Simple deterministic model of maintained drug self-administration behavior and its pharmacological applications, in *Computational Neuroscience of Drug Addiction* (Gutkin B and Ahmed AH, eds) pp 3–18, Springer, NY.
- Wise RA, Newton P, Leeb K, Burnette B, Pocock D, and Justice JB, Jr (1995) Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. *Psychopharmacology (Berl)* **120**:10–20.
- Yokel RA and Wise RA (1975) Increased lever pressing for amphetamine after pimozide in rats: implications for a dopamine theory of reward. *Science* **187**:547–549.
- Yokel RA and Pickens R (1974) Drug level of d- and l-amphetamine during intravenous self-administration. *Psychopharmacology (Berl)* **34**:255–264.

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