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The Affinity of D_2-Like Dopamine Receptor Antagonists Determines the Time to Maximal Effect on Cocaine Self-Administration

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ABSTRACT

Differences in the time to maximal effect (T_{max}) of a series of dopamine receptor antagonists on the self-administration of cocaine are not consistent with their lipophilicity (octanol-water partition coefficients at pH 7.4) and expected rapid entry into the brain after intravenous injection. It was hypothesized that the T_{max} reflects the time required for maximal occupancy of receptors, which would occur as equilibrium was approached. If so, the T_{max} should be related to the affinity for the relevant receptor population. This hypothesis was tested using a series of nine antagonists having a 2500-fold range of K_i or K_d values for D_2-like dopamine receptors. Rats self-administered cocaine at regular intervals and then were injected intravenously with a dose of antagonist, and the self-administration of cocaine was continued for 6 to 10 h. The level of cocaine at the time of every self-administration (satiety threshold) was calculated throughout the session. The satiety threshold was stable before the injection of antagonist and then increased approximately 3-fold over the baseline value at doses of antagonists selected to produce this approximately equivalent maximal magnitude of effect (maximum increase in the equiactive cocaine concentration, satiety threshold; C_{max}). Despite the similar C_{max}, the mean T_{max} varied between 5 and 157 min across this series of antagonists. Furthermore, there was a strong and significant correlation between the in vivo T_{max} values for each antagonist and the affinity for D_2-like dopamine receptors measured in vitro. It is concluded that the cocaine self-administration paradigm offers a reliable and predictive bioassay for measuring the affinity of a competitive antagonist for D_2-like dopamine receptors.

Introduction

The self-administration of dopamine receptor agonists represents a useful bioassay system for measuring the pharmacodynamic potencies of competitive antagonists of brain dopamine receptors (Roberts and Vickers, 1984; Norman et al., 2011b). In addition, the time course of the change in magnitude of the antagonist-induced elevation of the agonist satiety threshold may reflect the change in the antagonist concentration in the brain, i.e., its pharmacokinetics. Indeed, the time course of the diminution of the (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH23390) (a D_1 dopamine receptor antagonist)-induced effect on cocaine and apomorphine self-administration (Norman et al., 2011b) was consistent with its reported elimination half-life from plasma in rats (Kilts et al., 1985; Hietala et al., 1992). However, the approximate 30-min time to maximal effect (T_{max}) for SCH23390 is unlikely to reflect the peak brain concentration after an intravenous injection because positron emission tomography studies of [^{11}C]SCH23390 in humans show peak brain concentrations occurring by approximately 10 min (Farde et al., 1987). On the other hand, it is possible that the time course of antagonist-induced increases in the rate of cocaine self-administration reflects, at least in part, the antagonist's pharmacodynamic properties.

According to a pharmacological model of the regulation of cocaine self-administration behavior, the minimal maintained concentration of cocaine represents the satiety threshold (Tsibulsky and Norman, 1999; Norman and Tsibulsky,

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ABBREVIATIONS: SCH23390, (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; T_{max}, time to maximal effect; C_{max}, maximum increase in the equiactive cocaine concentration (satiety threshold); pA_3, negative logarithm of the antagonist dose (or concentration) required to induce a 3-fold increase in the agonist concentration ratio; V_d, volume of distribution; log D, distribution coefficient or octanol-water partition coefficient at a defined pH; log P, octanol-water partition coefficient.

2006). The cocaine satiety threshold remains constant throughout the maintenance phase of a self-administration session and, under these conditions, represents an equiactive cocaine concentration (Norman et al., 2011a). Because the cocaine concentration during self-administration is directly proportional to the striatal dopamine concentration (Nicolaysen et al., 1988), the satiety threshold is assumed to represent a specific fractional occupancy of dopamine receptors. Competitive antagonists increase equiactive agonist concentrations (Schild, 1947; Colquhoun, 2007) and increase the cocaine satiety threshold (Norman et al., 2011a). Therefore, the time course of antagonist-induced increases in the satiety threshold may reflect the time course of the antagonist occupancy of the relevant receptor population. Indeed, occupancy of a population of dopamine receptors by [^{3}H]SCH23390 in vitro increased over time until equilibrium was approached (Gifford et al., 1998). For a subsaturating concentration of ligand, the time at which equilibrium is approached should correspond to the maximal fractional occupancy and in a physiological system in vivo should correspond to T_{max}. Because the time to approach equilibrium is dependent on the ligand affinity, T_{max} should be proportional to the affinity of the antagonist for the receptors mediating the agonist-induced response. If so, the T_{max} for the antagonist-induced increase in the cocaine satiety threshold should be proportional to the antagonist affinity for the receptor population mediating the satiety response. This hypothesis was tested in rats using a series of competitive antagonists with K_i or K_d values for D_2 dopamine receptors spanning an approximately 2500-fold range. It is reported herein that there is a strong and significant correlation between the T_{max} for these antagonists on cocaine self-administration in rats in vivo and their reported in vitro affinities for D_2 dopamine receptors.

Materials and Methods

Cocaine Self-Administration Training. Male Sprague-Dawley rats (initial weight 180–200 and 400–500 g over the duration of these studies; Harlan, Indianapolis, IN) were housed individually on a 12-h 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* (Institute of Laboratory Animal Resources, 1996) and approved by the Institutional Animal Care and Use Committee at the University of Cincinnati. Surgical implantation and maintenance of intravenous catheters and cocaine self-administration training procedures were completed as described previously (Norman and Tsibulsky, 2006) for all rats.

Test chambers (Med Associates Inc., St. Albans, VT) were each equipped with an active and an inactive lever. Each chamber was situated inside of a laminated wooden compartment (43 × 61 × 35 cm) that provided sound attenuation and was equipped with a house light. Infusion pumps (model PHM-100; Med Associates Inc.) were situated outside of the compartments. Computers controlled unconditioned stimuli (drug injection) using a program written in Medstate Notation language (Med Associates, Inc.). After stable self-administration was acquired, the interinjection intervals as a function of the cocaine unit dose (over a range from 0.75 to 6 μmol/kg) were measured during a series of daily sessions. Every lever press when the infusion pump was not activated resulted in a cocaine injection. For all experiments reported here, during daily sessions run 3 days/week, rats self-administered a unit dose of 3 μmol/kg cocaine. After approximately 90 min of a stable rate of self-administration, a single dose of one of a series of dopamine receptor antagonists was injected intravenously, and the session was continued. During preliminary experiments, the dose of each antagonist that produced an approximately 3-fold maximal increase in the equiactive cocaine concentration (satiety threshold) (C_{max}) was determined [i.e., analogous to the antagonist pA_3 value (Schild, 1947, 1957)]. This magnitude of maximal effect was selected because it was reliably differentiated from the baseline values but the corresponding antagonist dose was not large enough to produce a cessation of self-administration behavior (Ettenberg et al., 1982; Norman et al., 2011b). The approximately equipotent dose of each antagonist was as follows: nemonapride, 10 nmol/kg, 0.004 mg/kg; (−)-eticlopride, 20 nmol/kg, 0.008 mg/kg; spiperone, 75 nmol/kg, 0.03 mg/kg; haloperidol, 100 nmol/kg, 0.04 mg/kg; aripiprazole, 200 nmol/kg, 0.09 mg/kg; molindone, 750 nmol/kg, 0.23 mg/kg; trifluprom azine, 750 nmol/kg, 0.3 mg/kg; olanzapine, 1500 nmol/kg, 0.47 mg/kg; and thioridazine, 6000 nmol/kg, 2.4 mg/kg.

Real-Time Calculation of Cocaine Levels in the Body. The cocaine levels in the body were calculated by monitoring the amount of cocaine that was administered to the animals and then using predetermined pharmacokinetic values to estimate the resulting cocaine levels in the animals over time. The calculated values for the whole-body cocaine levels depend on the amount injected per kilogram of body weight minus the amount eliminated per unit time. Therefore, the cocaine level in the body was calculated every second according to the simplified linear equation for the zero-order input and first-order elimination kinetics for a two-compartment model (Tsibulsky and Norman, 2005; Norman et al., 2011b). The volume of distribution (V_d) of cocaine was assumed to be constant and the calculated cocaine level (L) was assumed to be directly proportional to the cocaine concentration (C) according to the equation $C = L/V_d$.

Cocaine Concentration Ratios. Competitive antagonists increase agonist concentration ratios, and the magnitude of this shift is 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). The mean of the values for the level of cocaine at the time of each lever press (satiety threshold) during the maintenance phase (not including the initial loading phase) and before the injection of antagonist represented the baseline satiety threshold. 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. These cocaine concentration ratios minus 1 were plotted as a function of time after the injection of each antagonist.

Modeling and Statistical Analysis. Agonist concentration ratios were assumed to be proportional to the antagonist fractional occupancy of the receptor population underlying the agonist-induced satiety response. The maximal concentration ratio was the mean of the highest four to six values, and the T_{max} for each session was the mean of these same time values. A single peak logarithmic function was applied to the data, which provided a general description of the time course of antagonist effects for these antagonists. Linear regression analysis was applied to the plot of the log mean T_{max} values as a function of the mean log D (distribution coefficient) values and to the K_i or K_d values for D_2-like dopamine receptors and the correlation coefficients and statistical significance were reported. The log D values are a measure of lipophilicity and represent the ratio of the sum of the concentrations of ionized and un-ionized forms of the drugs partitioning into an immiscible mixture of water and octanol. This is similar to the partition coefficient (log P) but log D is pH-dependent and is a measure of lipophilicity in vivo. The mean log D values at pH 7.4 were calculated according to the equation $\log D = \log P - \log(1 + 10^{pK_a - pH})$, where pK_a is the acid dissociation constant (Scherrer and Howard, 1977). This equation is relevant to com-

pounds that are weak bases. The log P and pK_a values for this series of antagonists were obtained from the online CAS (a division of the American Chemical Society) REGISTRY database (http://www.cas.org/expertise/cascontent/registry/index.html) from 2009 and accessed using SciFinder. These values were verified by comparison with log D (7.4) values from the ChemSpider database (http://www.chemspider.com). The K_i or K_d values for the D_2-like dopamine receptor antagonists were obtained from the reviews by Seeman (1993, 2010) and the Collaborative Drug Discovery online database (http://www.collaborativedrug.com). Values for spiperone were obtained only from Hamblin et al. (1984) and Malmberg et al. (1996) because these studies were the only ones to use incubation times of at least 3 h, consistent with our T_{max} values for spiperone in vivo.

Drugs. Cocaine HCl was supplied by the Research Triangle Institute (Research Triangle Park, NC) through the National Institute on Drug Abuse drug supply program. Spiperone (base), haloperidol (base), (−)-eticlopride HCl, triflupromazine HCl, thioridazine HCl, and molindone HCl were purchased from Sigma-Aldrich (St. Louis, MO). Nemonapride (base) was purchased from Tocris Bioscience (Ellisville, MO). Olanzapine (Zyprexa IntraMuscular; Eli Lilly & Co., Indianapolis, IN) and aripiprazole (Abilify Injection; Bristol-Myers Squibb, Stamford, CT) were purchased from the Pharmacy Services at the University Hospital (Cincinnati, OH).

Results

As shown in Fig. 1A, after an initial series of rapid self-administrations, the rate of cocaine self-administration was relatively constant over a 90-min period. After the injection of eticlopride, there was an increase in the rate of cocaine self-administration. Over the subsequent 6 h, the rate of cocaine self-administration gradually returned to baseline rates. Figure 1B shows the calculated cocaine level at the time of every lever press shown in Fig. 1A. After the initial loading phase in which cocaine concentrations increased rapidly, the calculated cocaine level at the time of each lever press (satiety threshold) remained relatively constant over the 90-min period when the rate of self-administration was also constant. After the injection of eticlopride, there was a rapid increase in the cocaine satiety threshold that peaked after approximately 27 min, after which the satiety threshold declined toward baseline over the subsequent 6 h.

As shown in Fig. 2, in representative sessions there was a similar magnitude of peak increase (C_{max}) in the equiactive cocaine concentration in response to the injections of these four antagonists. However, the time to reach C_{max} (T_{max}) was markedly different among these antagonists. The mean ± S.E.M. T_{max} from the number of rats shown in parentheses was 5 ± 0.3 min (n = 5), 27 ± 1 min (n = 6), 57 ± 5 min (n = 5), and 157 ± 2 min (n = 5) for molindone, eticlopride, nemonapride, and spiperone, respectively.

There was a weak and not significant correlation (r = −0.42, p = 0.264) between the log D at pH 7.4 and the T_{max} values for this series of antagonists (Fig. 3). The contrast is readily observed by comparison of spiperone and molindone, which have similar mean log D values (1.74 and 1.83, respectively) but have the longest (157 min) and shortest (5 min) mean T_{max}, respectively, of this series of antagonists.

In contrast to the weak correlation between antagonist log D values and T_{max}, there was a strong and significant inverse correlation (r = −0.98, p < 0.001) between literature values for the K_i or K_d of these antagonists at D_2-like dopamine receptors and the T_{max} values for these antagonist-induced increases in the cocaine concentration ratio measured during maintained cocaine self-administration (Fig. 4).

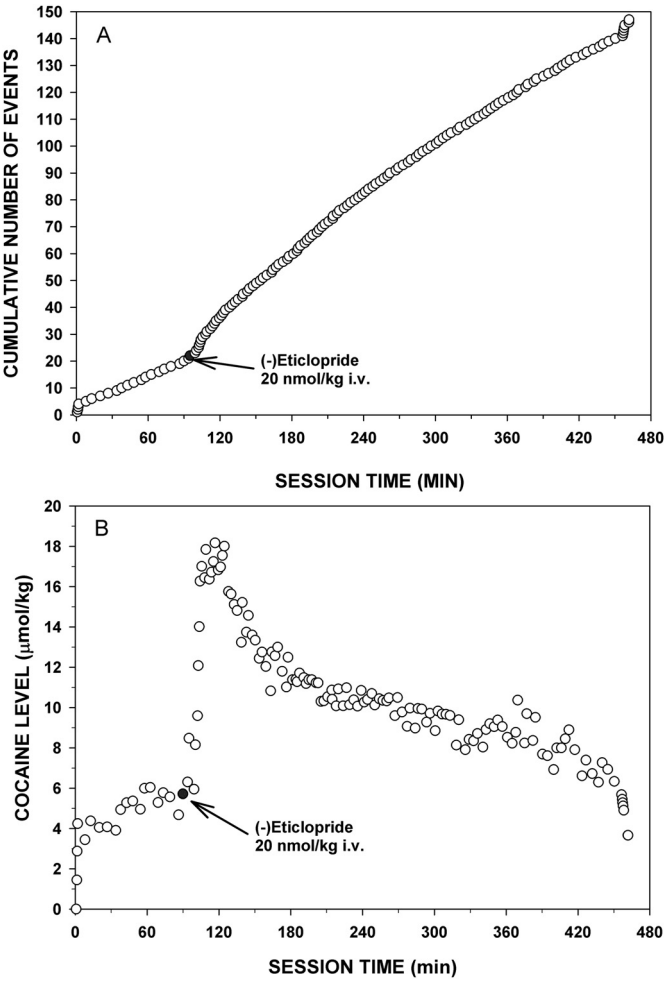

Fig. 1. Time course of the effect of a dopamine receptor antagonist on cocaine self-administration. Rats self-administered cocaine at a unit dose of 3 μmol/kg. After approximately 2 h, the D_2-like dopamine receptor antagonist (−)-eticlopride was rapidly injected intravenously. A, cumulative record of lever presses from a representative session. Each response is represented by a vertical increment, and the horizontal distance represents the interpress interval. After the initial loading phase, the interinjection intervals were stable during the maintenance phase. After the injection of eticlopride, self-administration accelerated. Approximately 6 h after the antagonist injection, access to cocaine was terminated, and responding was recorded until it was extinguished. B, calculated level of cocaine at the time of each self-administration shown in A. The baseline value is the mean of the cocaine levels at the time of self-administration (satiety threshold) during the maintenance phase of the session after loading is completed and before the injection of antagonist.

Discussion

The approximately 30-fold difference in T_{max} values across this series of antagonists is a striking example of the ability of the cocaine self-administration paradigm to differentiate between different antagonists. Explaining this marked difference may provide important insights into the mechanisms underlying the regulation of this behavior.

An obvious explanation for the differences in T_{max} could be related to the rapidity with which the different antagonists penetrate the blood-brain barrier. This is often related to the physicochemical properties of compounds, especially lipophi-

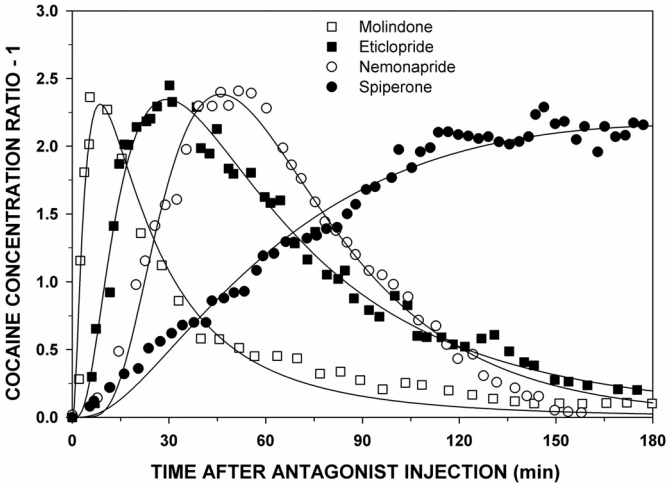

Fig. 2. The time courses of antagonist-induced increases in the cocaine satiety threshold are different. Symbols represent the proportional change in cocaine satiety threshold from the baseline value after the injection of antagonist. Time 0 is the time of antagonist injection. The doses of molindone, eticlopride, nemonapride, and spiperone were 750, 20, 10, and 75 nmol/kg, respectively. The data for eticlopride were from the same representative session shown in Fig. 1. The time to maximal response for these representative sessions were 5, 25, 45, and 180 min for molindone, eticlopride, nemonapride, and spiperone, respectively.

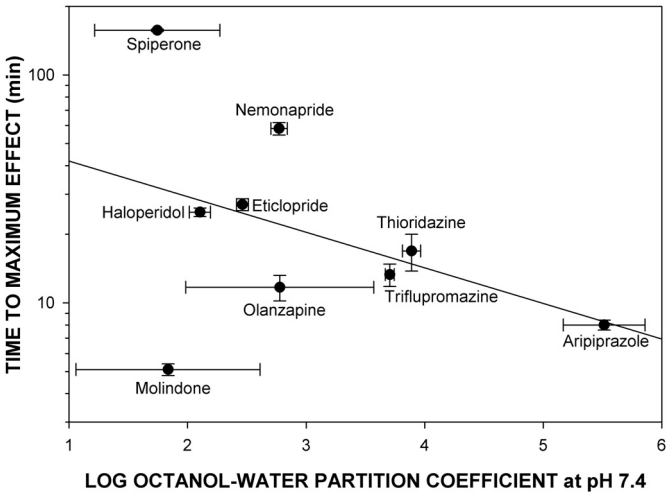

Fig. 3. The correlation of T_{max} for the antagonist-induced effect on cocaine self-administration and the antagonist log D. The mean log D at pH 7.4 values were calculated from the log P and pK_a values taken from the CAS REGISTRY online database. The linear regression line has a correlation coefficient of $r = -0.42$.

licity at physiological pH, but also may be related to differences in specific transporter processes for different small molecules. However, the differences in T_{max} are probably not due to the physicochemical differences within this series of antagonists. For example, spiperone and haloperidol are both butyrophenones and are, therefore, structurally similar yet have an approximately 6-fold difference in T_{max}. Likewise, the lipid solubility as measured by log D at pH 7.4 values, which may reflect in part the ability of a molecule to cross the blood-brain barrier, varies by more than 6000-fold across this series of antagonists. However, this measure of lipophilicity does not appear to correlate with T_{max}. This is readily apparent when spiperone and molindone, which have the most similar log D values among this series of antagonists and yet have a 30-fold difference in T_{max} values, are

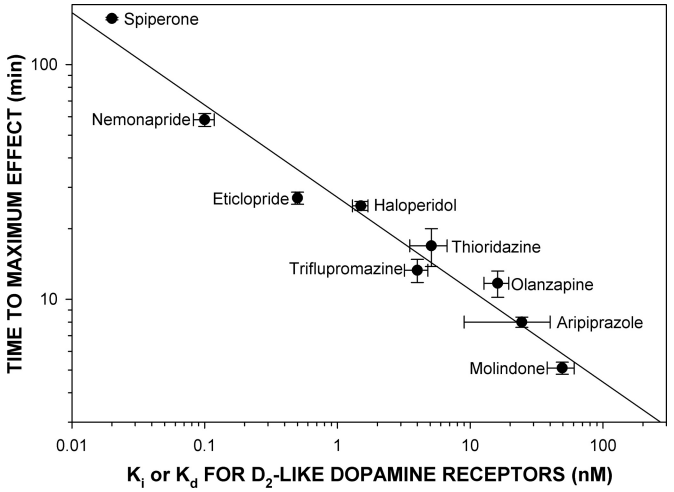

Fig. 4. The T_{max} of antagonist effect on cocaine self-administration correlates with antagonist affinity for D_2-like dopamine receptors. The symbols represent the mean ± S.E.M. for both the K_i or K_d and T_{max} values. The affinity values were taken from literature sources. The linear regression line has a correlation coefficient of $r = -0.98$.

compared. Therefore, the time to maximal effect may not represent the rate at which brain concentrations of these antagonists increase after intravenous injection. Indeed, in the previously published comparison of the effects of the D_1 and D_2 receptor antagonists SCH23390 and eticlopride on apomorphine and cocaine self-administration in rats (Norman et al., 2011b), it was noted that the 25- to 30-min T_{max} after intravenous injection was not consistent with the rapid distribution of these compounds to the human brain. It was speculated that the time course of the antagonist-induced increase in cocaine concentration ratios might represent the rate at which fractional occupancy of the relevant receptor population increases and that T_{max} would represent the time to approach equilibrium (Norman et al., 2011b). If so, it was predicted that T_{max} would be proportional to antagonist affinity for the relevant receptor population.

The strong correlation between T_{max} and affinity for D_2 dopamine receptors is consistent with the hypothesis that T_{max} represents the time for these antagonists to reach the maximal fractional occupancy at a receptor population that mediates the effects of cocaine. If so, then T_{max} may represent a bioreporter for the absolute pharmacodynamic potency (K_d) of these D_2 receptor antagonists. Thus, the affinity of any D_2 receptor antagonist could be determined by measuring its T_{max} on cocaine self-administration in vivo and comparing this to a standard curve such as that shown in Fig. 4. It is obvious that the accuracy of this method of determining the affinity of D_2 receptor antagonists is dependent on the reliability of both the T_{max} measurements in vivo and the antagonist K_i or K_d measurements in vitro. If the measurements of T_{max} after intravenous injections are demonstrated to be replicable among different laboratories, then the in vivo method used here may be a useful adjunct to the standard in vitro methods for measuring antagonist affinity. It should be noted that any reported antagonist K_d values measured in vitro that use higher affinity ligands and incubation times less than 3 h should be viewed with caution. The same caution concerning reported antagonist K_i values is also warranted when they are determined from competition binding assays in which the antagonist and/or the radioligand has

high affinity for a receptor population and the incubation time is less than several hours. This is because if the antagonist T_{max} values occur when the maximal fractional occupancy of a receptor population by a given dose is attained, then T_{max} values probably represent the time for the antagonist-receptor complexes to achieve equilibrium. Because the antagonist concentration should be declining in vivo, T_{max} would probably be the minimal time to approach equilibrium at a particular dose of antagonist.

The antagonist doses that were used in the present studies correspond to their pA_3 values, which reflect the in vivo potency of these antagonists. Of interest, the in vivo potencies of these antagonists do not correlate well with their K_i or K_d values measured in vitro. For example, the in vivo rank order of potency of this series of antagonists showed that nemonapride was the most potent (the lowest pA_3 value) and spiperone was approximately equipotent with haloperidol on a molar basis. In contrast, the clear differences in T_{max} between spiperone, nemonapride, and haloperidol did correlate with their in vitro K_i or K_d values. Thus, T_{max} rather than K_{dose} (or apparent pA_2) appears to more accurately reflect the absolute affinity for dopamine receptors. It is assumed that the in vivo potency of antagonists, as measured by K_{dose}, is related to K_d and the V_d ($K_{dose} = K_d \cdot V_d$). As K_{dose} can be determined by measuring C_{max} as a function of antagonist dose (Norman et al., 2011b), and K_d may be determined from the T_{max}, V_d can then be calculated. The differences between in vivo potency and in vitro affinity measures probably reflect wide differences in the apparent V_d among these antagonists. It is not clear why spiperone has a very large apparent V_d in vivo, but this may be due to spiperone being a substrate for the efflux drug transporter P-glycoprotein (Seelig, 1998; Wang et al., 2005) that would lower brain concentrations of spiperone and require larger plasma concentrations and concomitant larger doses to achieve effective concentrations in the brain.

In summary, the differences in T_{max} values for a series of competitive antagonists after intravenous injection correlate with their respective affinities for D_2-like dopamine receptors. The time course of the onset of effects on cocaine self-administration provides a method for determining the absolute potency of antagonists on receptors that mediate the cocaine-induced satiety response. Because the affinity of D_2 dopamine receptor antagonists correlated with their antipsychotic potency in humans (Creese et al., 1976; Seeman et al., 1976) this assay system may also represent a reliable in vivo bioassay system for measuring antipsychotic potency (Roberts and Vickers, 1984). The data reported herein further demonstrate that the cocaine self-administration paradigm represents a reliable high content in vivo bioassay system for measuring important pharmacodynamic as well as pharmacokinetic parameters for competitive dopamine receptor antagonists.

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Authorship Contributions

Participated in research design: A.B. Norman.

Conducted experiments: A.B. Norman, Tabet, M.K. Norman, and Fey.

Performed data analysis: A.B. Norman, Tabet, Fey, and Tsibulsky.

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

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