The affinity of D₂-like dopamine receptor antagonists determines the time to maximum effect on cocaine self-administration

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Abbreviations: Vd, volume of distribution; Cmax, maximal agonist concentration ratio; Tmax, time to maximum effect; pA3, negative logarithm of the antagonist dose (or concentration) required to induce a three-fold increase in the agonist concentration ratio; Log P, octanol-water partition coefficient; Log D, distribution coefficient or octanol-water partition coefficient at a defined pH.

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Abstract

Differences in the time to maximum effect ($T_{\text{max}}$) of a series of dopamine receptor antagonists on the self-administration of cocaine is not consistent with their lipophilicity (octanol-water partition coefficients at pH 7.4) and expected rapid entry into the brain after i.v. injection. It was hypothesized that the $T_{\text{max}}$ reflects the time required for maximal occupancy of receptors, which would occur as equilibrium was approached. If so, the $T_{\text{max}}$ should be related to the affinity for the relevant receptor population. This hypothesis was tested using a series of nine antagonists having a 2,500-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 i.v. with a dose of antagonist and the self-administration of cocaine continued for 6-10 hours. The level of cocaine at the time of every self-administration (satiety threshold) was calculated throughout the session. The satiety threshold was stable prior to the injection of antagonist and then increased approximately three-fold over the baseline value at doses of antagonists selected to produce this approximately equivalent maximum magnitude of effect ($C_{\text{max}}$). Despite the similar $C_{\text{max}}$ the mean $T_{\text{max}}$ varied between 5 min and 157 min across this series of antagonists. Furthermore, there was a strong and significant correlation between the in vivo $T_{\text{max}}$ 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., 2011a). 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 SCH23390 (a D₁ dopamine receptor antagonist)-induced effect on cocaine and apomorphine self-administration (Norman et al., 2011a) was consistent with its reported elimination half-life from plasma in rats (Kilts et al., 1985; Hietala et al., 1992). However, the approximately 30 min time to maximum effect (Tₘₐₓ) for SCH23390 is unlikely to reflect the peak brain concentration after an i.v. injection as positron emission tomography studies of [¹¹C]SCH23390 in humans shows peak brain concentrations occurring by approximately 10 min (Farde et al., 1987). Alternatively, 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 minimum maintained concentration of cocaine represents the satiety threshold (Tsibulsky and Norman, 1999; Norman and Tsibulsky, 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., 2011b). As 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., 2011b). 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 \[^3\text{H}\text{SCH23390}\] in vitro increased over time until equilibrium was approached (Gifford et al., 1998). For a sub-saturating 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 \(T_{\text{max}}\). As the time to approach equilibrium is dependent on the ligand affinity, \(T_{\text{max}}\) should be proportional to the affinity of the antagonist for the receptors mediating the agonist-induced response. If so, the \(T_{\text{max}}\) 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 2,500-fold range. It is reported herein that there is a strong and significant correlation between the \(T_{\text{max}}\) for these antagonists on cocaine self-administration in rats in vivo and their reported in vitro affinities for \(D_2\) dopamine receptors.
Methods

2.1. **Cocaine self-administration training**

Male Sprague-Dawley rats (from Harlan, Indianapolis, IN, initial weight 180 – 200 g and 400 – 500 g over the duration of these studies) were housed individually on a 12-h light-dark cycle (lights on at 6:00 a.m.) and food and water were available ad libitum. All studies were conducted in accordance with the NIH 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 i.v. catheters, cocaine self-administration training procedures were completed as described previously (Norman and Tsibulsky, 2006) for all rats.

Test chambers (Med Associates, Georgia, VT) were each equipped with an active and an inactive lever. Each chamber was situated inside of a laminated wooden compartment (43 x 61 x 35 cm) that provided sound attenuation and was equipped with a house light. Infusion pumps (model PHM-100, Med Associates Inc., Georgia, VT) were situated outside of the compartments. Computers controlled unconditioned stimuli (drug injection) using a program written in Medstate Notation® language (Med Associates, Inc., St. Albans, VT). After stable self-administration was acquired, the inter-injection intervals as a function of the cocaine unit dose (over a range from 0.75 – 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 three days per week, rats self-administered a unit dose of 3 μmol/kg of 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 i.v. and the session continued. During preliminary experiments, the dose of each antagonist that produced an approximately three-fold maximal increase in the cocaine satiety threshold (C_max) was determined (i.e. analogous to the antagonist pA3 value (Schild, 1947; 1957)). This magnitude of maximal effect was selected because it was reliably differentiated from the baseline values but was not so large as to produce a cessation of self-administration behavior as is observed at antagonist doses that produce large increases in the satiety threshold and the resulting rate of cocaine self-administration (Ettenberg et al., 1982; Norman et al., 2011a). The approximately equipotent dose of each antagonist was; 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), triflupromazine (750 nmol/kg; 0.3 mg/kg), olanzapine (1,500 nmol/kg; 0.47 mg/kg), and thioridazine (6,000 nmol/kg; 2.4 mg/kg).

2.2. 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 upon the amount injected per kg 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., 2011a). 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 $C = L/V_d$.

2.3. **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., 2011b) and the magnitude of the cocaine concentration ratio is directly proportional to the antagonist dose over a certain range of doses (Norman et al., 2011a). 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 prior to 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 one were plotted as a function of time after the injection of each antagonist.

2.4. **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 maximum concentration ratio was the mean of the highest 4-6 values and the $T_{\text{max}}$ 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 $T_{\text{max}}$ 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 $\log D = \log P - \log(1 + 10^{\text{pKa} - \text{pH}})$, where pKa is the acid dissociation constant (Scherrer and Howard, 1977). This equation is relevant to compounds that are weak bases. The Log P and pKa 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 from the Collaborative Drug Discovery online database (http://www.collaborativedrug.com). The values for spiperone were obtained only from Hamblin et al. (1984) and Malmberg et al. (1996) because these studies were the only ones to employ incubation times of at least 3 h, consistent with our $T_{\text{max}}$ values for spiperone in vivo.

2.5. 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), s(-)-eticlopride HCl, triflupromazine HCl, thioridazine HCl and molindone
HCl were purchased from Sigma (St. Louis, MO). Nemonapride (base) was purchased from Tocris (Ellisville, MO). Olanzapine (Zyprexa IntraMuscular, Lilly) and aripiprazole (Abilify Injection, Bristol-Myers Squibb) were purchased from the Pharmacy Services at the University Hospital, Cincinnati.
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. Following the injection of eticlopride there was an increase in the rate of cocaine self-administration. Over the subsequent six hours the rate of cocaine self-administration gradually returned to baseline rates. Fig. 1B shows the calculated cocaine level at the time of every lever press shown in Fig. 1A. After the initial loading phase where 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 towards baseline over the subsequent six hours.

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 the C_{max} (T_{max}) was markedly different between these antagonists. The mean ± SEM 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 $T_{\text{max}}$, respectively, of this series of antagonists.

In contrast, to the weak correlation between antagonist log D values and $T_{\text{max}}$ 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 $T_{\text{max}}$ values for these antagonist-induced increases in the cocaine concentration ratio measured during maintained cocaine self-administration (Fig. 4).
Discussion

The approximately 30-fold difference in \( T_{\text{max}} \) 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 \( T_{\text{max}} \) 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 lipophilicity at physiological pH, but also may be related to differences in specific transporter processes for different small molecules. However, the differences in \( T_{\text{max}} \) are not likely 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 \( T_{\text{max}} \). Similarly, the lipid solubility as measured by log D at pH 7.4 values, which may reflect in part a molecule’s ability to cross the blood-brain barrier, varies by more than 6,000-fold across this series of antagonists. However, this measure of lipophilicity does not appear to correlate with \( T_{\text{max}} \). This is readily apparent when comparing spiperone and molindone which have the most similar log D values among this series of antagonists and yet have a 30-fold difference in \( T_{\text{max}} \) values. Therefore, the time to maximum effect may not represent the rate at which brain concentrations of these antagonists increase after i.v. 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., 2011a) it was noted that the 25-30 min
T\textsubscript{max} after i.v. 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\textsubscript{max} would represent the time to approach equilibrium (Norman et al., 2011a). If so, it was predicted that T\textsubscript{max} would be proportional to antagonist affinity for the relevant receptor population.

The strong correlation between T\textsubscript{max} and affinity for D\textsubscript{2} dopamine receptors is consistent with the hypothesis that T\textsubscript{max} represents the time for these antagonists to reach the maximum fractional occupancy at a receptor population that mediates the effects of cocaine. If so, then T\textsubscript{max} may represent a bioreporter for the absolute pharmacodynamic potency (K\textsubscript{d}) of these D\textsubscript{2} receptor antagonists. Consequently, the affinity of any D\textsubscript{2} receptor antagonist could be determined by measuring its T\textsubscript{max} on cocaine self-administration in vivo and comparing this to a standard curve such as shown in Fig. 4. Obviously, the accuracy of this method of determining the affinity of D\textsubscript{2} receptor antagonists is dependent on the reliability of both the T\textsubscript{max} measurements in vivo and the antagonist K\textsubscript{i} or K\textsubscript{d} measurements in vitro. If the measurements of T\textsubscript{max} after i.v. injections are demonstrated to be replicable amongst different laboratories, then the in vivo method employed 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\textsubscript{d} values measured in vitro that employ higher affinity ligands and incubation times less than three hours should be viewed with caution. The same caution concerning reported antagonist K\textsubscript{i} values is also warranted when they are determined from competition binding assays where 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 $T_{\text{max}}$ values occur when the maximum fractional occupancy of a receptor population by a given dose is attained, then $T_{\text{max}}$ values likely represent the time for the antagonist-receptor complexes to achieve equilibrium. As the antagonist concentration should be declining in vivo, $T_{\text{max}}$ would likely be the minimum 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. Interestingly, 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 nemonapride being the most potent (the lowest $pA_3$ value) and spiperone being approximately equipotent with haloperidol on a molar basis. In contrast, the clear differences in $T_{\text{max}}$ between spiperone, nemonapride and haloperidol did correlate with their in vitro $K_i$ or $K_d$ values. Thus, $T_{\text{max}}$ rather than $K_{\text{dose}}$ (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 $K_{\text{dose}}$, is related to $K_d$ and the volume of distribution ($V_d$) ($K_{\text{dose}} = K_d \cdot V_d$). As $K_{\text{dose}}$ can be determined by measuring $C_{\text{max}}$ as a function of antagonist dose (Norman et al., 2011a) and $K_d$ may be determined from the $T_{\text{max}}$, $V_d$ can then be calculated. The differences between in vivo potency and in vitro affinity measures likely 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 $T_{\text{max}}$ values for a series of competitive antagonists after i.v. 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. As 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.

Contributed new reagents or analytic tools: Not applicable.

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

Wrote or contributed to the writing of the manuscript: A.B. Norman, Tabet, M.K. Norman, Tsibulsky, Millard.
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of reversed-phase high-performance liquid chromatography with electrochemical detection.

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Footnotes

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Legends for Figures

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 D2-like dopamine receptor antagonist (-)eticlopride was rapidly injected i.v. Panel A represents the cumulative record of lever presses from a representative session. Each response is represented by a vertical increment and the horizontal distance represents the inter-press interval. After the initial loading phase, the inter-injection intervals were stable during the maintenance phase. After the injection of eticlopride, self-administration accelerated. Approximately six h after the antagonist injection, access to cocaine was terminated and responding was recorded until it was extinguished. Panel B represents the calculated level of cocaine at the time of each self-administration shown in Panel 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. The regression line through these values has a slope approaching zero, indicating that the levels are relatively stable over this phase of the session.

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 zero 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 maximum response for these representative sessions were 5, 25, 45 and 180 min for molindone, eticlopride, nemonapride and spiperone, respectively.
Fig. 3. The correlation of $T_{\text{max}}$ for the antagonist-induced effect on cocaine self-administration and the antagonist octanol-water partition coefficient (log D). The mean log D at pH 7.4 values were calculated from the Log P and pKa values taken from the CAS Directory online data base. The linear regression line has a correlation coefficient ($r$) of -0.42.

Fig. 4. The $T_{\text{max}}$ of antagonist effect on cocaine self-administration correlates with antagonist affinity for D$_2$-like dopamine receptors. The symbols represent the mean ± SEM for both the $K_i$ or $K_d$ and $T_{\text{max}}$ values. The affinity values were taken from literature sources. The linear regression line has a correlation coefficient ($r$) of -0.98.
Fig. 1A and 1B
Fig. 2.
Fig. 3

TIME TO MAXIMUM EFFECT (min)

LOG OCTANOL-WATER PARTITION COEFFICIENT at pH 7.4
Fig. 4.