Published in final edited form as:

J Neurosci Methods. 2011 January 15; 194(2): 252–258. doi:10.1016/j.jneumeth.2010.10.017.

Using the self-administration of apomorphine and cocaine to measure the pharmacodynamic potencies and pharmacokinetics of competitive dopamine receptor antagonists

Andrew B. Norman, Michael R. Tabet, Mantana K. Norman, and Vladimir L. Tsibulsky

Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA.

Abstract

Competitive dopamine receptor antagonists accelerate psychomotor stimulant self-administration. According to pharmacological theory of competitive antagonism antagonists raise the equiactive agonist concentration. In the self-administration paradigm this is assumed to be the satiety threshold or C_{min} . The magnitude of the proportional increase in satiety threshold (agonist concentration ratio) as a function of antagonist dose should reflect the antagonist pharmacodynamic potency. The time course of this effect should reflect the rate of change of antagonist occupancy of receptors and, therefore, antagonist concentration i.e. pharmacokinetics. Rats self-administered apomorphine or cocaine at a stable rate and were then injected i.v. with one of four competitive D_1 -like or D_2 -like dopamine receptor antagonists and the session continued. The agonist concentrations at the time of each self-administration (satiety thresholds) were calculated during the session. The antagonists accelerated self-administration of both agonists with a concomitant increase in the calculated satiety thresholds. The maximum agonist concentration ratio was proportional to the dose of antagonist. The time courses of the changes in agonist concentration ratio were independent of the agonist and of the dose of antagonist. Schild analysis of the maximum agonist concentration ratio as a function of the antagonist dose allowed apparent pA_2 (or K_{dose}) to be measured. Antagonist K_{dose} values should provide a quantitative basis for receptor identification in behavioral pharmacology. The assay system may also measure the pharmacokinetics of antagonist elimination from the brain. Agonist self-administration represents a sensitive in vivo pharmacological assay system that provides information useful for pharmacokinetic/pharmacodynamic modeling of antagonist effects.

Keywords

pharmacokinetics; pharmacodynamics; equiactive agonist concentration; Schild analysis; pA_2 ; satiety threshold; C_{min}

© 2010 Elsevier B.V. All rights reserved.

Corresponding author: Andrew B. Norman, Ph.D., Department of Psychiatry and Behavioral Neuroscience, College of Medicine, University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH 45267, USA. andrew.norman@uc.edu, Tel: (513) 558-6654, Fax: (513) 558-0042.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Statement of Conflicts of Interest

None.

1. Introduction

Pharmacokinetic (PK)/pharmacodynamic (PD) models have been proposed to explain the regulation of agonist self-administration by animals (Yokel and Pickens, 1974; Wise, 1999; Tsibulsky and Norman, 1999; Ahmed and Koob, 2005; Norman and Tsibulsky, 2006). In these models the concentration of cocaine at the time of self-administration is an important pharmacodynamic parameter. This parameter has been termed the satiety threshold (Tsibulsky and Norman, 1999; Norman and Tsibulsky, 2006), which is the minimum cocaine concentration ($C_{\min}$) during the maintenance phase of a self-administration session and is assumed to be constant.

D₁-like (Koob et al., 1987) and D₂-like (Yokel and Wise, 1975) dopamine receptor antagonists typically increase the rate of self-administration of cocaine and related stimulants. Furthermore, the potencies of several antipsychotic drugs (assumed to be D₂-like receptor antagonists) to increase the rate of cocaine self-administration by 25% correlated with their antipsychotic potencies (Roberts and Vickers, 1984). This latter approach to using self-administration behavior as a pharmacological assay system, while setting an important precedent, did not take full advantage of established pharmacological techniques for the measurement of the absolute potency of competitive receptor antagonists. Based on 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; Arunlakshana and Schild, 1959; Colquhoun, 2007). In the pharmacological model of the self-administration paradigm (Tsibulsky and Norman, 1999), the satiety threshold represents an equiactive agonist concentration. Therefore, according to the pharmacological theory of competitive antagonism, the dose of antagonist required to increase the satiety threshold by a factor of two would represent the dose required to produce 50% fractional occupancy of the receptor population mediating the agonist-induced satiety response i.e. K_{dose} . This is a measure of the absolute potency of the antagonist, which is also the apparent antagonist pA_2 (negative logarithm of the antagonist concentration (or dose) required to produce a 2-fold increase in the agonist concentration (or dose) required to induce a defined magnitude of response). Occasional reports have provided pA_2 values that have been used to identify the receptor subtypes mediating the discriminative stimulus effects of opiates (Bertalmio and Woods, 1987; Woods et al., 1992), and the self-administration of alfentanil (Bertalmio and Woods, 1989), heroin (Rowlett et al., 1998) and benzodiazepines (Paronis and Bergman, 1999), all of which are direct receptor agonists. The theory of competitive antagonism assumes that the competitive antagonist and the agonist bind reversibly to the same site. Therefore, indirect monoamine receptor agonists such as cocaine may not be suitable for this type of investigation. However, the direct dopamine receptor agonist apomorphine is also self-administered by rats (Baxter et al., 1974; Roberts et al., 1977; Yokel and Wise, 1978) making this agonist an appropriate model system.

The potency of antagonists measured by their pA_2 values has provided a basis for receptor classification (Rang, 2006). In addition, the magnitude of a competitive antagonist-induced increase in satiety threshold should be directly proportional to the concentration of antagonist at its site of action. If so, then the time course of the change in the magnitude of the satiety threshold should reflect the change in the concentration of the antagonist in vivo, i.e. its pharmacokinetics.

The data presented herein demonstrate that the drug self-administration assay can be used to determine both the pharmacodynamic potency of competitive D₁-like and D₂-like dopamine receptor antagonists in vivo and the pharmacokinetics of the same antagonists at their site of action, presumably the brain.

2. Materials and Methods

2.1. Cocaine self-administration training

Male Sprague-Dawley rats (from SASCO, Wilmington, MA, 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. These rats that stably self-administered cocaine also self-administered the direct dopamine receptor agonist apomorphine when it was substituted for cocaine. Antagonist effects were typically first tested on apomorphine self-administration, and then were tested again on cocaine self-administration. There was no evidence that the order of apomorphine or cocaine sessions or the order of antagonists influenced the magnitude or duration of antagonist effects.

During daily sessions, run 5 days per week, rats self-administered a unit dose of 150 or 3000 nmol/kg of apomorphine or cocaine, respectively. After approximately 90–120 min of a stable rate of self-administration, a single i.v. dose of either the selective D₁-like dopamine receptor antagonist SCH23390, the selective D₂-like dopamine receptor antagonist (–)eticlopride, or (in the studies using cocaine) the less subtype selective and lower affinity antagonists triflupromazine and olanzapine were rapidly administered and the session continued. To determine whether the effects on cocaine self-administration of these antagonists were due to their effects in the brain, a potent antagonist of D₂-like dopamine receptors, domperidone, was used that has approximately equal affinity for D₂ dopamine receptors as does eticlopride, but does not cross the blood-brain barrier and so has no central effects (Barone, 1999). The dose of domperidone was 10-fold higher than the dose of eticlopride that produced a detectable effect on apomorphine and cocaine self-administration.

2.2. Real-time calculation of cocaine levels in the body

The apomorphine and cocaine levels in the body were calculated by monitoring the amount of agonist that was administered to the animals and then using predetermined pharmacokinetic values to estimate the resulting agonist levels in the animals over time. The calculated values for the whole body agonist levels depend upon the amount injected per kg body weight minus the amount eliminated per unit time. The agonist's volume of distribution (V_d) was assumed to be constant. Therefore, the agonist level in the body (L) was calculated every Δt seconds 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):

$$\begin{aligned} L_{c1} &= L_{c0} \cdot (1 - k_{10} \cdot \Delta t - k_{12} \cdot \Delta t) + L_{p0} \cdot k_{21} + V_{01} \cdot \Delta t \\ L_{p1} &= L_{p0} \cdot (1 - k_{21} \cdot \Delta t - k_{20} \cdot \Delta t) + L_{c0} \cdot k_{12} \end{aligned}$$

where L_{c1} and L_{p1} are agonist levels at the time t_1 in central and peripheral compartments, respectively, L_{c0} and L_{p0} are agonist level at the time t_0 , $\Delta t = t_1 - t_0$ is a computation interval and the value for Δt was set at 1 s, k_{10} , k_{20} , k_{12} and k_{21} are the agonist elimination rate constants and V_{01} is the rate of agonist injection and $V_{01} \cdot \Delta t$ represents the amount of agonist administered. The group mean value for cocaine k_{10} was approximately 0.0014 s^{-1} ($k = \ln(2)/t_{1/2}$, $t_{1/2}$ was 500 s), which was calculated as described previously (Tsibulsky and Norman, 1999) and $k_{12} = 0.035 \text{ s}^{-1}$ ($t_{1/2} = 20 \text{ s}$) and $k_{21} = 0.028 \text{ s}^{-1}$ ($t_{1/2} = 25 \text{ s}$) which are

within the range of previously reported literature values for these parameters (Booze et al., 1997). The group mean value for apomorphine k_{10} was approximately 0.0017 s^{-1} ($t_{1/2} = 420 \text{ s}$), which was also calculated from plots of inter-injection intervals as a function of apomorphine unit dose, which is consistent with literature values for this parameter (Melzacka et al., 1979; Paalzow and Paalzow, 1986), and values for k_{12} and k_{21} were the same as used for cocaine. In this two-compartment model it is assumed that there is no elimination of agonist from the peripheral (second) compartment and, therefore, $k_{20} = 0$.

2.3. Agonist concentration ratios

The mean of the values for the level of apomorphine or 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 calculated agonist levels (L) were assumed to be directly proportional to the agonist concentrations (C) according to the equation $C=L/V_d$. The level of agonist 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 agonist concentration ratio. These agonist concentration ratios minus one were plotted as a function of time after the injection of antagonist.

2.4. Pharmacokinetic 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 time to the maximum response ($T_{\max}$) for each session was the mean of these same time values. It was assumed that the subsequent decrease in the agonist concentration ratios was a first-order process. The effect decay $t_{1/2}$ was calculated using non-linear regression analysis according to a monoexponential function, which was applied to the values after the time of the maximum concentration ratio.

Because the maximum agonist concentration ratio was found to be approximately linearly proportional to antagonist dose, the concentration ratio minus one was assumed to be linearly proportional to the antagonist concentration at any time after dynamic equilibrium was approached. This allowed the application of pharmacokinetic models to the antagonist-induced changes in the agonist concentration ratio. The bimodal antagonist-induced changes in agonist concentration ratio as a function of time were analyzed using a series of single and multiple-compartment pharmacokinetic models. These models provided reasonable fits to the data (for illustration purposes, one of these models is shown in Figs. 2 and 3). The analysis of these data sets using these relatively complex pharmacokinetic models will be reported elsewhere.

Linear regression analysis was applied to the data on the Schild plots and the slope and intercept on the abscissa of the linear regressions were assessed.

3. Results

3.1. The effect of antagonists on apomorphine self-administration

After the initial series of self-administrations short intervals, there was an abrupt decrease in the rate of self-administration of the same unit dose also with regular intervals (Fig. 1A). This abrupt change in inter-injection intervals corresponded to the end of the loading phase and the start of the maintenance phase of the session when the level of apomorphine at the time of each administration remained relatively stable (Fig. 1B). The inter-injection intervals remained stable for at least 2 h and this represented the baseline rate of self-administration.

Following the injection of the antagonist there was an increase in the rate of apomorphine self-administration, which gradually returned towards baseline values over the subsequent 4–6 h (Fig. 1A). As shown in Fig. 1B, the increase in the rate of self-administration corresponded to an increase in the levels of apomorphine at the time of each lever press. The peak level of apomorphine was observed at approximately 30 min after the injection of antagonist after which time the level of apomorphine at the time of each response gradually declined in an apparent exponential manner towards the levels observed prior to the injection of antagonist (Fig. 1B). When access to apomorphine was terminated, there was an abrupt increase in the rate of lever pressing behavior until the behavior ceased after approximately 30 min (Fig. 1A).

3.2. Antagonists increase the equiactive agonist concentration

It is assumed that each lever press occurs at an equiactive concentration of agonist. In the presence of a competitive antagonist, the degree to which the equiactive agonist concentration is increased (concentration ratio) should be directly proportional to the antagonist concentration. Therefore, the data from Fig. 1B provided the apomorphine concentration ratio at the time of each lever press after the injection of antagonist, which is shown in Fig. 2A as the dose of 30 nmol/kg of eticlopride. Also shown in Fig. 2A are the time courses of the effect of eticlopride at doses of 5, 10 and 20 nmol/kg. The times to maximum response ($T_{\max}$) were 31, 32, 32 and 31 min at the doses of 30, 20, 10 and 5 nmol/kg of eticlopride, respectively.

The apomorphine concentration ratio over time should reveal the time-dependent changes in antagonist concentration. The mean values for the effect decay $t_{1/2}$ were not significantly different across this range of eticlopride doses (Table 1). The simplest model that approximated each data set was generated by a single compartment pharmacokinetic model with first-order input. An example of the fit of this model to the data generated from each dose of eticlopride is shown in Fig. 2A.

The D_1 dopamine receptor antagonist SCH23390 similarly increased the rate of self-administration of apomorphine with a concomitant increase in the apomorphine concentration at the time of each lever press (data not shown). The apomorphine concentration ratio minus one as a function of time after the injection of SCH23390 is shown in Fig. 2B. Similar to the effects of eticlopride, there was an antagonist dose-dependent increase in the maximum concentration ratio. The $T_{\max}$ values were 24, 31, 27 and 35 min for the doses of 30, 20, 10 and 5 nmol/kg, respectively. The mean values for the effect decay $t_{1/2}$ were not significantly different across this range of SCH23390 doses (Table 1). The best fit to the same pharmacokinetic model used to analyze the eticlopride data is also shown for each dose of SCH23390 (Fig. 2B).

3.3. Schild analysis of antagonist pharmacodynamic potencies on apomorphine self-administration

As shown in Fig. 3, when the peak concentration ratio was plotted as a function of the antagonist dose according to the method of Schild, the magnitude of the peak apomorphine concentration ratio increased linearly with the antagonist dose and with a slope close to unity for both eticlopride and SCH23390. On this plot, the intercept on the abscissa represents the dose of antagonist that would produce a peak concentration ratio of two and that would occupy 50% of the receptors underlying the response i.e. the apparent pA_2 , or antagonist K_{dose} . For eticlopride, the apparent pA_2 value was 8.0 ($K_{\text{dose}} = 9.1$ nmol/kg). For SCH23390, the apparent pA_2 value was 8.2 ($K_{\text{dose}} = 6.7$ nmol/kg).

3.4. Antagonists increase the equiactive cocaine concentration

These antagonists also accelerated the self-administration of cocaine similar to their effects on apomorphine self-administration. As shown in Fig. 3, eticlopride (Fig. 4A) and SCH23390 (Fig. 4B) also increased the cocaine concentration ratio. Both antagonists produced a dose-dependent increase in the maximum concentration ratio. The $T_{\max}$ values were 26, 31 and 31 min at eticlopride doses of 30, 20 and 10 nmol/kg, respectively. The $T_{\max}$ values across the tested range of SCH23390 doses were 33, 25, 34 and 39 min at doses of 30, 20, 10, and 5 nmol/kg, respectively.

The effect decay $t_{1/2}$ values for the cocaine concentration ratio were not significantly different across the ranges of antagonist doses (Table 2). Furthermore, the values for eticlopride and SCH23390 were not significantly different ($p > 0.05$, two-way ANOVA) from the respective values observed when apomorphine was self-administered (compare corresponding values in Table 1). The 5 nmol/kg dose of eticlopride was not tested due to an expected low magnitude of response close to the lowest level of detection. For this low dose of SCH23390, although $C_{\max}$ could be measured, the effect decay $t_{1/2}$ could not be reliably resolved due to the low magnitude of response. The dopamine receptor antagonists triflupromazine and olanzapine produced a similar acceleration of cocaine self-administration and a concomitant increase in the equiactive cocaine concentration. However, relative to eticlopride and SCH23390 higher doses of these other antagonists were required to produce a similar magnitude of effect.

3.5. Schild analysis of antagonist pharmacodynamic potencies on cocaine self-administration

Despite cocaine being an indirect agonist, the peak cocaine concentration ratio also increased linearly with antagonist dose (Fig. 5). This was demonstrated by a slope of the linear regression analysis of the Schild plot that approached unity for these four antagonists. For eticlopride and SCH23390, the apparent pA_2 values were 7.9 ($K_{\text{dose}} = 12.6$ nmol/kg) and 8.1 ($K_{\text{dose}} = 7.9$ nmol/kg), respectively, which were similar to those for these same antagonists when measured during the self-administration of apomorphine (Fig. 3). The potencies of triflupromazine and olanzapine were markedly lower than those of the other two antagonists having apparent pA_2 values of 7.1 ($K_{\text{dose}} = 80$ nmol/kg) and 6.1 ($K_{\text{dose}} = 700$ nmol/kg), respectively.

3.6. The lack of effect of a peripherally acting dopamine receptor antagonist

In order to determine whether the eticlopride-, triflupromazine and olanzapine-induced increases in the agonist concentration ratios reflect the brain concentrations of the antagonists, their effect was compared with that of domperidone. A relatively high dose of the potent, but not centrally active, D_2 -like dopamine receptor antagonist domperidone had no detectable effect on the rate of apomorphine self-administration. In the representative session shown in Fig. 6, the mean $\pm$ SEM inter-injection interval was 9.1 ± 0.5 min ($n = 11$ intervals) and 7.8 ± 0.8 min ($n = 32$ intervals) before and after, respectively, the injection of domperidone. Similarly, domperidone had no significant effect on the rate of self-administration of cocaine (data not shown). The mean $\pm$ SEM inter-injection interval before and after the injection of domperidone during the self-administration of cocaine at a unit dose of 3,000 nmol/kg was 6.0 ± 0.1 min and 5.4 ± 0.6 min ($n = 4$ rats), respectively.

4. Discussion

The lack of effect on agonist self-administration of the potent D_2 -like dopamine receptor antagonist domperidone, which does not cross the blood-brain barrier in vivo (Barone 1999),

demonstrates that the receptors mediating the cocaine-induced and apomorphine-induced satiety responses are in the brain.

The time course of the change in agonist satiety threshold in response to these series of antagonists could reflect the pharmacokinetics of the antagonist in the brain. Indeed, the relatively rapid initiation of the SCH23390-induced increase in the apomorphine satiety threshold is consistent with the rapid entry of SCH23390 into the brain of rats and the time course of the decline of effect is consistent with the elimination $t_{1/2}$ previously measured in rat plasma (Hietala et al., 1992). Interestingly, previous studies reported the presence of SCH23390 persisted in brain for several hours after plasma levels were undetectable (Kilts et al., 1985; Hietala et al., 1992). Similarly, the present studies demonstrate that the effects of SCH23390 and eticlopride on agonist self-administration decline gradually and are still detectable after several hours. Therefore, the time course of the diminution of effect of SCH23390 is consistent with its known pharmacokinetics in rats. The antagonist $T_{\max}$ and elimination $t_{1/2}$ values were similar across antagonist doses and appeared to be independent of whether the self-administered agonist was apomorphine or cocaine. These findings are consistent with the time course of the change in agonist satiety threshold representing the pharmacokinetics of these antagonists. However, the absolute values of the $T_{\max}$ for SCH23390 (24–27 min) appear to be relatively long and are not consistent with the rapid $T_{\max}$ (approximately 5–10 min) for [^{11}C]SCH23390 in human brain after i.v. injection measured using Positron Emission Tomography (Farde et al., 1987). It seems unlikely that the distribution of drugs to the brain would be slower in rats than in humans, which means that the rate of increase in the agonist concentration ratio induced by SCH23390 (and eticlopride) may not reflect the rate of increase in the concentration of these antagonists in the brain. If so, then the time course of the increase in agonist concentration ratio may represent the rate at which fractional occupancy of the relevant receptor populations increases and $T_{\max}$ would represent the time to approach equilibrium. Indeed, the time for [^3H]SCH23390 to approach equilibrium at 37°C in rat striatal tissue in vitro is approximately 30 min (Gifford et al., 1998), consistent with the value for $T_{\max}$. Under these circumstances $T_{\max}$ would measure aspects of the pharmacodynamic potencies of these antagonists and, if so, it is predicted that $T_{\max}$ will be proportional to antagonist affinity. Further investigation will be necessary to test this hypothesis. Once dynamic equilibrium is achieved then the agonist concentration ratio should be directly proportional to the antagonist concentration and changes in this ratio should reflect the pharmacokinetics of the antagonist.

If the maximum concentration ratio is close to equilibrium conditions, the linear increase in the peak agonist concentration ratio as a function of antagonist dose provides a measure of the pharmacodynamic potency of these antagonists. When the agonist concentration ratio is two this should correspond to the dose of the antagonist required to occupy 50% of the receptors that mediate the satiety response, *i.e.* the apparent pA_2 (or K_{dose}) of the antagonist. The antagonist pA_2 or K_{dose} value should be the product of its affinity constant measured in vitro and its apparent volume of distribution (V_d); $\text{K}_{\text{dose}} = \text{K}_D \cdot V_d$. As the V_d of the antagonist can usually be assumed to be constant across a range of doses, the K_{dose} of antagonists should be constant for all responses mediated by the same receptors in vivo. If so, then knowing the K_D and the K_{dose} allows the apparent V_d for an antagonist to be estimated. In the case of SCH23390, assuming a K_D of 0.3–0.5 nM and with a K_{dose} of 7–8 nmol/kg the apparent V_d would be approximately 14–27 L/kg. For eticlopride assuming a K_D of 0.2–0.5 nM and with a K_{dose} of 9–12 nmol/kg the apparent V_d would be approximately 18–40 L/kg. Trifluoromazine has intermediate affinity for D_2 dopamine receptors with a K_D of approximately 2.8 nM (Richelson and Nelson, 1984) and a K_{dose} of 80 nmol/kg, while olanzapine is not selective for D_1 and D_2 dopamine receptors and has lower affinity for these receptors with K_D values ranging from approximately 7–46 nM

(Seeman, 2010) and a K_{dose} of 700 nmol/kg. Therefore, the apparent V_d for triflupromazine would be approximately 28 L/kg and for olanzapine would be in the range of 15–100 L/kg. It is possible that the rank order of antagonist potencies for a specific receptor measured in vitro and in vivo may be different if there are significant differences in their apparent V_d . However, because the V_d of an antagonist should be constant, the rank order of in vivo potencies for a series of antagonists should also be constant for responses mediated by the same receptor population.

The low K_{dose} of eticlopride and SCH23390 were similar for apomorphine and cocaine, indicating that these self-administered drugs produce their effects through the same populations of both D_2 -like and D_1 -like dopamine receptors, respectively. Interestingly, despite being an indirect dopamine receptor agonist, the linear increase with a slope close to unity in the cocaine concentration ratio as a function of antagonist dose indicates that the dopamine concentration at the receptors mediating the cocaine-induced satiety response should be directly proportional to cocaine concentration. This is consistent with the linear increase in brain dopamine concentrations as a function of cocaine dose that were measured in rats using in vivo microdialysis (Wise et al., 1995).

An important assumption of the Schild equation is that the measurements of antagonist-induced shifts in the agonist concentration ratio are made at equilibrium (Kenakin, 1997; Colquhoun, 2007). Because both agonist and antagonist concentrations are constantly changing in vivo it is difficult to determine whether equilibrium is achieved. The reasonable approximation to the data of a slope of unity on the Schild plot may indicate that the major assumptions of the Schild method may be satisfied. An important caveat is that non-equilibrium situations can produce Schild plots with slopes of unity but provide erroneous measures of pA_2 (Kenakin, 1997). Whether this caveat will prove to be a significant limitation of this method will only become apparent from additional studies.

Typically, the agonist concentration ratio can increase over several orders of magnitude of antagonist concentrations when using isolated tissue preparations in vitro (Kenakin, 1997). In contrast, in the behavioral assay used here, the range of agonist concentration ratios that can be used appears to be limited to less than one order of magnitude. This is because at higher doses of D_1 -like and D_2 -like dopamine receptor antagonists that would be expected to produce higher agonist concentration ratios, self-administration is instead suppressed by all of these antagonists. This phenomenon has been reported previously in rats for the non-specific dopamine receptor antagonist flupenthixol (Ettenberg et al., 1982). The mechanism by which this suppression of self-administration occurs is not well understood at present and is beyond the scope of the present investigation. However, this phenomenon represents a practical limitation of this behavioral assay system that must be accommodated for assessment of antagonist pharmacodynamic potencies and pharmacokinetics.

In summary, these studies demonstrate that classical pharmacological principles that have been previously applied to competitive antagonists in vitro might also be applicable in vivo. This method may be useful for investigating PK/PD models of antagonist effects in brain.

Acknowledgments

Supported by United States PHS grant DA018538 from the National Institute on Drug Abuse. We thank William R. Buesing for excellent technical assistance. We are also grateful to Nora E. Stevenson for providing excellent program coordination.

References

- Ahmed SH, Koob GF. Transition to drug addiction: a negative reinforcement model based on an allostatic decrease in reward function. *Psychopharmacology*. 2005; 180:473–490. [PubMed: 15731896]
- Arunlakshana O, Schild HO. Some quantitative uses of drug antagonists. *Br J Pharmacol Chemother*. 1959; 14:47–58.
- Barone JA. Domperidone: a peripherally acting dopamine 2-receptor antagonist. *Ann Pharmacother*. 1999; 33:429–440. [PubMed: 10332535]
- Baxter BL, Gluckman MI, Stein L, Scerni RA. Self-injection of apomorphine in the rat: Positive reinforcement by a dopamine receptor stimulant. *Pharmacol Biochem Behav*. 1974; 2:387–391. [PubMed: 4858099]
- Bertalmio AJ, Woods JH. Differentiation between mu and kappa receptor-mediated effects in opioid drug discrimination: apparent pA_2 analysis. *J Pharmacol Exp Ther*. 1987; 243:591–597. [PubMed: 2824753]
- Bertalmio AJ, Woods JH. Reinforcing effect of alfentanil is mediated by mu opioid receptors: apparent pA_2 analysis. *J Pharmacol Exp Ther*. 1989; 251:455–460. [PubMed: 2553919]
- Booze RM, Lehner AF, Wallace DR, Welch MA, Mactutus CF. Dose-response cocaine pharmacokinetics and metabolite profile following intravenous administration and arterial sampling in unanesthetized, freely moving male rats. *Neurotoxicol Teratol*. 1997; 19:7–15. [PubMed: 9088006]
- Colquhoun D. Why the Schild method is better than Schild realized. *Trends Pharmacol Sci*. 2007; 28:608–614. [PubMed: 18023486]
- Ettenberg A, Pettit HO, Bloom FE, Koob GF. Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. *Psychopharmacology*. 1982; 78:204–209. [PubMed: 6296898]
- Farde L, Halldin C, Stone-Elander S, Sedvall G. PET analysis of human dopamine receptor subtypes using ^{11}C -SCH 23390 and ^{11}C -raclopride. *Psychopharmacology*. 1987; 92:278–284. [PubMed: 2957716]
- Gifford AN, Gatley SJ, Volkow ND. Evaluation of the importance of rebinding to receptors in slowing the approach to equilibrium of high-affinity PET and SPECT radiotracers. *Synapse*. 1998; 28:167–175. [PubMed: 9450517]
- Hietala J, Seppala T, Lappalainen J, Syvalahti E. Quantification of SCH 39166, a novel D1 dopamine receptor antagonist, in rat brain and blood. *Psychopharmacology*. 1992; 106:455–458. [PubMed: 1349751]
- Kenakin, TP. Competitive antagonism. In: Kenakin, T., editor. *Pharmacologic analysis of drug-receptor interaction*. Third edition. Philadelphia, PA: Lippincott-Raven; 1997. p. 331–373.
- Kilts CD, Dew KL, Ely TD, Mailman RB. Quantification of R-(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-methyl-3-benzazepine in brain and blood by use of reversed-phase high-performance liquid chromatography with electrochemical detection. *Biomed Applications*. 1985; 342:452–457.
- Koob GF, Le HT, Creese I. The D₁ dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. *Neurosci Lett*. 1987; 79:315–320. [PubMed: 2958724]
- Melzacka M, Wiszniowska G, Daniel W, Vetulani J. Behavioral effects and cerebral pharmacokinetics of apomorphine in the rat: dependence upon the route of administration. *Pol J Pharmacol Pharm*. 1979; 31:309–317. [PubMed: 574957]
- Norman AB, Tsibulsky VL. The compulsion zone: A pharmacological theory of acquired cocaine self-administration. *Brain Res*. 2006; 1116:143–152. [PubMed: 16942754]
- Paalzow LK, Paalzow GH. Concentration-response relations for apomorphine effects on heart rate in conscious rats. *J Pharm Pharmacol*. 1986; 38:28–34. [PubMed: 2869123]
- Paronis CA, Bergman J. Apparent pA_2 values of benzodiazepine antagonists and partial agonists in monkeys. *J Pharmacol Exp Ther*. 1999; 290:1222–1129. [PubMed: 10454498]
- Rang HP. The receptor concept: Pharmacology's big idea. *Br J Pharmacol*. 2006; 147:S9–S16. [PubMed: 16402126]

- Richelson E, Nelson A. Antagonism by neuroleptics of neurotransmitter receptors of normal human brain in vitro. *Eur J Pharmacol.* 1984; 103:197–204. [PubMed: 6149136]
- Roberts DCS, Vickers G. Atypical neuroleptics increase self-administration of cocaine: An evaluation of a behavioral screen for antipsychotic activity. *Psychopharmacology.* 1984; 82:135–139. [PubMed: 6141584]
- Roberts DC, Corcoran ME, Fibiger HC. On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol Biochem Behav.* 1977; 6:615–620. [PubMed: 122445]
- Rowlett JK, Wilcox KM, Woolverton WL. Self-administration of cocaine-heroin combinations by rhesus monkeys: antagonism by naltrexone. *J Pharmacol Exp Ther.* 1998; 286:61–69.
- Schild HO. pA, a new scale for the measurement of drug antagonism. *Br J Pharmacol Chemother.* 1947; 2:189–206. [PubMed: 20258355]
- Schild HO. Drug antagonism and pA_x. *Pharmacol Rev.* 1957; 9:242–246. [PubMed: 13465304]
- Seeman P. Dopamine D2 receptors as treatment targets in schizophrenia. *Clin Schizophr Relat Psychoses.* 2010; 1:56–73. [PubMed: 20643630]
- Tsibulsky VL, Norman AB. Satiety threshold: A quantitative model of maintained cocaine self-administration. *Brain Res.* 1999; 839:85–93. [PubMed: 10482802]
- Tsibulsky VL, Norman AB. Real time computation of in vivo drug levels during drug self-administration experiments. *Brain Res Brain Res Protoc.* 2005; 15:38–45. [PubMed: 15878149]
- Wise RA. Cognitive factors in addiction and nucleus accumbens function: some hints from rodent models. *Psychobiology.* 1999; 27:300–310.
- Wise RA, Newton P, Leeb K, Burnette B, Pocock D, Justice JB. Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. *Psychopharmacology.* 1995; 120:10–20. [PubMed: 7480530]
- Woods JH, Winger G, France CP. Use of in vivo apparent pA₂ analysis in assessment of opioid abuse liability. *Trends Pharmacol Sci.* 1992; 13:282–286. [PubMed: 1509522]
- Yokel RA, Pickens R. Drug level of d- and l-amphetamine during intravenous self-administration. *Psychopharmacologia.* 1974; 34:255–264. [PubMed: 4819978]
- Yokel RA, Wise RA. Increased lever pressing for amphetamine after pimozide in rats: Implications for a dopamine theory of reward. *Science.* 1975; 187:547–549. [PubMed: 1114313]
- Yokel RA, Wise RA. Amphetamine-type reinforcement by dopaminergic agonists in the rat. *Psychopharmacology.* 1978; 58:289–296. [PubMed: 98800]

Figure 1.

Time course of the effect of a dopamine receptor antagonist on apomorphine self-administration. Rats self-administered apomorphine at a unit dose of 150 nmol/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 (inverted triangles). After the injection of (–)eticlopride, self-administration accelerated (closed circles). Approximately six h after the antagonist injection, access to apomorphine was terminated and responding was recorded

(small circles) until it was extinguished. **Panel B** represents the calculated level of apomorphine at the time of each self-administration shown in Panel A. The baseline value is the mean of the apomorphine 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.

Figure 2.

The dose-dependent effects of antagonist-induced increases of the apomorphine satiety threshold. The data are from sessions conducted as shown in Figure 1. The concentration ratios were calculated using the mean baseline values of the satiety threshold in each session. The best fit to each data set was generated by a pharmacokinetic model with first order absorption into a single compartment.

Figure 3.

The absolute pharmacodynamic potencies of (–)eticlopride and SCH23390 on apomorphine satiety threshold. The concentration ratio values represent the mean maximum concentration ratio at each dose of antagonist calculated from sessions conducted as described in Figure 1 and analyzed as shown in Figure 2. The pA_2 (and K_{dose}) values for the antagonists were calculated according to the method of Schild. The corresponding pA_2 values for (–)eticlopride and SCH23390 were 8.0 and 8.2, respectively and the slopes of the linear regressions were 1.2 and 1.3, respectively.

Figure 4.

The dose-dependent effects of antagonist-induced increases of the cocaine satiety threshold. The data are from sessions conducted in the same way as when the self-administered drug was apomorphine. The concentration ratios were calculated using the mean baseline values of the satiety threshold in each session. The best fit to each data set was generated by the same pharmacokinetic model used in Fig. 2.

Figure 5.

The absolute pharmacodynamic potencies of antagonists on the cocaine satiety threshold. The concentration ratio values represent the mean maximum concentration ratio at each dose of antagonist calculated from sessions conducted as described in Figure 1 and analyzed as shown in Figure 4. The pA_2 (and K_{dose}) values for the antagonists were calculated according to the method of Schild. The corresponding pA_2 values for SCH23390, (-)-eticlopride, triflupromazine and olanzapine were 8.1, 7.9, 7.1, 6.1, respectively and the slopes of the linear regressions were 1.0 1.1, 0.9, 1.0, respectively.

Figure 6.

The lack of effect of domperidone on apomorphine self-administration. In the representative session shown the rat self-administered the agonist and the inter-injection intervals were stable. Domperidone (100 nmol/kg i.v.) was administered and the session resumed. There was no detectable effect on the inter-injection intervals following the injection of antagonist.

Table 1

Effect decay half-life of antagonists measured during the self-administration of apomorphine.

	(-)-Eticlopride	SCH23390
30 nmol/kg	43.2 ± 4.8 (3, 6)	48.3 ± 8.5 (5, 8)
20 nmol/kg	48.3 ± 4.6 (5, 9)	41.8 ± 2.7 (8, 16)
10 nmol/kg	50.9 ± 6.8 (4, 4)	70.9 ± 16.2 (7, 7)
5 nmol/kg	52.2 ± 4.5 (4, 4)	61.1 ± 19.5 (5, 5)

Values for the antagonist effect $t_{1/2}$ are in units of min and represent the mean ± SEM from the number of rats (the first number in parentheses). The second number in parentheses represents the total number of sessions. For each antagonist there were no significant differences in the $t_{1/2}$ values across antagonist doses ($p > 0.05$, one-way ANOVA).

Table 2

Effect decay half-life of antagonists measured during the self-administration of cocaine.

Antagonist and dose administered	Half-life (min)
(-)-Eticlopride: 30 nmol/kg	67.4 ± 11.5 (5, 5)
20 nmol/kg	73.8 ± 9.1 (5, 5)
10 nmol/kg	47.1 ± 8.6 (6, 7)
SCH23390: 30 nmol/kg	50.1 ± 17.2 (3, 3)
20 nmol/kg	84.7 ± 30.6 (3, 3)
10 nmol/kg	53.8 ± 9.3 (4, 6)
5 nmol/kg	ND (7, 7)
Triflupromazine: 400 nmol/kg	39.8 ± 7.5 (5, 5)
200 nmol/kg	53.3 ± 8.2 (5, 5)
100 nmol/kg	43.8 ± 11.0 (5, 5)
50 nmol/kg	40.3 ± 10.7 (5, 5)
Olanzapine: 2,000 nmol/kg	28.6 ± 12.5 (3, 3)
1,500 nmol/kg	35.3 ± 5.4 (5, 5)
600 nmol/kg	38.4 ± 10.4 (4, 8)
200 nmol/kg	25.5 ± 6.6 (4, 4)

Values for the antagonist effect $t_{1/2}$ represent the mean ± SEM from the number of rats (the first number in parentheses). The second number in parentheses represents the total number of sessions. For each antagonist there were no significant differences in the $t_{1/2}$ values across antagonist doses ($p > 0.05$, one-way ANOVA). Not determined (ND) because the low magnitude of response was at the limits of detection.