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Effects of Endogenous Dopamine on Measures of [^{18}F]N-Methylspiroperidol Binding in the Basal Ganglia: Comparison of Simulations and Experimental Results From PET Studies in Baboons

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ABSTRACT The effect of endogenous dopamine on PET measures of radioligand binding is important to the measurement of receptor density (or availability) and neurotransmitter interactions in vivo. We recently reported that pretreatment with amphetamine, a drug which stimulates dopamine release, significantly reduced NMS binding in the baboon brain as determined by the product λk_3 derived from the graphical analysis method for irreversible systems (λ is the ratio of the forward to reverse plasma to tissue transport constants and k_3 is proportional to receptor density) (Dewey et al.: *Synapse* 7:324–327, 1991). The purpose of this work is twofold: to evaluate the sensitivity and stability of the analysis method used for the NMS data and from simulation studies which include the competitive effects of dopamine on NMS binding to predict the effect of dopamine on the in vivo PET experiment. Using a measured plasma [^{18}F]-NMS input function from a control study in a baboon, simulation data was numerically generated explicitly allowing competition between NMS and dopamine in the calculation. This data was analyzed using the same techniques as used for the experimental data and the results were compared to in vitro calculations. The following conclusions were reached: 1) The effect of dopamine on specific binding was found to be greater in vivo than in vitro because the in vitro equilibrium experiment is controlled only by the relative Kd's of tracer and dopamine while the in vivo experiment also depends upon the halftime of tracer in tissue which is controlled by the tissue-to-plasma transport constant; 2) Experimental evidence from rodent studies (Seeman et al.: *Synapse* 3:96–97, 1989) and the agreement between PET studies (Wong et al.: *Science* 234:1558–1563, 1986a) and postmortem human studies (Seeman et al.: *Science* 225:728–731, 1984) in schizophrenics suggest that NMS is not likely to be affected by normal levels of endogenous dopamine. From the calculations reported here the effective in vivo Kd of dopamine for the NMS binding site would have to be on the order of or greater than 100 nM, assuming a synaptic dopamine concentration of 20 nM, in order that this concentration of dopamine have little effect on NMS binding.

INTRODUCTION

Two recent PET measurements of the concentration of dopamine D2 receptors in the brains of untreated schizophrenic patients have given conflicting results. Wong et al. (1986a), using the higher affinity radioligand [^{11}C]N-methylspiroperidol (NMS), found an elevated concentration of D2 receptors, in vivo, confirming measurements made in post-mortem brain tissue (Seeman et al., 1984, 1987). In contrast Farde et al. (1987, 1990) using the lower affinity ligand, [^{11}C]raclopride, found no elevation in the concentration of D2 receptors in schizophrenic patients relative to normal subjects. Whether or not receptor density (Bmax) is elevated in

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schizophrenic patients is an important medical and scientific issue and a NIMH workshop was recently convened to examine reasons for this discrepancy (Andreasen et al., 1988).

One of the variables that could account for the discrepancy in results is the different sensitivities of the radiotracers utilized to competition for the D2 receptor from endogenous dopamine. In fact, Seeman et al. (1989, 1990) have presented evidence that the presence of endogenous dopamine leads to an underestimation of Bmax and that the underestimation is greater for the lower affinity radioligand [^{3}H]raclopride than for [^{3}H](NMS). Another problem encountered in PET is the estimation of nonspecific binding (that is ligand bound to tissue but not to receptors). Seeman et al. (1990) have reported that although the density of D2 receptors is underestimated with raclopride in the presence of dopamine, if the cerebellum is used as a normalization for nonspecific binding, the apparent value for the D2 density is elevated since nonspecific binding is greater in the basal ganglia than in the cerebellum. This could account for the higher control densities reported in PET experiments using raclopride.

In contrast to the postulated high sensitivity of raclopride to the synaptic concentration of dopamine, it has been supposed that PET measurements with NMS are insensitive to changes in dopamine concentration. For example, Seeman et al. (1989) reported that in vitro D2 densities determined in human brain tissue with [^{3}H]NMS were unchanged by additional dopamine (100 nM). In order to assess the in vivo effect of changes in endogenous dopamine concentrations on [^{18}F]NMS binding in the baboon brain we investigated the effects of d-amphetamine with PET (Dewey et al., 1991). Amphetamine which increases striatal dopamine concentrations (Butcher et al., 1988; Kuczenski and Segal, 1989; Sharp et al., 1987) decreased striatal binding of [^{18}F]NMS in the 3 baboons studied. This result suggests that NMS binding can be sensitive to endogenous dopamine.

We consider in this paper the advantages and limitations of the method developed by Wong et al. (1986b) which was used to analyze the data from [^{18}F]-NMS studies (test/retest and control/amphetamine) in the baboon brain as well as the limitations of compartmental analysis applied to NMS data. Applying Wong's method of analysis to numerically simulated data, the predicted effect of dopamine on NMS binding in vivo was investigated and compared to in vitro calculations. Although it was found that the effect of dopamine on NMS binding is greater in vivo than in vitro, experimental evidence including the work of Hall et al. (1990), Wong et al. (1986a–c), and Seeman et al. (1989) indicates that normal levels of endogenous dopamine do not affect NMS binding. From the simulation results we find that for an endogenous dopamine concentration of 20 nM, the effective Kd of dopamine competing for the NMS binding site must be $\geq$100 nM if endogenous dopamine is to have little effect on NMS binding.

MATERIALS AND METHODS

Experimental

Five separate experiments with [^{18}F]NMS (5.9–12.8 mCi; 2.0–3.0 Ci/μmol) were done each consisting of a control and either a second control (test/retest) or a pretreatment study with d-amphetamine (1 mg/kg). The control and pretreatment (or test/retest) studies were done on different days. Animals were prepared for PET scanning and arterial blood sampling (including metabolite correction) as described by Dewey et al. (1990). Tracer distribution in brain tissue was followed for 170 min with a CTI tomograph (931-08/12; 15 slice, 6.5 mm slice thickness with an in plane resolution of 6.0 $\times$ 6.0 mm (FWHM)). In one study (#104) amphetamine was given intravenously over a time period of 25 min prior to the injection of [^{18}F]NMS. In the other two amphetamine studies (#105, #110), the amphetamine was given as a bolus 2–3 min prior to the injection of tracer (Dewey et al., 1991). Regions of interest were chosen for the basal ganglia and the cerebellum as described by Dewey et al. (1990, 1991).

Theory

The equations relating changes in concentration with time for bound tracer and competitor (such as endogenous dopamine) B* and B, respectively, and free (F*, F) tracer and competitor are given by (assuming known initial values for free and bound of both species)

$$\begin{aligned}
\frac{dF^*}{dt} &= -k^*_{on}(Bmax - B^* - B)F^* + k^*_{off}B^* \\
\frac{dB^*}{dt} &= \tilde{k}^*_{on}(Bmax - B^* - B)F^* - k^*_{off}B^* \\
\frac{dF}{dt} &= -k_{on}(Bmax - B^* - B)F + k_{off}B \\
\frac{dB}{dt} &= k_{on}(Bmax - B^* - B)F - k_{off}B.
\end{aligned} \tag{1}$$

Eq(1) is written in terms of concentrations of tracer and competitor. Under equilibrium conditions these equations become

$$\begin{aligned}
(Bmax - B^* - B)F^* &= Kd^* B^* \\
(Bmax - B^* - B)F &= Kd\, B.
\end{aligned} \tag{2}$$

Bmax is the total receptor concentration and Kd refers to an equilibrium dissociation constant. See Appendix A for definitions. Eq(2) can be solved for B and B* if either free (F, F*) or total ligand (free + bound) (T, T*) is specified for each species.

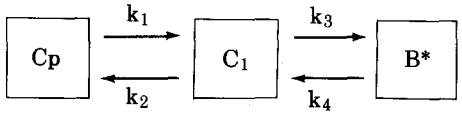
In the PET experiment, the radioligand is supplied by

capillary plasma. The equations relating transport of ligand between plasma and tissue and binding and dissociation of ligand and receptor in the presence of endogenous ligand can be written as (assuming a known fixed total concentration of dopamine given by T = B + F)

$$\begin{aligned}\frac{dF^*}{dt} &= k_1\,Cp(t) - \tilde{k}_2F^* - \tilde{k}^*_{on}\,(Bmax - B^* - B)F^* \\ &\quad + k^*_{off}B^* \\ \frac{dB^*}{dt} &= \tilde{k}^*_{on}\,(Bmax - B^* - B)F^* - k^*_{off}B^* \qquad (3) \\ \frac{dB}{dt} &= k_{on}\,(Bmax - B^* - B)(T - B) - k_{off}B\end{aligned}$$

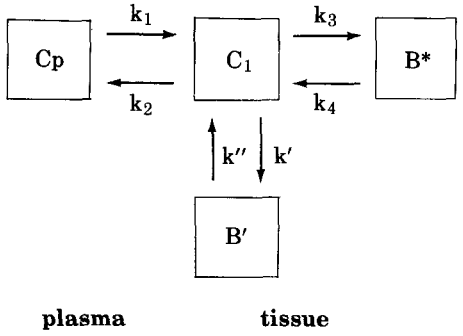
where Cp(t) is plasma ligand (NMS) concentration and k_1 and $\tilde{k}_2$ are the influx and efflux constants. Eq(3) represents a model of the synaptic concentration of antagonist (NMS) which explicitly includes competition between the tracer and dopamine for the receptor. The differential equations for bound dopamine and antagonist (dB/dt and dB*/dt) are coupled through the term (Bmax − B* − B). On the other hand, the (3 or 4 compartment) models generally used in fitting NMS data assume a constant free receptor concentration for which the concentration of tracer is small, that is, Bmax − B* ≈ Bmax. Furthermore these models (for example, Mintun et al., 1984) assume that dopamine does not interfere with the binding of NMS with the D2 receptor so that (Bmax − B* − B) ≈ Bmax. The 3 compartment model shown below for binding of NMS to the D2 receptor neglects the presence of other receptor types which bind NMS. Hall et al. (1990) report that 30 to 35% of total binding in in vitro studies with [^{3}H]-NMS in human putamen and rat striatum, respectively, is due to binding to serotonin receptors. If a second tissue compartment (representing serotonin receptors and/or other reversible binding) is included we have the 4 compartmental model. These models also do not separately treat "nonspecific" binding but rather consider the free ligand to be some fraction of the free plus nonspecifically bound ligand. Mintun et al. (1984) introduced the quantity "f_2" to represent the free fraction in tissue. This treatment of nonspecific binding as a fraction of non-receptor bound ligand is valid when nonspecific binding is much more rapid than the other binding or transport processes (Logan et al., 1987). (The additional nonspecific binding Seeman et al. (1990) report for basal ganglia could be rapid so as to be incorporated into the constant f_2 or if somewhat slower but still reversible, incorporated into B′ of the 4 compartment model.) The free fraction f_2 is incorporated into the efflux constant $\tilde{k}_2$ and the binding constant $\tilde{k}_{on}$ (Mintun et al., 1984) so that the constant k_3 shown below is given by $f_2\tilde{k}^*_{on}$ (Bmax − B* − B) and F* becomes f_2C_1 (also $k_2 = f_2\tilde{k}_2$ and $k^*_{on} = f_2\tilde{k}^*_{on}$) where C1 is the sum of the free and nonspecifically bound ligand.

3 COMPARTMENT MODEL



Including another compartment for NMS binding with transfer constants k′ and k″ gives

4 COMPARTMENT MODEL



where B′ represents additional binding of NMS (not to the D2 receptor) and $k_4 = k^*_{off}$.

The analysis methods applied to the experimental and simulated data are discussed below. The purpose of the simulation studies was to determine the predicted effect of dopamine on NMS binding by generating data using Eq(3) (or a modification of Eq(3) with an additional reversible binding component) in which dopamine is allowed to compete for the receptor and then analyzing this data using the standard techniques described below.

Analysis methods

The general procedure for applying these models to data is to determine the ratio of transport constants ($k_1/k_2 = \lambda$) from a region such as the cerebellum which does not contain D2 receptors to reduce the number of parameters to be determined from the receptor ROI (region of interest) time radioactivity data (Mintun et al., 1984; Wong et al., 1986b). For NMS an additional constraint that can be applied is that the receptor-ligand dissociation is so small that it can be neglected (or set to a very small value); this appears to be a reasonable assumption since we had previously observed no washout of radioactivity in baboon basal ganglia after 8 hours (Arnett et al., 1985) with [^{18}F]-spiroperidol. With these assumptions (fixing the ratio k_1/k_2 to the cerebellum value and setting k^*_{off} to a small value), the model fit to baboon striatum using the 3 compartment model (varying k_1 and k_3 to obtain the best fit in a least squares

sense) is poor. While the 4 compartment model gives a good fit to the data, the uncertainties in most of the parameters are relatively large and we found the test/retest reproducibility of the model parameters to be poor. Because of these difficulties, we use the procedure developed by Wong et al. (1986b) to determine the receptor-ligand association parameter which is based on the graphical method developed by Patlak et al. (1983, 1985), Fenstermacher et al. (1979), Blasberg et al. (1979), and Gjedde (1981, 1982; Gjedde et al., 1986). When ligands are irreversibly bound (valid for NMS for the duration of the PET experiment), Patlak et al. (1983) has shown that a plot of ROI(t)/Cp(t) vs. $\int_0^t Cp(t')dt'/Cp(t)$ becomes linear after some time with slope Ki. Assuming that the free receptor concentration (Bmax − B* − B) is reasonably constant for the duration of the experiment,

$$Ki = \frac{k_1 k_3}{(k_2 + k_3)} = \frac{k_1 \lambda k_3}{k_1 + \lambda k_3}. \quad (4)$$

Other independent classes of receptors which bind the ligand reversibly and have reached their steady state distribution will not contribute to the matrix product which makes up the slope Ki but will enter into the intercept (see Patlak et al., 1983; Wong et al., 1986b). When the reversible components have effectively reached a steady state, a linear portion of this plot is observed. The advantage of this type of analysis is that the presence of other kinds of binding do not have to be treated explicitly. The receptor parameter k_3 can be determined from Eq(4) if the other two parameters are known (Wong et al., 1986b,c). The transport constant k_1 can be determined from the initial part of the uptake curve by compartmental analysis. Although k_1 is a function of blood flow (see Patlak and Fenstermacher, 1975; Lassen and Gjedde, 1983), the ratio k_1/k_2 (λ) is independent of flow (see Appendix B). If λ determined from the cerebellum applies to the basal ganglia, k_3 can be found in terms of k_1, λ, and Ki, that is

$$k_3 = \frac{Ki\ k_1}{\lambda(k_1 - Ki)}. \quad (5)$$

However, recent results by Seeman et al. (1990) cast doubt on the validity of using a common value of λ for both basal ganglia and cerebellum since the nonspecific binding in basal ganglia was found to be greater than that in the cerebellum. If this additional nonspecific binding is rapid so as not to be separable from the transport process, "f_2" will be smaller, giving a larger value of λ. An alternative approach which we have also used is to report results in terms of the product λk_3 which is independent of both blood flow and the fraction "f_2" (nonspecific binding) which cancels from k_3 and k_2 (see Appendix B).

The parameter that is of primary interest in these experiments is the receptor availability which is contained in k_3 (or λk_3). However, it is derived from Ki which is not in general proportional to the number of free receptors but is a more complicated function of this parameter. The limiting factor is the sensitivity of Ki to changes in receptor number. The sensitivity of Ki to changes in k_3 and in k_1 can be assessed by taking the normalized derivative of Ki with respect to each one (Kim et al., 1990). A normalized derivative of 1 means that any change in the system is directly proportional to a change in the signal (Ki in this case). The normalized derivative of Ki with respect to k_1 is

$$\frac{\partial Ki/\partial k_1}{Ki/k_1} = 1 - \frac{k_1}{(k_1 + \lambda k_3)} \quad (6a)$$

and with respect to k_3

$$\frac{\partial Ki/\partial k_3}{Ki/k_3} = 1 - \frac{\lambda k_3}{(k_1 + \lambda k_3)}. \quad (6b)$$

If $\lambda k_3 >> k_1$, Ki is a function of k_1 with a small dependence on k_3 (a situation that is called "flow limited"). These functions are assessed for data from the control studies of Tables IB and IIB.

A 3 compartment model (4 parameter) with two transport constants (k_1 and k_2) and two binding constants (k_3 and k_4) was necessary to fit data from the cerebellum (see Logan et al., 1989). Data from cerebellar regions of interest were also analyzed by a graphical method which gives directly the volume of distribution (for a 3 compartment model this is given by $k_1/k_2(1 + k_3/k_4)$) as the slope of a plot of $\int_0^T ROI(T)/ROI(T)$ vs. $\int_0^T Cp(t)dt/ROI(T)$ where ROI(T) represents radioactivity in the cerebellum at time T (Logan et al., 1990).

Simulation studies of the effect of endogenous dopamine

Simulations of the effect of endogenous dopamine on radioligand binding using Eq(3) require values for the concentration of endogenous dopamine, the Kd of dopamine at the D2 receptor, as well as individual rate constants (k_{on} and k_{off}). Literature values for the concentration of endogenous dopamine vary over a 10-fold range. Most of the measurements represent extracellular concentrations determined by microdialysis: 7 to 9 pmol/cc (Becker, 1990), 10 pmol/cc (Reid et al., 1990), 20–50 pmol/cc (Church et al., 1987; Sharp et al., 1986; Zetterström et al., 1983). Ross (1991) estimates values of ≈50 pmol/cc based on increased binding of D2 agonist (N-n-propylnorapomorphine) after treatment with γ-butyrolactone to inhibit firing of dopaminergic neurons. A Kd of 5 nM for dopamine with the D2 receptor has been reported by Seeman et al. (1990). However Ross (1991) estimates the Kd to be on the order of the synaptic dopamine concentration, which would be ≈50 nM. Bennett and Yamamura (1985) quote a value of

$\approx 10^6$ M^{-1} sec^{-1} for the association constant for most neurotransmitter receptors. This gives $k_{on} = 0.06$ $nM^{-1}min^{-1}$ with k_{off} varying from 0.3 to 6 min^{-1} for Kd's of 5 and 100 nM, respectively. Due to the uncertainties in all these values, calculations were done with the dopamine concentration and Kd ranging from 5 to 100 nM. For a fixed total dopamine concentration, Eq(3) was solved numerically using the arterial plasma input function from experiment 100 and kinetic parameters given in Table IV assuming a specific activity (NMS) of 2.5 Ci/μmol. The specific activity was used to convert the plasma radioactivity into a concentration to solve Eq(3). The calculated total tissue concentration (T*) was converted to radioactivity-time data. The background dopamine concentration was assumed to be in equilibrium at the time of injection of tracer since equilibrium was rapidly established (within 1 min). The simulated data were then analyzed in terms of Ki (for times from 30 to 170 min) in order to determine the predicted effect of dopamine. The validity of this technique is confirmed by the observed linearity after some initial time of the plots in Figure 3. Dopamine does not distort the linearity of the plot but lowers Ki due to an apparent reduction in Bmax resulting from competition with endogenous dopamine.

Using a constant dopamine concentration oversimplifies the processes occurring in the synapse which include quantal release of dopamine and presynaptic reuptake, as well as binding to one or more states of D1 and D2 receptors with more than one affinity for each. However since the experimental (PET) measurement consists of an average over many synapses a constant value may be a reasonable approximation to a normal baseline condition.

To simulate the effect of a transient elevation of dopamine, loss of excess dopamine from the synapse after the introduction of a specified concentration was incorporated into Eq(3) removing the restriction that the total dopamine concentration remain constant. With this modification Eq(3) becomes Eq(7).

$$\begin{aligned}
\frac{dF^*}{dt} &= k_1 Cp(t) - \tilde{k}_2 F^* - \tilde{k}^*_{on}(Bmax - B^* - B)F^* + k^*_{off}B^* \\
\frac{dB^*}{dt} &= \tilde{k}^*_{on}(Bmax - B^* - B)F^* - k^*_{off}B^* \\
\frac{dF}{dt} &= -k_5 F - k^*_{on}(Bmax - B^* - B)F \\
\frac{dB}{dt} &= k_{on}(Bmax - B^* - B)F - k_{off}B.
\end{aligned} \tag{7}$$

TABLE IA. Test/retest results from baboon cerebellum

Baboon	Experiment No.	Type of study	λ[1]	K_R[2]
Oral	51	Control	3.47	6.9
	52	Control	4.36	7.2
Peace	71	Control	4.39	8.8
	78	Control	4.84	9.5

[1] k1/k2.
[2] For a 3 compartment model (2 tissue compartments) $K_R = \frac{k1}{k2}\left(1 + \frac{k3}{k4}\right)$.

Loss of free dopamine from the synapse is controlled by parameter k_5.

RESULTS

Experimental

Test/retest

Although both graphical and compartmental methods of analysis were used, the graphical analysis yielded far more stable results. For that reason all results (except λ) are reported in terms of parameters derived from the graphical analysis. The limitation of using the graphical method (Ki) is that it is not directly proportional to free receptor density. Using Eqs(6a) and (6b) the sensitivity of Ki to changes in k_3 and k_1 is assessed (Table III). The average normalized derivative of Ki with respect to k_1 is 0.45 and to k_3 is 0.51. This means that a 10% reduction in k_3 results in ≈5% reduction in Ki assuming k_1 constant. Therefore an observable change in Ki (that which exceeds experimental error) represents a relatively large change in receptor density while small changes are lost in the "noise." This limits the sensitivity of the measurement regardless of whether k_1 is used to eliminate the dependence on blood flow. In spite of this limitation, the method appears to be adequate to register changes due to amphetamine pretreatment.

The analyses of the experimental PET data assessing the reproducibility of NMS results in the same baboon are reported in Tables IA and IB. For the baboon cerebellum the results are reported as the steady state distribution volume and λ. The steady state distribution volumes (reported as K_R in Table IA) were determined graphically for times between 15 and 120 min. Because a 3 compartmental model was required to fit data from the cerebellum, (indicating the presence of something other than rapid nonspecific binding) λ had to be determined from a compartmental fit to cerebellar data. (For a 2 compartment model λ is equal to the distribution volume.) There was uncertainty in the determination of λ due to the difficulty of separating the 2 (or possibly more) components of the binding. We have not observed

TABLE IB. Test/retest results from baboon striatum

Baboon	Experiment No.	Type of study	Ki[1]	k1[1]	λk3[1]	k3[1]
Oral	51	Control	0.22	0.43	0.45	.130
	52	Control	0.21	0.40	0.42	.096
Peace	71	Control	0.25	0.60	0.43	.098
	78	Control	0.26	0.62	0.45	.093

[1] Min^{-1}.

TABLE IIA. Control/amphetamine results from cerebellum

Baboon	Experiment No.	Type of study	λ[1]	K_R[2]
Leah	103	Control	2.79	5.0
	104	Amphet[3]	2.08	4.0
Bright	108	Control	3.10	5.9
	110	Amphet	3.59	7.5
Clovis	100	Control	4.25	8.1
	105	Amphet	3.83	5.7

[1]k1/k2.
[2]For a 3 compartment model (2 tissue compartments) $K_R = \frac{k1}{k2}\left(1 + \frac{k3}{k4}\right)$.

this in human cerebellum data which can be very well described by a 2 compartment model. For n = 8, we found λ to be 3.2 for normal controls (Logan et al., 1991) which is similar to the value reported by Wong et al. (1986b) using [^{11}C]NMS. This difference between the human and baboon studies could be related to the use of anesthetic in the animal studies. λ is the only parameter from the cerebellum used in the analysis of data from basal ganglia. From Table IA there is variation in both λ and in the distribution volume between the test/retest studies. Table IB contains values for Ki, k_1, λk_3, and k_3 for basal ganglia which was calculated using λ from the cerebellum. Due to variability in λ, there is considerable variability in k_3. However, there is much less variability in λk_3 ($\approx$7% for test/retest) which is a decided advantage of using this parameter for comparison of experimental data.

Some of the difficulties of using the compartmental analysis method are evident from a compartmental analysis of data from basal ganglia ROI of experiment 52. Two different (but indistinguishable) fits to a 4 compartmental model were found. In one case λ was fixed at the cerebellum value of 4.4 giving $k_1 = 0.45$, $k_3 = 0.094, k' = 0.17, k'' = 0.05\ min^{-1}$. In the other case $\lambda = 6$, with $k_1 = 0.44$, $k_3 = 0.071$, $k' = 0.13$, $k'' = 0.05$. Both sets of parameters are consistent with a Ki value of 0.21 (indicating that the 4th compartment does not contribute to Ki). Clearly the uncertainty in k_3 from the 4 compartment fit is large given the uncertainty in λ. For this particular data set, a 3 compartment model fits the data reasonably well if both k_1 and k_2 were allowed to vary; however, λ for the optimized k_1 and k_2 was found to be $\approx$20 which is considerably larger than the values found for the cerebellum and therefore probably not physiologically meaningful. Lack of reproducibility of receptor parameters derived from compartmental models is seen by comparing results of a 4 compartment model fit to data from the first of the test/retest studies in Oral (experiment 51) which gave a k_3 value of 0.08 min^{-1} (for $\lambda = 6$) which differs by $\approx$16% from the value for experiment 52 even though Ki from Table IB is in good agreement for both experiments. The variability in these model fits illustrates the hyper-sensitivity of parameters characteristic of an ill-conditioned system (Kim et al., 1990).

Effects of amphetamine on NMS binding

Figure 1 illustrates uptake in striatum for control/amphetamine pretreatment studies (#100/#105). The Patlak-Gjedde analysis for this experiment is illustrated in Figure 2. From Table IIB all three studies show a decrease in Ki and λk_3 (for λk_3 % changes with amphetamine are 12% [#104], 28% [#110], and >90% [#105]). Two of the three cases show a decrease in Ki accompanied by a decrease in k_3. In one case an increase in k_3 is observed with a small decrease in Ki. This is due to the smaller value of λ found for the pretreated study and this is more likely to be a result of uncertainties in parameters from which k_3 is calculated than a real elevation in k_3. In the second case k_3 was smaller than the control and in the last case specific binding was reduced to such an extent that k_3 (or free receptor concentration) is close to zero. There was no significant change in k_1 between control and amphetamine experiments. Compartmental model fits to experiments 100/105 are illustrated in Figure 1. Note, however, that large standard errors were found in the receptor parameters.

Simulation studies

Studies with a constant dopamine background

The effect of a constant endogenous dopamine concentration on Ki is simulated in Figure 3 using the input function from experiment 100 with $k_1 = .50$, $\lambda = 6$, $k^*_{on} = 0.004\ nM^{-1}\ min^{-1}$, Bmax = 20 pmol/g, $k_{on} = 0.06\ nM^{-1}\ min^{-1}$, Kd = 5 nM. The value used for λ was slightly larger than λ found for the cerebellum to allow for the possibility that λ in the basal ganglia is larger due to an increase in rapid nonspecific binding. The

TABLE IIB. Control/amphetamine results from striatum

Baboon	Experiment No.	Type of study	Ki[1]	k1[1]	λk_3[1]	k_3[1]
Leah	103	Control	.090	0.21	0.16	.056
	104	Amphet[2]	.083	0.21	0.14	.065
Bright	108	Control	.160	0.38	0.28	.089
	110	Amphet[3]	.133	0.41	0.20	.055
Clovis	100	Control	.205	0.50	0.35	.082
	105	Amphet[3]	.020	0.43	0.02	.005

[1]Min^{-1}.
[2]Amphetamine give over 25 min *prior to* [^{18}F]-NMS.
[3]Amphetamine given as a bolus 2-3 min before NMS.

TABLE III. Normalized derivative for K_1 and K_3

Expt No.	$1-\lambda k_3/(k_1 + \lambda k_3)$	$1-k_1/(k_1 + \lambda k_3)$
103	0.57	0.43
108	0.42	0.42
100	0.41	0.41
51	0.49	0.55
52	0.49	0.51
71	0.58	0.42
78	0.58	0.42
Avg	0.51	0.45

TABLE IV. The effect of endogenous dopamine on Ki[1]

Dopamine (pmol/cc)	Ki (with dopamine)/Ki (without dopamine)			
	Kd = 5	Kd = 10	Kd = 50	Kd = 100
5.0	.89 (.94)	.91 (.94)	.96 (.97)	.99 (.99)
10.0	.76 (.87)	.81 (.89)	.92 (.94)	.95 (.96)
25.0	.46 (.66)	.60 (.72)	.82 (.86)	.90 (.91)
50.0	.24 (.37)	.36 (.48)	.70 (.74)	.81 (.83)
100.0	.10 (.18)	.20 (.26)	.53 (.57)	.69 (.70)

[1]Parameters used in the calculations were k1 = .5 min^{-1}, k_{on}^* = .004 nM^{-1} min^{-1}, Bmax = 20 pmol/cc, k_{off}^* = .0005 min^{-1}, k_{on} = .06 nM^{-1} min^{-1}, k_{off} varied from 0.3 to 6.0 min^{-1} (Kd ranged from 5 to 100 nM). Values in parentheses are for k_{on}^* = .002 nM^{-1} min^{-1} and Bmax = 40 pmol/cc. specific activity was 2.5 Ci/μmol. Total dopamine concentration varied from 0 to 100 pmol/cc. Results are reported as the ratio of Ki determined from data files generated using the kinetic parameters given above with the competitor (dopamine) to Ki determined without the competitor in the simulation. Ki for the control case was 0.23 min^{-1} (Bmax = 20 pmol/cc) and .234 (Bmax = 40 pmol/cc).

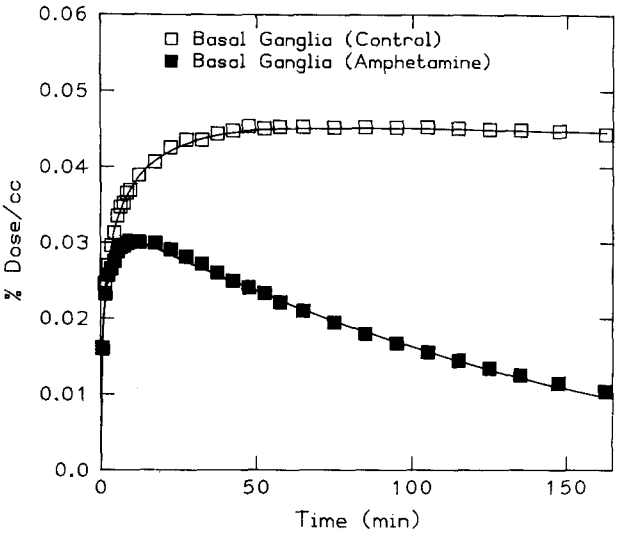


Fig. 1. ROI from striatum for control study 100 and from study 105 after amphetamine pretreatment. The fit to data points for the control study was for the 4 compartment model with k_{off}^* (k_4) fixed at .0005 min^{-1}. The optimized parameters were k_1 = .48, k_2 = .06, k_3 = .045, k′ = .065, k″ = .032 in units of min^{-1}. In this case λ = 8 and as a result k_3 is smaller than the value reported in Table IIB although the product λk_3 is the same (.36). There is, however, considerable uncertainty associated with the determination of this number of parameters. The model fit for the pretreatment study was to a 3 compartment model in which the dissociation constant was not fixed but allowed to vary. Although this gives a good fit to the data, it clearly is not a correct model. In this case k_1 (which is probably the only meaningful parameter in this analysis) was found to be .44 min^{-1}.

dissociation constant for NMS (k_{off}^*) was fixed at a small value, .0005 min^{-1}. Calculations were also done with k_{on}^* = 0.002 nM^{-1} min^{-1}, and Bmax = 40 nM (values given in parentheses in Table IV) to see the dependence on Bmax while maintaining constant the product k_{on}Bmax. The ratios of Ki determined with dopamine to Ki without dopamine are reported in Table IV. For a dopamine concentration of 5 pmol/cc (Kd = 5 nM) and Bmax = 40 pmol/cc the Ki is reduced by 6% and the apparent Bmax is reduced by 11% and for Bmax = 20 pmol/cc the decrease in Ki and Bmax are 11% and 20%, respectively. The change in Bmax was calculated by taking the ratio of k_3 values determined from Ki. Although this represents a relatively large change in Bmax, the observed change in Ki (Fig. 3) is much less. This apparent reduction in binding is considerably more than would be predicted from equilibrium in vitro calculations with the same amount of competitor (see discussion and Table VIA). For larger values of Kd, Ki was found to be less sensitive to increasing dopamine concentrations (Table IV). This is expected since the larger Kd reduces the number of receptors occupied with a given concentration of dopamine and the Ki is therefore much less sensitive to the presence of competitor. There was no noticeable effect on Ki due to variations of k_{on} from 0.006 to 3 $nM^{-1}min^{-1}$.

To determine if the additional reversible binding such as that represented by serotonin receptors affects results, calculations were also done including the 4th compartment in Eq(3) with k′ = 0.08 and k′ = 0.04 min^{-1}. For the control case (no dopamine) the slope for the 3 compartment model was the same whether the time period of evaluation was 30 to 90 min or 30 to 170 min. With the 4th compartment the slope evaluated for 30 to 170 min was the same as for the 3 compartment model but the slope for 30 to 90 min was 5% higher. Also the slope (0.23 min^{-1}) was less than what would be predicted assuming no occupancy of receptors (that is for k_3 = 0.08 min^{-1} which would give Ki = 0.244 min^{-1}). Due to the specific activity and parameters used in the calculations, the concentration of specifically bound NMS was 2 pmol/cc at 165 min which lowered the calculated Ki. It is necessary to evaluate Ki at times that allow the reversible components to reach an effective steady state as well as to consider possible effects due to occupancy of receptors by unlabeled tracer. These considerations should be taken into account when analyzing experimental data.

If the simulations are done with a constant dopamine concentration of 20 nM which is intermediate in the range of values reported for baseline measurements (Becker 1990; Church et al., 1987; Reid et al., 1990; Sharp et al., 1986; Zetterström et al., 1983) k_{on}^* (NMS) must be increased from 0.004 to 0.01 $nM^{-1}min^{-1}$ (for Kd = 5 nM) to generate the same uptake curve obtained without dopamine using k_{on}^* = 0.004. If Kd is increased

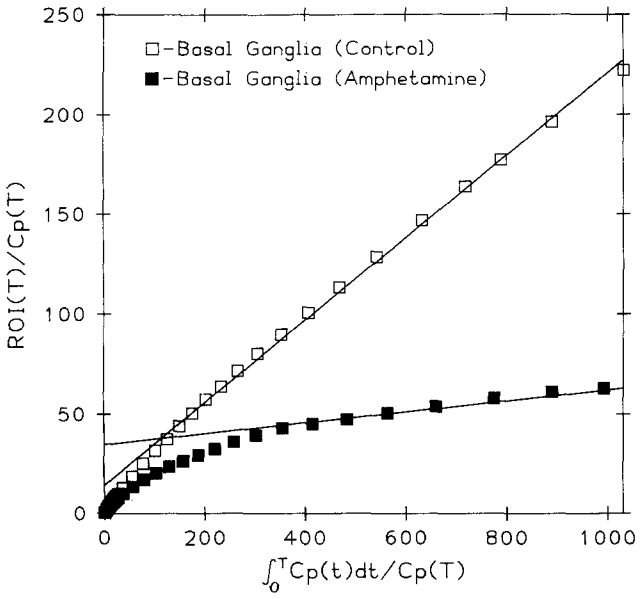


Fig. 2. Patlak analysis of the data in Figure 1. The slope (Ki) was found to be .205 min^{-1} for the control study and .02 min^{-1} for the pretreatment study.

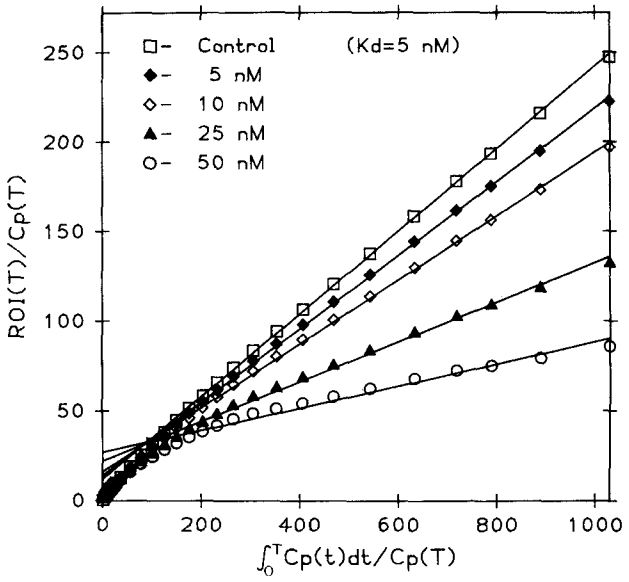


Fig. 3. Analysis of simulation data in terms of Ki for different concentrations of endogenous dopamine. The parameters used in the simulation are given in Table IV for $\lambda = 6$, Kd = 5 nM. Bmax was fixed at 20 pmol/g and dopamine concentration is indicated. Simulation data was generated from Eq(3).

to 10 nM, k^*_{on} becomes 0.0079. Only when Kd is 100 nM does k^*_{on} become 0.0046 which is only slightly larger than the original value. This implies that if the background dopamine concentration is on the order of 20 nM, the effective in vivo Kd of dopamine in competition with NMS would have to be ≥100 nM for the background endogenous dopamine to have little effect on NMS binding. Even for a dopamine concentration of 5 nM a dopamine Kd of 75 nM is required so that NMS $k^*_{on} = .0041$.

Effect of a transient dopamine pulse

A second set of calculations (Eq(7)) was done to simulate a transient elevation in endogenous dopamine due to amphetamine varying both the halftime of the transient peak in tissue ($t_{1/2}$) and the time (relative to injection of tracer) (Δt) at which it was introduced into the calulation. A constant background dopamine concentration of 20 nM was assumed. From calculations described previously, a Kd of at least 100 nM was necessary for this concentration of dopamine to have only a small effect on NMS uptake. Using Kd = 100 nM, a transient dopamine pulse was introduced at times $\Delta t = -30$ (30 min before injection of tracer), $\Delta t = 0$ (at the same time as tracer) and $\Delta t = 30$ min after injection. Values used for the half-time in tissue were $t_{1/2} = 10$ and 30 min with peak dopamine concentrations of 150, 500, and 1,000 pmol/g. The initial (transient) dopamine concentration was assumed to be all free. The results are given in Table V as the ratio of Ki with competitor under conditions described above to Ki with a constant dopamine background of 20 nM and Kd = 100 nM. This shows that the effect depends on the amount of dopamine released, the halftime in tissue, and relative time of injection of tracer. From Table V the effect is seen to be substantially lost if the peak occurs 30 min or more

TABLE V. The effect of a transient elevation in dopamine in Ki[1]

$t_{1/2}$	Dopamine (nM)	Ki(dopamine)/Ki(control) ($\Delta t = 0$)
10	1,000	0.63
30		0.36
10	500	0.73
30		0.54
10	150	0.94
30		0.89
		($\Delta t = 30$)
10	1,000	0.89
30		0.77
10	500	0.93
30		0.84
10	150	0.98
30		0.94
		($\Delta t = -30$)
10	1,000	0.91
30		0.54
10	500	0.97
30		0.93
10	150	1.00
30		0.91

[1]Dopamine concentrations of 1,000, 500, and 150 nM were introduced at the same time as injection of tracer ($\Delta t = 0$), 30 min after ($\Delta t = 30$) or 30 min before ($\Delta t = -30$). Loss of dopamine from synapse was allowed by introducing the half-time of free dopamine in tissue $t_{1/2}$. E_q (7) was used for dopamine concentrations that exceeded the background value of 20 nM and Eq(3) was used when transient dopamine concentrations had fallen to the background value. The transient dopamine was assumed to be initially all free. Kd for dopamine was fixed at 100 nM. Ki for the control case was 0.22 min^{-1} which included a constant dopamine background of 20 nM.

TABLE VIA. In vitro calculations of the effect of endogenous dopamine

	B* (with dopamine)/B*(without dopamine): dopamine concentration (pmol/cc) (Bmax = 20 pmol/g Kd = 5 nM)				
Kd*	10	20	40	50	60
.075[1]	0.99	0.99	0.98	0.97	0.97
.125	0.99	0.99	0.97	0.96	0.94
1.38[2]	0.96	0.90	0.75	0.69	0.63

TABLE VIB. In vitro calculations of the effect of endogenous dopamine[1]

	B* (with dopamine)/B*(without dopamine) dopamine concentration (pmol/cc): (Bmax = 11 pmol/g²Kd = 5 nM)				
Kd*	10	20	40	50	60
.075[2]	0.99	0.98	0.95	0.94	0.93
.125	0.99	0.97	0.93	0.90	0.89
1.38[3]	0.88	0.77	0.58	0.51	0.46

[1]Using Eq (2) with 1.5 pmol/cc tracer.
[2]Kd (NMS) and Bmax determined by Seeman et al. (1989).
[3]Kd for Raclopride determined by Seeman et al. (1989).

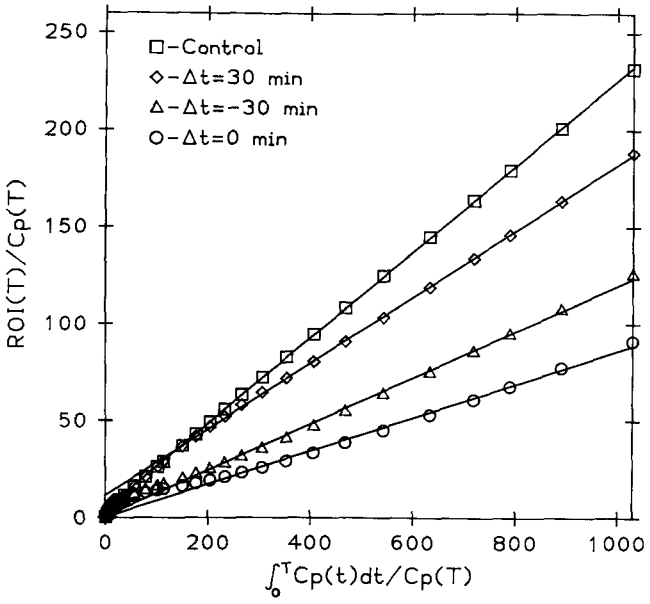


Fig. 4. Analysis of simulated data in terms of Ki for a peak dopamine concentration of 1,000 pmol/cc with a halftime in tissue of 30 min and Kd of 100 nM and a background dopamine concentration of 20 nM.

before injection of tracer with a halftime of 10 min. For a peak concentration of 1,000 nM with $t_{1/2}$ = 10 min and Δt = −30, Ki is greater than 90% of the control value.

Simulations also show the effect is smaller if the dopamine peak occurs some time after introduction of NMS. Since the dissociation of NMS from the receptor is slow, the only effect dopamine can have is to prevent NMS from binding. (However, in the case of raclopride which has a more rapid dissociation, an increase in endogenous dopamine occurring after the tracer has reached a steady state can have a marked effect on binding. This could be observed as a decrease in the distribution volume.) With short halftimes, the effect of even large concentrations of dopamine on NMS uptake can be considerably less than what would be predicted for a constant concentration. Figure 4 illustrates graphical analysis of data simulated with $t_{1/2}$ = 30 min and Kd = 100 nM. Although free receptor concentration is increasing due to loss of excess of dopamine from the synapse, all data sets have a clearly defined linear region.

In vitro calculations comparing tracer ligands with different Kd's

A comparison of the effect of endogenous dopamine on ligand binding in vitro is given in Tables VIA and VIB for Kd* values of .075, .125, and 1.38 nM in the presence of various concentrations of competitor with Kd = 5 nM. The results are reported as the ratio of ligand bound in the presence of competitor to ligand bound without competitor (T = 0) calculated from Eq(2) for a loading dose of T* = 1.5 pmol/cc as a function of total competitor concentration using Bmax = 20 pmol/cc and Bmax = 11 pmol/cc. The Kd* value of .075 nM corresponds to the value determined by Seeman et al. (1989) for NMS in vitro and Kd* = 1.38 nM corresponds to the in vitro value for raclopride (Seeman et al., 1990). Kd = 5 nM for dopamine was determined by Seeman et al. (1990). The Kd* value of .125 is consistent with values used in the calculations reported here. The presence of endogenous dopamine has a much smaller effect on binding for the ligand with a Kd* of .075 or .125 nM (binding is only reduced by 7–11% for an endogenous dopamine concentration 60 pmol/cc with Bmax = 11 pmol/cc) but a much greater effect on binding (reduced by 50% for the same dopamine concentration) for Kd* = 1.38.

DISCUSSION

We have observed that in vivo increases in endogenous dopamine concentration stimulated by amphetamine can affect the binding of [^{18}F]NMS in basal ganglia. In order to understand the effects of a constant background of dopamine on [^{18}F]NMS binding, as well as the effects of a transient pulse of dopamine as expected from an amphetamine experiment, we have simulated PET data from NMS experiments under both conditions. These simulations indicate that the presence of a constant dopamine concentration during the course of an experiment has a greater effect on specific binding for NMS in the PET in vivo experiment than for the in vitro equilibrium experiments. The reason is that the in vitro equilibrium experiment is controlled by the relative Kd's of the tracer ligand and competitor (dopamine) while the in vivo experiment also depends upon the half-time of the radioligand in tissue which is determined by the transport (influx and efflux) con-

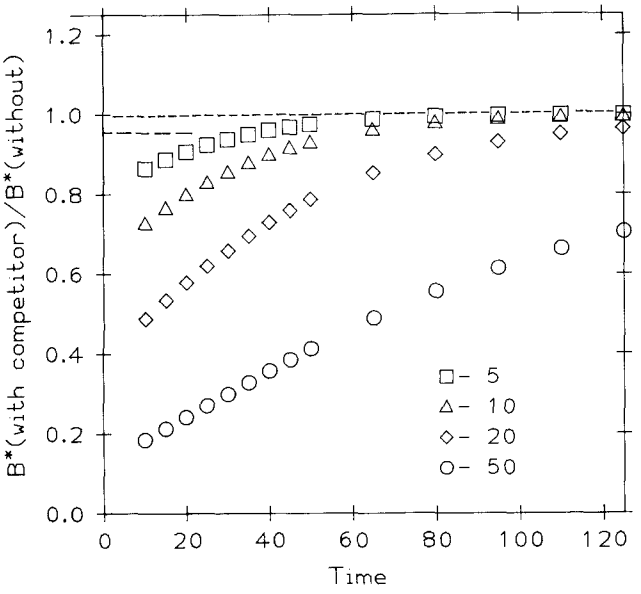


Fig. 5. In this simulation 1.5 pmol/cc of NMS was introduced directly into the tissue compartment as free ligand with $k_2 = 0$ so that no ligand was lost, along with varying amounts of dopamine as specified in pmol/g. The parameters used were $k^*_{on} = .004$ $nM^{-1}min^{-1}$ and $k^*_{off} = .0005$ min^{-1} for NMS and $k_{on} = .06$ $nM^{-1}min^{-1}$ and $k_{off} = .3$ min^{-1} for dopamine. The results are expressed as the ratio of bound NMS in the presence of dopamine to the amount bound without dopamine. The presence of dopamine greatly slows the rate at which NMS approaches its equilibrium value. The equilibrium value of the ratio of NMS bound in the two cases is .99 for a dopamine concentration of 20 pmol/cc and is indicated by the upper dashed line. For a dopamine concentration of 50 pmol/cc the equilibrium value is .96 and is indicated by the lower dashed line.

stants. As a result, a concentration of endogenous dopamine that would have little effect on an in vitro experiment can have a significant effect on NMS binding in vivo. The greater sensitivity of the in vivo simulations can be understood from Figure 5 in which 1.5 pmol of NMS was introduced into the tissue compartment as free ligand (with k_2 in Eq(3) set to 0 so that no ligand is lost from tissue) along with varying amounts of free dopamine. The ratio of bound NMS in the presence of competitor (dopamine with Kd = 5 nM) to the value without competitor is plotted vs. time. The presence of the competitor greatly slows the overall rate at which NMS binds to receptor. Even in the presence of 5 pmol/cc of competitor the amount of tracer bound does not approach the value bound without competitor (that is $\approx$1.0 in the plot) for about 40 min. Therefore in the in vivo experiment when the rate of loss of NMS from tissue is a competing factor, considerably less ligand will be bound than in the absence of the competitor. If the calculations shown in Figure 5 are followed to equilibrium, the ratio of B* (with dopamine)/B* (without dopamine) is 0.96 (see Fig. 5) for 50 pmol/cc dopamine so that, as expected, an in vitro experiment with NMS would show only a small dependence on dopamine. The postulated effect of endogenous dopamine on the PET experiment has been previously considered by Friedman et al. (1984) although assuming a slow loss from tissue and equilibrium conditions which do not hold for NMS.

Calculations with the transient dopamine peaks for Kd = 100 nM, illustrate the variability in results to be expected under different physiological conditions. Kuczenski and Segal (1989) observed that both the magnitude and duration of dopamine elevation were related to the dose of amphetamine given. There is undoubtedly considerable variation in individual response to an amphetamine dose as evidenced by the variation in our experimental results (Table IIB) for which ratios of Ki(amphetamine) to Ki (control) are 0.92, 0.83, and 0.1. This is consistent with results in humans in which the physiological response to an amphetamine stimulus is tremendously variable from one individual to the next (Angrist, 1987).

The in vitro results in Tables VIA and VIB illustrate that specific binding of radiotracer in the presence of dopamine depends upon the relative Kd's of tracer and dopamine (in contradiction to a recent statement to the contrary by Farde et al., 1989). Raclopride is affected by the presence of endogenous dopamine to a much greater extent than NMS due to its larger Kd. However, from the simulations reported here, both NMS and raclopride can be significantly affected by the presence of dopamine when the loss of NMS from tissue (k_2) is also important. We cannot directly address the effect of dopamine on the in vivo PET experiment with raclopride. However, using the kinetic constants determined by Farde et al. (1989), ($k_1 = 0.17$, $k_2 = 0.4$, $k_{on} = 0.015$, $k_{off} = 0.1$ and Bmax = 22 nM) with an input function synthesized to generate curves similar to the published striatal curves and Eq(3), we find that specific binding of raclopride is decreased by 11% in the presence of 5 nM dopamine (Kd = 5 nM) at the time-radioactivity maximum (total tracer uptake was simulated to be .3 pmol/g). For the in vitro equilibrium case the uptake is reduced by 5% for the same Kd and Kd*.

In contrast to the in vitro results of Table VIA, it is apparent (see Table IV) that synaptic concentrations of dopamine greater than 5 pmol/cc will have a distinct effect on the in vivo uptake of NMS if the Kd of dopamine is on the order of 5 nM. If the in vivo Kd is larger, the effect of dopamine can be significantly less. Seeman's observation that the striatum to cerebellum radioactivity ratio in rats given [^{3}H]spiperone to be unaffected by prior treatment with resperine indicates that a normal concentration of dopamine does not greatly affect NMS binding. Furthermore Wong et al. (1986a) report values for Bmax determined with NMS in vivo in normal and schizophrenics that are consistent with those determined by Seeman et al. (1984) in postmortem tissue. This also implies that NMS binding is not affected by normal endogenous dopamine levels and also that possible errors due to the use of λ from cerebellum in determination of k_3 are unimportant. Further evidence

that NMS is not affected by normal levels of endogenous dopamine comes from work by Hall et al. (1990) who investigated in vitro competition studies with [^{3}H]NMS and dopamine as well as D2 antagonists including raclopride. They interpret the large inhibition constants found in the competition studies with [^{3}H]NMS (generally several times larger than those found for [^{3}H]-raclopride) as implying that the binding site of NMS on the receptor does not exactly coincide with the active (dopamine) site. This is also consistent with the model developed by Rognan et al. (1990) for the attachment sites of antagonists on the D2 receptor. They propose four main areas on the receptor to be related to antagonist binding (not all antagonists bind to all four areas). One region common to haloperidol and spiperone but not to the benzamides such as sulpiride is proposed to be responsible for the high affinity of these drugs. Rognan et al. (1990) also found the benzamides to be more potent in inhibiting agonist induced behavior in mice due to apomorphine. From this model the site associated with the high affinity of spiperone is apparently not the same as the agonist site. This suggests that the Kd of dopamine in competition with NMS could be considerably larger than that determined for interaction with the dopamine-D2 binding site, resulting in a lesser effect on NMS binding.

The method chosen for the analysis of the experimental data was based on the graphical method of Wong et al. (1986b); however, we found that for the NMS baboon data the most stable parameter for comparison of experiments was λk_3 rather than k_3 as used by Wong. This method is limited by the sensitivity of Ki to changes in Bmax and as a result there are limits to the reliability of Bmax and other parameters derived from Ki. In spite of this, we have found the Wong method to be more stable than the standard nonlinear fit to compartmental models which in the case of the 3 compartment model gave parameter values for λ from basal ganglia which were considerably larger than those from the cerebellum while the 4 compartment model (which is more likely to represent the physiology) was associated with large uncertainties in parameter values as evidenced by the two different but indistinguishable fits to the same set of data.

The conclusions to be drawn from the simulation studies reported here are that the efflux (tissue to plasma) constant which determines the halftime of the ligand in tissue is at least as important a factor as Kd when considering results of competition between a drug and an endogenous neurotransmitter for a receptor and that the effective Kd of dopamine in competition with NMS is probably on the order of or greater than 100 nM if endogenous dopamine concentrations are 20 nM. As a result a more sensitive tracer for changes in endogenous dopamine would be a ligand such as raclopride which according to Hall et al. (1990) competes with dopamine for the dopamine-D2 binding site.

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APPENDIX A

B^* is the concentration of specifically bound radioligand.

B is the concentration of specifically bound competitor.

F^* is the concentration of free radioligand.

F is the concentration of free competitor.

K_d^* is the equilibrium dissociation constant = $k^*_{off}/\tilde{k}^*_{on}$ for radioligand.

k^*_{on} is the radioligand receptor association constant.

k^*_{off} is the radioligand-receptor dissociation constant.

K_d is the equilibrium dissociation constant for competitor = k_{off}/k_{on}.

Bmax is the total concentration of receptors.

"f_2" is the free fraction of ligand $= \dfrac{1}{1+k'_{NS}/k''_{NS}}$ where k'_{NS} and k''_{NS} refer to association and dissociation constants for rapid nonspecific binding (Logan et al., 1987).

$k_3 = f_2\tilde{k}^*_{on}(\text{Bmax} - B^* - B) = k^*_{on}(\text{Bmax} - B^* - B)$.

$F^* = f_2 C1$.

C1 is sum of free and nonspecifically bound ligand.

k' and k'' are association constant and dissociation constant for binding not to D2 receptor.

B' is concentration of binding controlled by constants k' and k''.

k_1 is the plasma-to-tissue influx constant.

$k_2 = \tilde{k}_2 f_2$ is the observed tissue-to-plasma efflux constant.

$\lambda = k_1/k_2$.

Ki is the slope of the graphical analysis for irreversible systems described in general terms by Patlak et al. (1983).

APPENDIX B

The average concentration of drug within a capillary supplied with an arterial input of concentration Cp and flow F′ is given by (Patlak and Fenstermacher, 1975; Lassen and Gjedde, 1983)

$$Cc = Cp(1. - e^{-k_1'/F'})\frac{F'}{k_1'} + C_1 \frac{k_2'}{k_1'} \left(1. - \frac{1}{k_1'/F'}(1 - e^{-k_1'/F'})\right) \quad (1B)$$

where C_1 is the concentration in tissue surrounding the capillary, k_1' and k_2' are the permeability surface constants for transport into and out of tissue respectively. For compounds like NMS these constants contain factors that take into account nonspecific binding so they are not equal (Mintun *et al.*, 1984). The uptake of tracer by tissue (assuming no binding) can be described by

$$\frac{dC_1}{dt} = k_1'Cc - k_2'C_1. \quad (2B)$$

Substituting for Cc in Eq(2B) gives

$$\frac{dC_1}{dt} = F'(1. - e^{-k_1'/F'})Cp + \frac{k_2'}{k_1'}F'(1. - e^{-k_1'/F'})C_1, \quad (3B)$$

which can also be written as

$$\frac{dC_1}{dt} = k_1\, Cp - k_2\, C_1 \quad (4B)$$

where

$$k_1 = F(1. - e^{-k_1'/F'}) \quad (5B)$$

and

$$k_2 = \frac{k_2'}{k_1'}F'(1. - e^{-k_1'/F'}) \quad (6B)$$

so that the ratio is the same for both, *i.e.*, $k_1/k_2 = k_1'/k_2'$ with no dependence on F′.

Also

$$\lambda k_3 = \frac{k_1}{\tilde{k}_2 f_2}f_2\tilde{k}_3 = \frac{k_1}{\tilde{k}_2}\tilde{k}_3$$

where $\tilde{k}_3 = \tilde{k}_{on}Bmax$.