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MEASUREMENTS, IN VIVO, OF PARAMETERS OF THE DOPAMINE SYSTEM

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The purpose of this paper is to discuss methods of measuring important parameters of the dopamine system in the living animal by use of PET techniques. We will discuss the methods and then compare the results of some of these measurements.

One primary concern is the density and binding affinity of post-synaptic neuroreceptors. These have been measured, in vivo, by use of binding assays on synaptosome preparations; but the values, while relevant, are not necessarily the same as in living tissue. In addition, there is some evidence (1,2,3) that receptor densities may change, in vivo, as a result of drug manipulations or disease. A second concern is the activity of neurons, in vivo, this is generally related to the turnover of neurotransmitter and can also be related to the uptake of precursor compounds by the neurons. As we shall see, if the transmitter and neuroleptic compound compete for the same binding sites (on the receptor molecule) these two effects are interwoven and are not easily isolated.

MASTER

Since the primary measurement in positron tomography is of activities, concentrations, and temporal behavior we must somehow relate a model of these observables to the parameters we wish to measure. We have chosen to do this by use of the equilibrium model of ligand binding first advanced by Clark (4) and modified by Triggle (5). It certainly is true that the most general case would include the kinetic parameters of the uptake and binding process. However, if the kinetics of binding are controlled by the flow of the radiopharmaceutical from the brain; and if this process is slow enough for equilibrium between ligand and receptor to be maintained; then the equilibrium model will describe the relationship between receptor and drug. This appears to be the case under normal circumstances for many neuroleptic drugs. Leyson et al. (6) found that the binding of both halperidol and spiroperidol to striatal microsomes in vitro was complete within 5 minutes. They also found that the dissociation half lives were complex and consisted of a short component (0.55 min for halperidol, and 2.4 min for spiroperidol) and long component (99 minutes for halperidol and 25 minutes for spiroperidol). On the other hand, Kulmala et al (7) found (for bromospiroperidol) that the increase and decrease of the drug in the brain, in vivo, varied slowly over a time scale of hours; a similar observation was made for spiroperidol by Laduron et al (8). Therefore, it appears that the movement of neuroleptic drugs from the brain is indeed slow enough to allow equilibrium to be maintained between ligand and receptor, especially after some time for the initial washout and translocation in the brain. We will examine this assumption in more detail later in this paper. To test the consequences of equilibrium binding and the possible use of the model for measurement of receptor densities by emission tomography we have modified Clark's calculation in several ways. We have included binding behavior at low doses, simultaneous competitive binding of

two ligands to the same receptor, and the binding of a single ligand to a pair of binding sites of differing association constants. In this note we will describe the solutions of the equations and some comparisons of the predictions of the model with data, as well as its application to tomographic measurements.

MATHEMATICAL MODEL:

We will define the basic equation:

$$A + R = AR \tag{1}$$

Where A is a ligand (in this case the neuroleptic drug) R is the receptor AR is the ligand-receptor complex Ka is the equilibrium constant for the reaction

In this case:

$$Ka = (AR)/((A)(R)) \tag{2}$$

and

$$Ka = 1/Ki$$

where Ki is the usual dissociation constant for receptor binding, i.e. the concentration of ligand corresponding to 50% binding. If Ro is the total concentration of receptor, Ao is the total concentration of ligand, and X is the amount of bound ligand, then at equilibrium:

$$(A) = (Ao - X)$$

$$(R) = (Ro - X)$$

$$(AR) = X$$

and $$Ka = X / ((Ao - X)(Ro - X)) \qquad (3)$$

A useful parameter is the fraction of receptors which are occupied, Ya, where

$$Ya = (AR) / ((R) + (AR)) = X / Ro$$

From equations (2) and (3):

$$Ya = X / Ro = (Ka)(Ao - X)(Ro - X)/((Ro - X) + X)$$

From which we get by rearrangement:

$$Ya = 1 / (1 + (1/(K (Ao - X))) \qquad (4)$$

Then : $$X = (Ro)(Ya)$$

A second useful parameter is the fraction of ligand bound to receptor which we shall designate as Fa

$$Fa = X / Ao \qquad (5)$$

It is, of course feasible to solve equation (3) directly for X as was done by Eckelman et al (9). However, for the more complex case of several ligands or receptors, it will be easier to use this formalism. In practice we will assign values of Ro and K, and for a range of values of free drug concentration (Ao - X) we will calculate Ya, X, Ao, and Fa. Figure 1a and 1b indicate the behavior of Ya and Fa as a function of total drug concentration, Ao, for a variety of values receptor density, Ro.

If we now consider the case of two ligands (A and B) competitively binding to the same receptor with association constants, Ka and Kb, then we have two simultaneous equations:

$$A + R = RA$$

$$B + R = RB$$

These can be solved in the same manner as above to give:

$$Ya = 1 / (1 + (1 / (((Ka)(Ao - X)) + ((Bo - Z)(Kb))/((Ka)(Ao -X))) \quad (6)$$

Which is essentially the solution given by Triggle (5).

Where Ka and Kb are the equilibrium constants, Ao and Bo are total concentrations, and X and Z are the bound concentrations of ligands A and B respectively. The amounts of free ligands (Ao - X) and (Bo - Z), equilibrium constants (Ka and Kb), and receptor density (Ro), are input parameters.

Then:

$$X = (Ro)(Ya) \quad (7)$$

$$Ao = X + (Ao - X) \quad (8)$$

$$Fa = X / Ao \quad (9)$$

and

$$(Bound/Free)_A = \frac{X}{A_o - X} = K\ (R_o - X - Z) \quad (10)$$

Similar solutions can readily be obtained for ligand B by use of the reciprocity theorem, i.e. by simply interchanging X,Z,A,B,and a,b in the

equations. In our case we will consider ligand B to be the endogenous dopamine in the brain, and will assume that the concentration of free dopamine stays approximately constant during the experiment. Figure 2 indicates the effect (on Ya and Fa) of varying the concentration of ligand B. There are several important points we should note about the behavior of the functions Fa and Ya, as can be seen by examination of Figs. 1 and 2. First, the fraction of ligand bound, Fa, does not increase continuously with decreasing ligand concentration but approaches an assymptotic limit. Unfortunately, at very low ligand concentrations there is a high percentage of non specific binding, which reduces the length of the plateau. From equations (3) and (5) we can see that the limiting value occurs when X is negligible compared to Ro. At that point the ratio of bound to free ligand, X / (Ao - X), equals (Ka)(Ro) (Ro-RB). Therefore, there will be no change in Fa beyond this point.

It is obvious from equation (10) that the limiting value of the ratio of bound/free drug will be defined by K,Ro and concentration of competetive ligand B. In that case, measurements made with a neuroleptic drug at low concentrations will not reveal more than the amount of free receptor rather than total receptor concentration. Holt and Schubert (10) showed that, for dopamine active neuroleptics, the binding in the cerebellum was essentially nonspecific and that in the striatum was specific; They proposed that the ratio of the amount of drug bound per gram in the striatum to that in the cerebellum was a measure of the specific binding, and this convention has been widely used since that time. The striatal binding includes both specific, displaceable binding to dopamine receptors and non specific, non displaceable binding. The bound drug in the cerebellum is non displaceable and non specific. Therefore, the fraction of bound drug, Fa, should equal the ratio of the differences in concentration in the striatum and cerebellum to the

concentration in the striatum (for dopamine active neuroleptic drugs). In principle, the displaceable binding could be measured by performing a displacement reaction and saturating the receptors with nonradioactive drug. In practice, it is much more feasible to use the striatum/cerebellum concentration ratio; we will estimate the error induced by this approximation later in this paper. It is patently obvious that the concentration of the drug in the cerebellum can also serve as a measure of non specific binding in the frontal cortex and other areas of the brain. However, it will not serve as a measure of binding to secondary, displaceable sites. Second, the fraction of receptors bound, Ya, approaches 1.0 at high concentrations of ligand when the specific binding ratio, Fa, approaches zero. Therefore, measuring receptor density non invasively by displacement would require the measurement of a small difference in ligand concentration. This, of course, is the least accurate method of approach. In fact, if the specific binding ratio is 0.1 then a 1% error in measurement of the ligand concentration before and after displacement could lead to a 20% error in receptor density; this is comparable or greater than the potential error introduced by using the striatum/cerebellum ratio for measurement of specific binding ratio. Third, specific binding and receptor occupancy could be strongly dependent on the concentration of the second ligand if the value of [B]Kb is comparable to [A]Ka (see Fig. 2). Since studies of the binding of all neuroleptic drugs , in vivo, will be in the presence of endogenous neurotransmitters it is important to evaluate the magnitude of the interaction of the endogenous material with the receptors. The case of two binding sites competing for the same ligand is a trivial extension of these equations. Let us define the concentration of binding sites as Rx for type X and Ry for type Y. Then the concentration of ligand A in each site is given by the solution of the simultaneous equations:

$$A + X = AX \qquad K = Kx$$

$$A + Y = AY \qquad K = Ky$$

At very dilute concentrations, the ratio of the concentration of ligand bound to each site is given by:

$$AX/AY = (Kx)(Rx)/(Ky)(Ry)$$

If site Y is non specifically dispersed and consists of a small number of tightly bound complexes the observed specific binding will disappear and the total/free ratio will approach 1 at low concentrations of ligand.

COMPARISON WITH DATA

There are three relatively complete sets of data on the binding, in vivo, of neuroleptic drugs which are antagonistic to the dopamine system. One of these was obtained by DeJesus et al. (11) on the binding of ^{3}H-Sp and ^{77}Br-BrSp in mice, the next was due to Laduron et al. (8) on the binding of H-3 spiroperidol in rats, and the last was due to Kulmala et al. (7) on the binding of Br-77 bromospiroperidol in rats.

The first point we will test is the validity of our approximation that the specific binding ratio can be obtained by using the concentration in the cerebellum as a measure of non specific binding and free drug. Table 1.,taken from the data in the literature (7,8) shows the amount of neuroleptic found in the striatum, frontal cortex, and cerebellum in rats at various times after injection of a low and high concentration of the drug .We have also listed in the table the values of specific binding fraction, Fa calculated by assigning the specifically bound drug concentration as (total - nondisplaceable) and

compared these values to those calculated by assigning it to (striatal concentration - cerebellar concentration). As can be seen from the table, the approximation becomes valid for bromospiroperidol, in rats, after 3 hours. However, it does not become valid for spiroperidol for some time and is still in error by 20% after 8 hours. This reflects the kinetics of the approach to equilibrium and is likely to be both species and drug dependent, requiring evaluation for each set of experiments. In particular, the kinetics may also be dependent on the concentration of injected drug.

Table 1.

Specific Binding Fraction of Br-77 bromospiroperidol in rats (7)

Tissue	Time After Injection	$Fa^{(a)}$	$Fa^{(b)}$
Striatum	3 hrs	0.73+/- .05	0.71 +/- .05
	4 hrs	0.85 .06	0.76 .05
	5 hrs	0.88 .09	0.81 .08

Specific Binding Fraction of H-3 spiroperidol in rats (8)

Striatum	1 hr.	0.00	0.78
	2 hrs	0.37	0.82
	4 hrs	0.57	0.90
	8 hrs	0.69	0.88
	16 hrs	0.83	0.90

(a) calculated from (total - displaceable) ligand

(b) calculated from (striatal conc. - cerebellar conc.) of ligand

We can also test the behavior of the specific binding of radiobrominated spiroperidol (BrSp) and tritiated spiroperidol (Sp) in vivo against our model by using the data of DeJesus et al. (11). Fig. 3. illustrates the specific binding ratio found by DeJesus et al for these compounds in mice. The solid curves are the values calculated for these compounds. In Figure 4 we can see the behavior of the specific binding at very low drug loadings (11). The rapid fall off at low doses is indicative of a low concentration of sites, ubiquitously distributed in the brain. Figs. 5 and 6 illustrate the values of chi-square obtained between calculated and measured values as a function of receptor density and dopamine content. It is of interest to note that a receptor density of 130+/-25 picomoles/gram of tissue and a dopamine concentration of 5.0 nanomoles/gram gives the best fit to this data when using published values of the association constant for BrSp (0.385×10^{12} g/mole) and DA (1.0×10^{9} g/mole).

In principle, this could be the basis of the method used to measure receptor densities with PET. A single measurement could be made of the striatal and cerebellar quantities at equilibrium. These, and the resulting specific binding ratio will define the receptor density if Ka,Kb, and the DA concentration are known. Unfortunately we do not know the DA concentration in vivo at any given time. It would require a pair of measurements at widely spaced concentrations to determine both parameters. This might be done by injecting the drug at a relatively high dose and following the washout along the equilibrium isotherm, however the washout after equilibrium is a very slow process, so this does not appear feasible.

A second approach to a method of measurement is suggested by comparing the data and calculations (made using the model) as shown in Figs. 7, 8, 9, and 10. In these figures we have shown the data obtained by DeJesus et al.

(11) showing the concentration of BrSp and Sp observed in the striatum and cerebellum of mice as a function of injected dose.

In order to use the model for calculations of these values we have to determine a method of quantifying the relationship between injected dose and the concentration of free drug in the brain. If the barrier between the two compartments is treated as a lipid phase membrane interspersed between two aqueous compartments which allows extraction and transfer of solute between the compartments at equilibrium, then the ratio of the concentration of solute between the inner and outer compartment is given by:

$$C(\text{inner})/C(\text{outer}) = k(\text{outer})/k(\text{inner})$$

where k is the the equilibrium constant for extraction of solute from the aqueous solutions to the lipid membrane. Since the blood concentration of BrSp and Sp is of the order of 0.3%/gram this ratio appears to be about 30/1 for our data.

The extraction coefficient for these two compartment systems is defined as the ratio of concentration of solute in the inner compartment to the sum of concentrations of both compartments. Our data would then yield an extraction coefficient (at equilibrium) of 96%. This is greater than reported values of first pass extraction coefficients of ^{18}F- Sp of 70 - 90%, but equilibrium values should be higher.

In order to calculate the values of the cerebellar concentrations in Figs. 7-10 we have used this value of the extraction coefficient and an average blood concentration of 0.3%/g. As can be noted by inspection of the figures this gives a very reasonable fit to the data. This simple model also implies that the washout of drug from the brain is directed by the very slow

decrease in concentration of the drug in the blood after the initial drop to ~0.3%/g..

The striatal concentrations are calculated by the model using the free (i.e. cerebellar) concentrations as a base. Figures 7-10 are the results of calculations made with differing values of DA concentration. As can be noted, the curves can be forced to fit the data on the linear portion of the curves, but the "fit" is very sensitive to DA concentration in the initial section. When the values of CHI2 are calculated as a function of DA concentration the typical parabolic behavior (See Fig. 6) indicates that a value of 5.0 nanomoles per gram is most suitable for the SYNAPTIC DA concentration. Fig. 10 shows the results of a calculation with this concentration and a receptor density of 130 picomoles/gram of tissue. The mean standard deviation of all of the data points from the calculated values in Fig. 10 is 9.0%.

We can also use the model to determine the fraction of occupied receptors, and have done so using the same parameters as for Fig. 10. These results are given in Fig. 11. In the figure we see the calculated values of the fraction of receptors occupied by Sp,DA, and free receptor.

The first major point of interest in Fig. 11 is that at low doses of Sp or BrSp, almost all of the receptors are occupied by DA; while at high doses over 90% of the receptors are occupied by the drug.

The second major point is that the shape of the striatal concentration curve is strongly dependent on SYNAPTIC DA concentration at low drug doses since most of the receptors are occupied by endogenous DA.

This then provides a method of measurement. If two separate measurements are made, one at drug loadings of 10-30 micrograms/kg and one at loadings of 150 - 200 micrograms/kg we can use the model to obtain values of BOTH the receptor densities and synaptic DA concentration. Alternately, a measurement

made at low drug loadings, on the plateau of the specific binding curve will yield the density of free receptors, and a measurement made at high loadings will yield the total receptor density. From these, one can infer the number of receptors occupied by DA and hence the synaptic DA concentration. This latter method would require that there is no loss of specificity for the drug at low concentrations. Wagner has reported that methyl spiroperidol does not lose specificity at low concentrations(12) and this drug could thus be used in that manner.

MEASUREMENTS WITH PET

Our method of approach with PET is to first determine if we can obtain data on primates comparable to that obtained in mice and to determine the applicability of the model to primates. Therefore we have started a series of studies, using positron emitting isotopes to label BrSp, of the striatal and cerebellar concentrations and of the time necessary to reach transient equilibrium.

Since our PET 6 system has recently become operative we have only been able to perform a single set of measurements. This was done in a rhesus monkey (15 kg.) at a dose loading of 7 micrograms/kg. Because of the uncertainties in scheduling and starting an initial experiment we elected to use ^{76}Br for this experiment. Consequently, about one milicurie of ^{76}Br-p-bromospiroperidol was injected after adjustment with non radioactive BrSp to make up the drug loading. The image obtained by X-Ray Computer Axial Tomography (CAT) of a monkey in a 4mm slice corresponding to PET level 6 was superimposed on the PET image to identify the caudate nuclei. There was reasonable uptake in the caudate nuclei and a number of other areas of high concentration, although the caudate nuclei had the greatest concentration.

One objective in this experiment was to study the time course of washout from the brain to estimate the time necessary for equilibrium to be reached. It was found that the measured striatum/cerebellum ratio gradually increased to 1.88/1.0 and was still increasing at 230 minutes post injection. In conjunction with the presence of other areas of high concentration we feel that this indicates a slower approach to equilibrium than in our experiments on small animals. We do not know whether this is characteristic of primates, dose dependence of the kinetics, or simply a poor experiment.

A second objective was to determine whether the extraction coefficient was the same as found in small animals. Our studies with mice would have predicted a concentration of drug in the cerebellum of 1.25 picomoles/gram at a loading of 7 micrograms/kg. We find that in this experiment the cerebellar concentration varied from 1.5 picomoles/g 20 minutes post injection to 1.0 picomoles/g after 4 hours. At the standard time of 2 hours (used for all the murine experiments) it was 1.35 picomole/gram. This is an encouraging correspondence.

The third objective was to determine the specificity of binding. Our small animal data had indicated that the maximum specific binding fraction of BrSp was 0.875, as indicated by limiting striatal/cerebelllar ratio of 8/1. Since this experiment was performed at low resolution (1.5 cm), the ratio of concentrations of a small point source, such as the caudate nucleus, to a source, such as the cerebellum, would have to be corrected for partial volume effects. The correction can be calculated from the size of the caudate nuclei observed in the CAT scans and is approximately 2.25/0.5. This yields a corrected ratio of about 7.6/1 or about a specific finding fraction of 0.8. We feel that the agreement is fortuitous, but the results do indicate a reasonable amount of specificity. Our future studies will be performed in the high resolution mode to minimize the correction factor.

CONCLUSIONS

We can draw several conclusions from these studies:

1. The model is applicable in small animals and indicates that the use of a radioactive neuroleptic drug can yield information on both receptor densities and on synaptic dopamine concentrations, in vivo.
2. The concentration of free ligand in the brain can be estimated by a simple relation between levels of drug in the blood and an extraction coefficient.
3. The first studies on primates with PET and BrSp indicate that the method and model should be as feasible as the small animal studies.

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FIGURE CAPTIONS

Figure 1. Variation of computed Specific Binding Ratio and Fraction of Receptor Bound as a function of total ligand concentration [A] for various values of Receptor Densities [R].

Figure 2. Variation of computer specific binding ratio and fraction of receptors bound as a function of total ligand concentration [A] for various concentrations of competitive ligand [B].

Figure 3. Variation of the ratio of bound drug to free drug for Sp and BrSp in the caudate nuclei of mice as a function of the concentration of free drug. The curves are calculated using the parameters in the text.

Figure 4. This is identical to Fig. 3, but also includes values for Sp and BrSp obtained at low concentrations.

Figure 5. Variation in chi-squared as a function of receptor densities [R].

Figure 6. Variation of chi-squared as a function of DA concentration for the most probable value of receptor density [R].

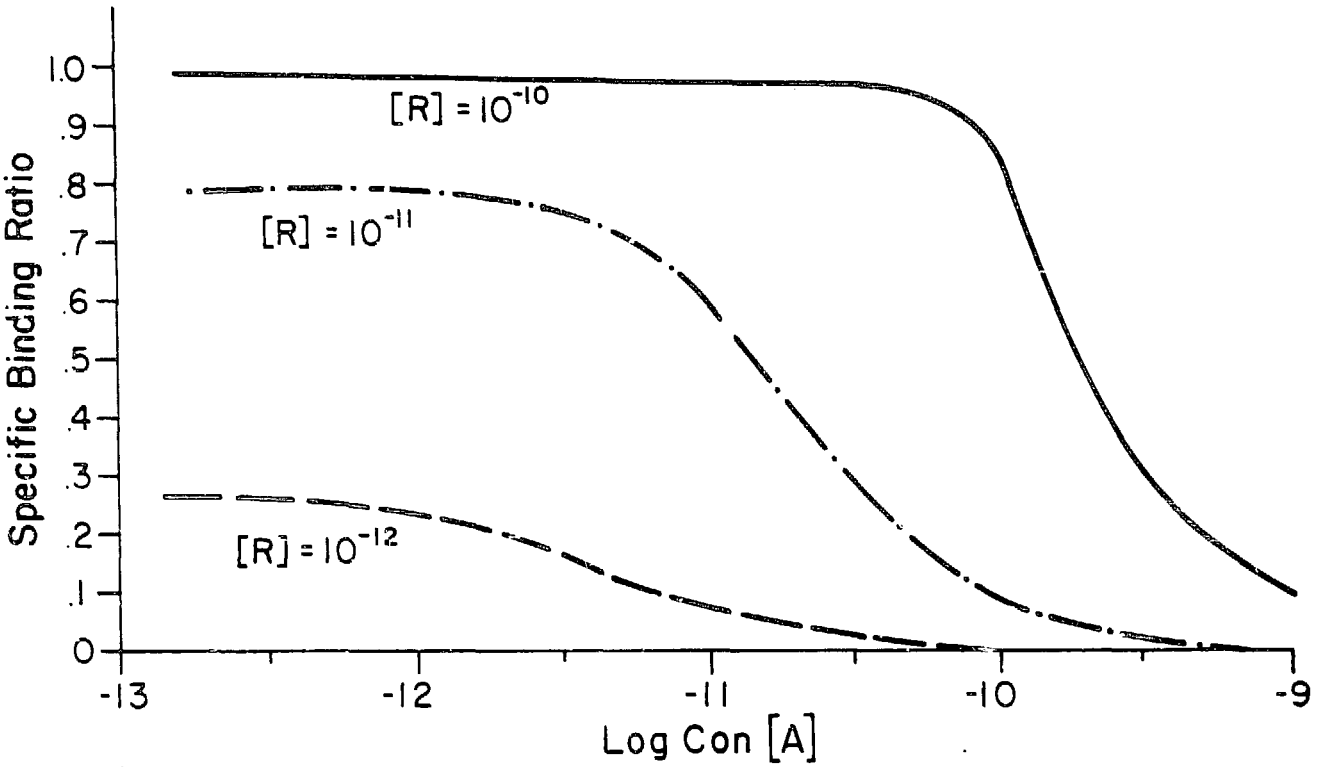
Figure 7. Comparison of measured and calculated values of striatal and cerebellar concentrations; calculated for DA concentration equals 0.

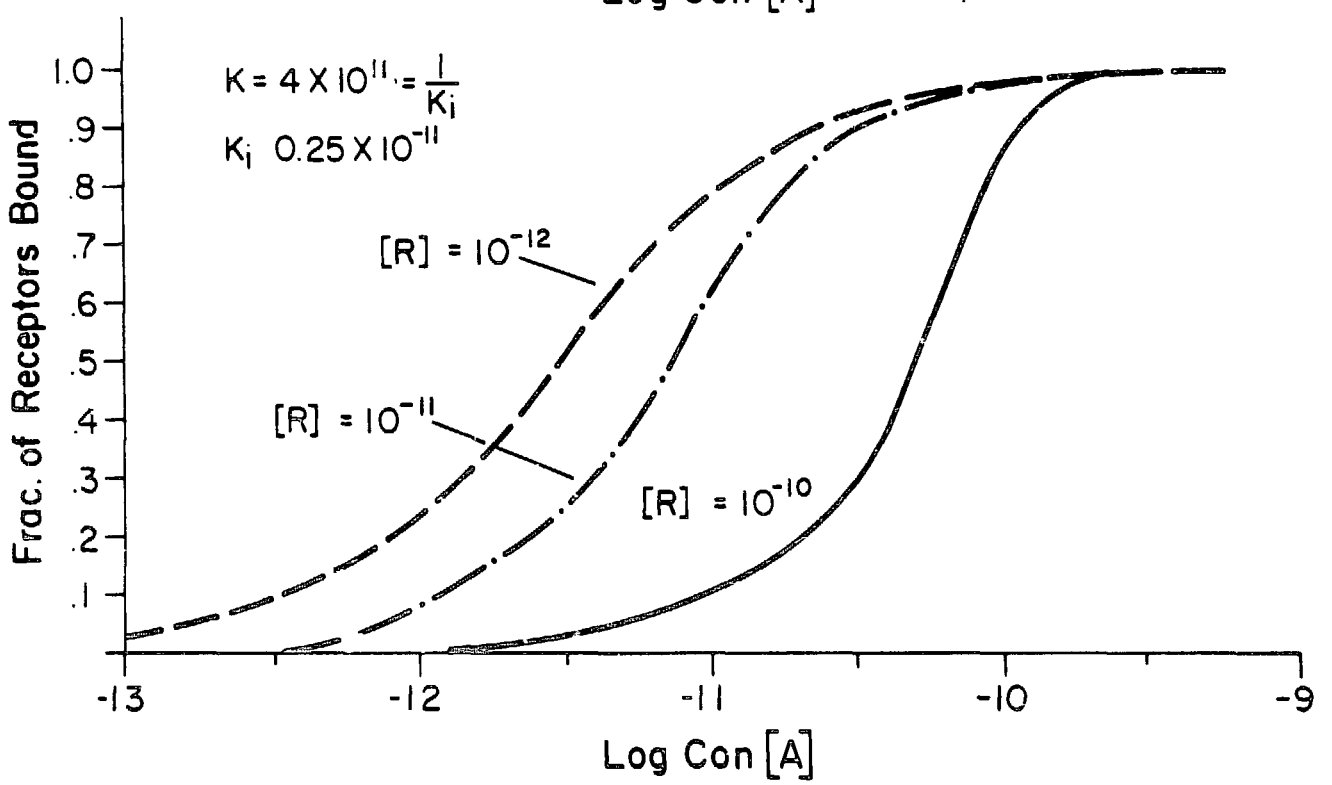
Figure 8. Same as Fig. 7 but calculated for [DA] = 0.05×10^{-8} molar.

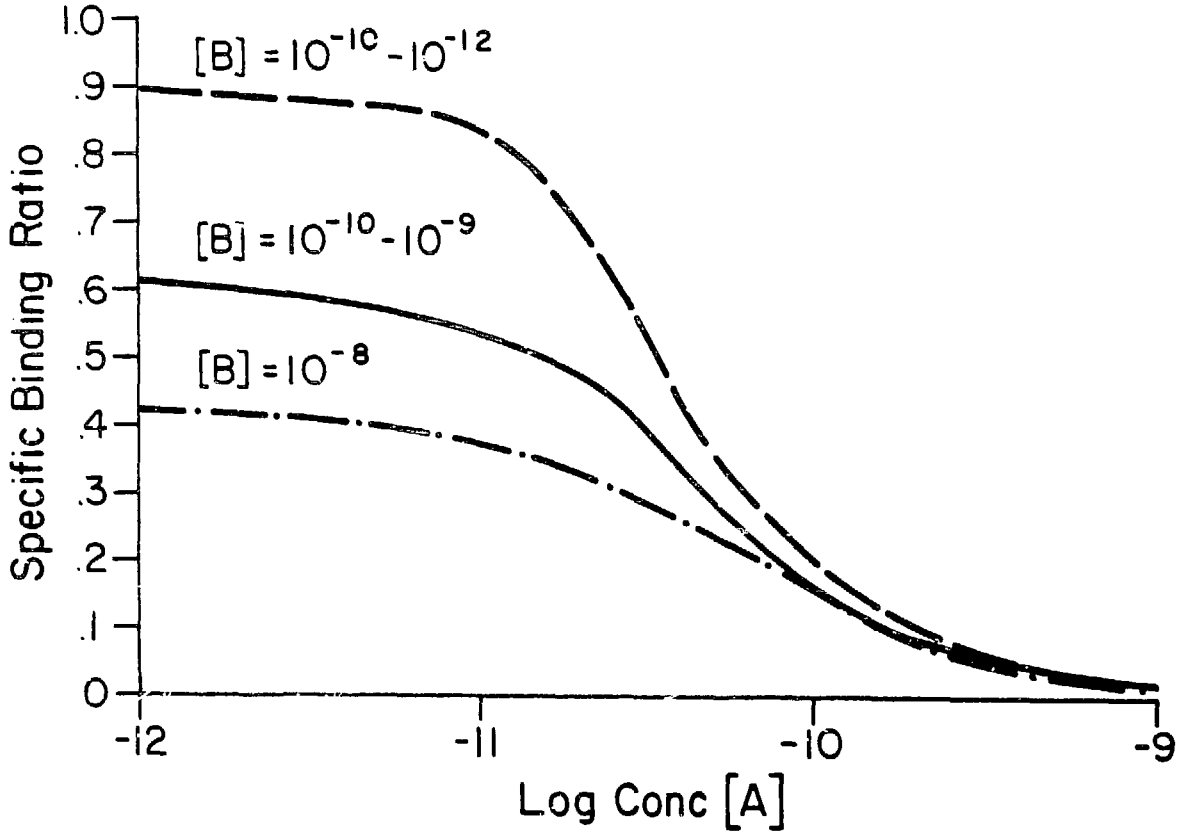
Figure 9. Same as Fig. 7 but calculated for [DA] = 2.0×10^{-8} molar.

Figure 10. Same as Fig. 7 but calculated for [DA] = 0.5×10^{-8} molar, this is the optimal value indicated in Fig. 6.

Figure 11. Fraction of free receptors, Sp and DA occupied receptors as a function of injected dose. These values are obtained [illegible]ng the calculation of the curves of Fig. 10.


Specific Binding Ratio
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
[R] = 10^-10
[R] = 10^-11
[R] = 10^-12
-13
-12
-11
-10
-9
Log Con [A]


Frac. of Receptors Bound
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
K = 4 X 10^11 = 1/Ki
Ki 0.25 X 10^-11
[R] = 10^-12
[R] = 10^-11
[R] = 10^-10
-13
-12
-11
-10
-9
Log Con [A]


Specific Binding Ratio
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
[B] = 10^-10 - 10^-12
[B] = 10^-10 - 10^-9
[B] = 10^-8
-12
-11
-10
-9
Log Conc [A]

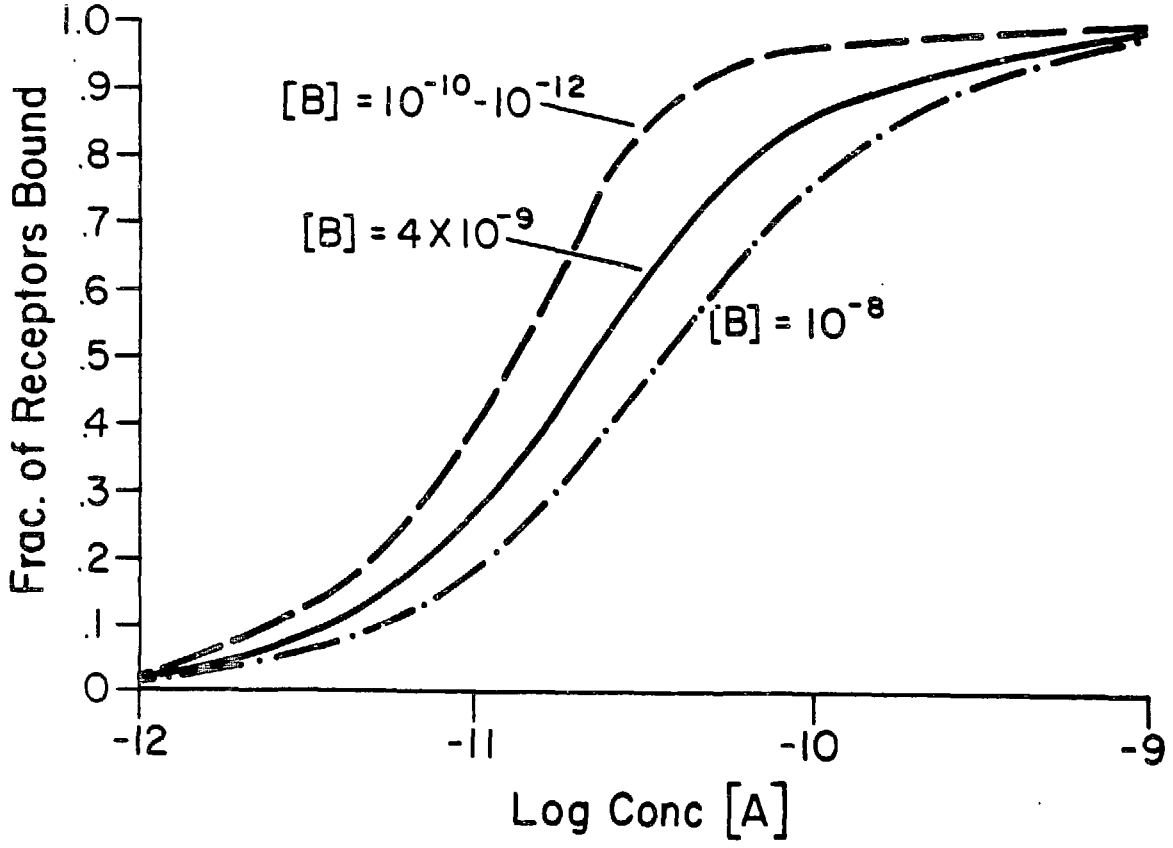
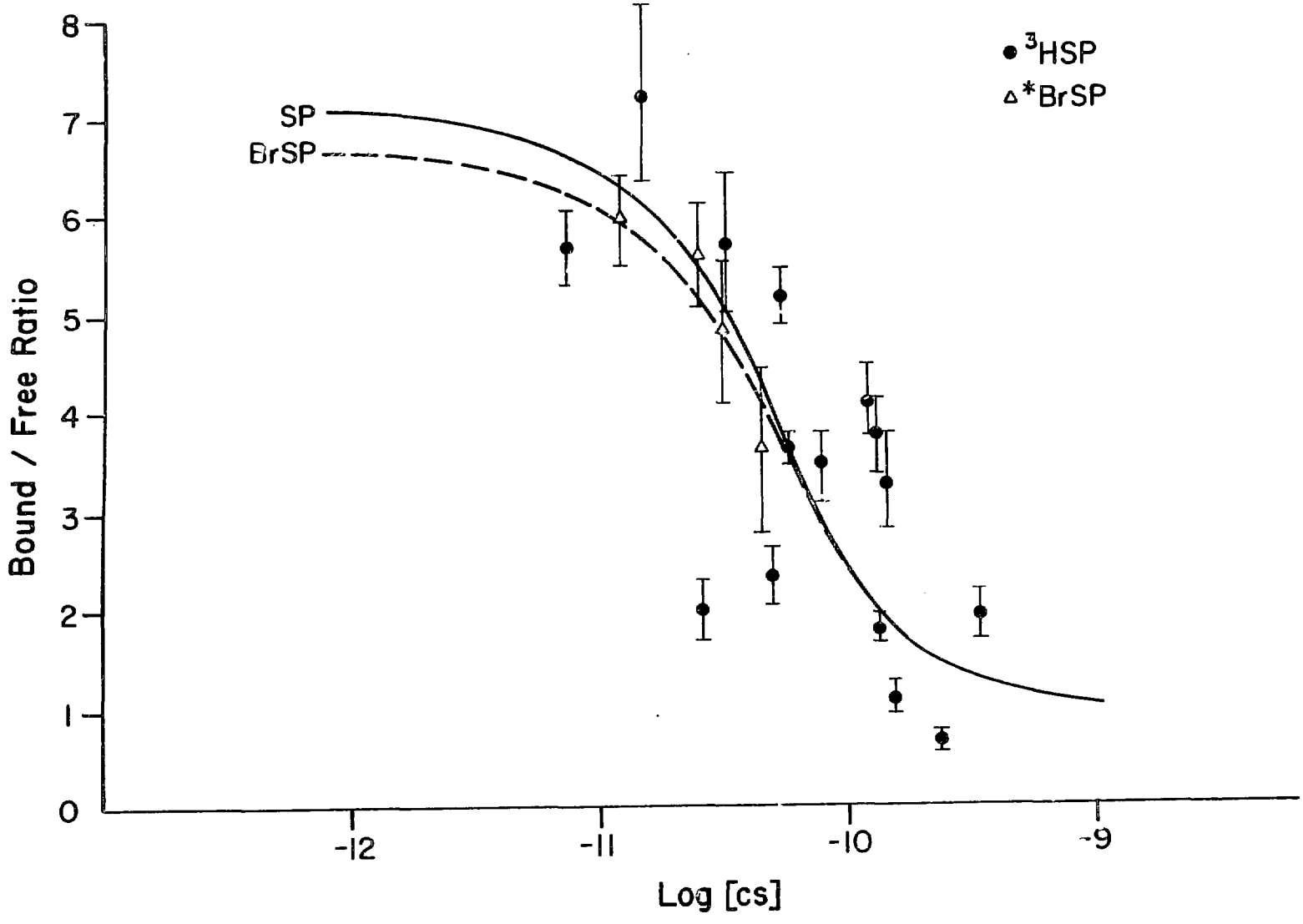
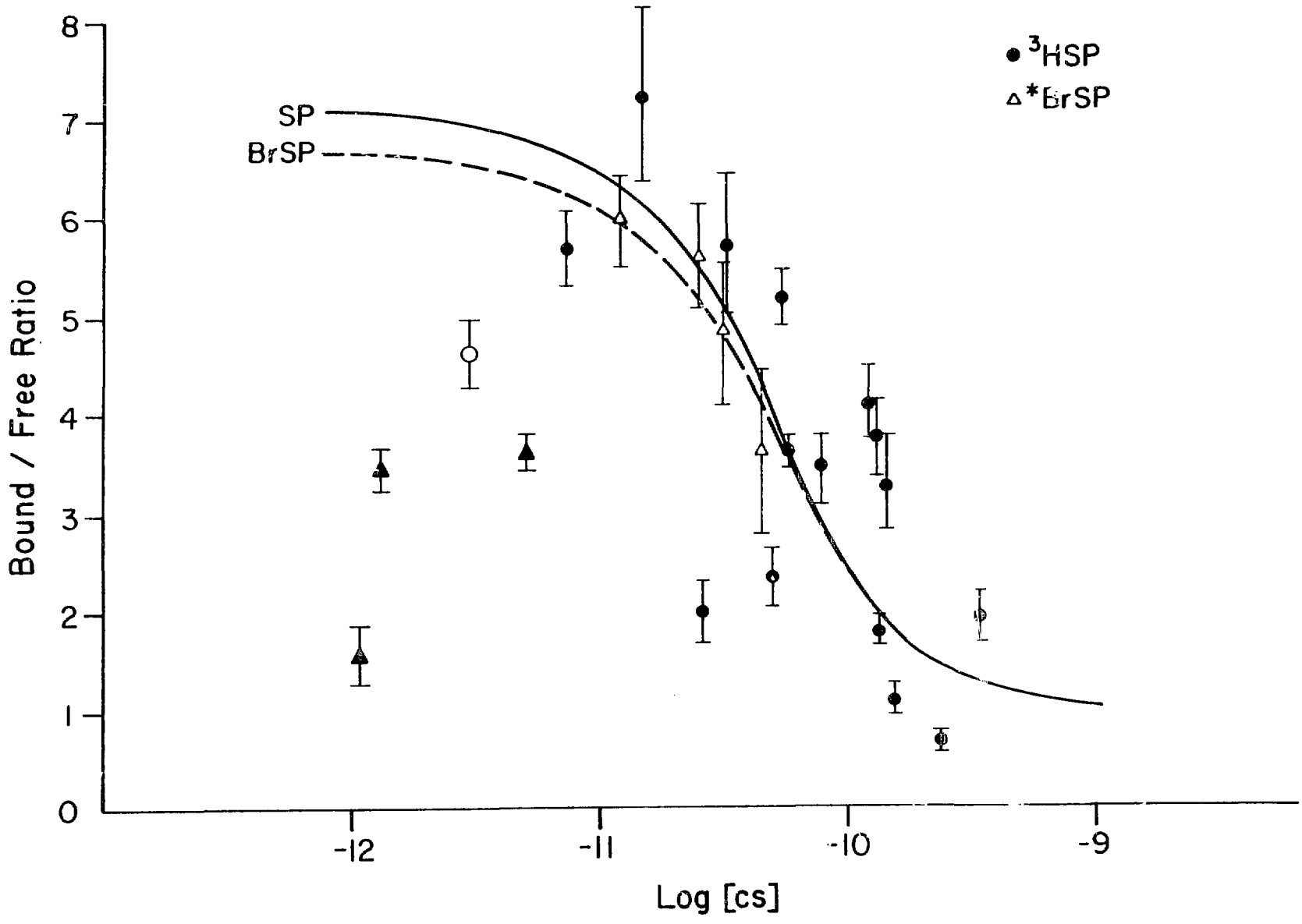
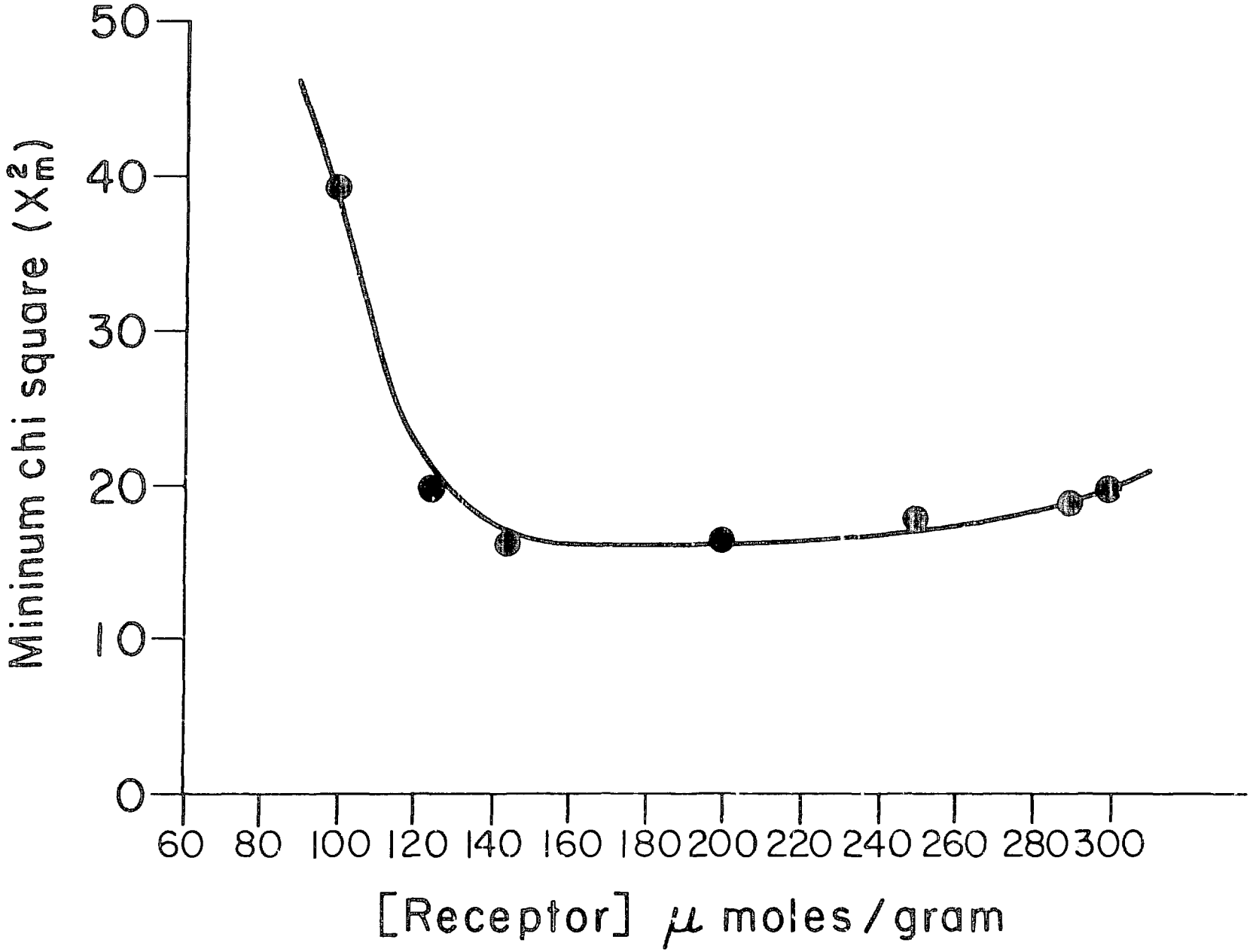
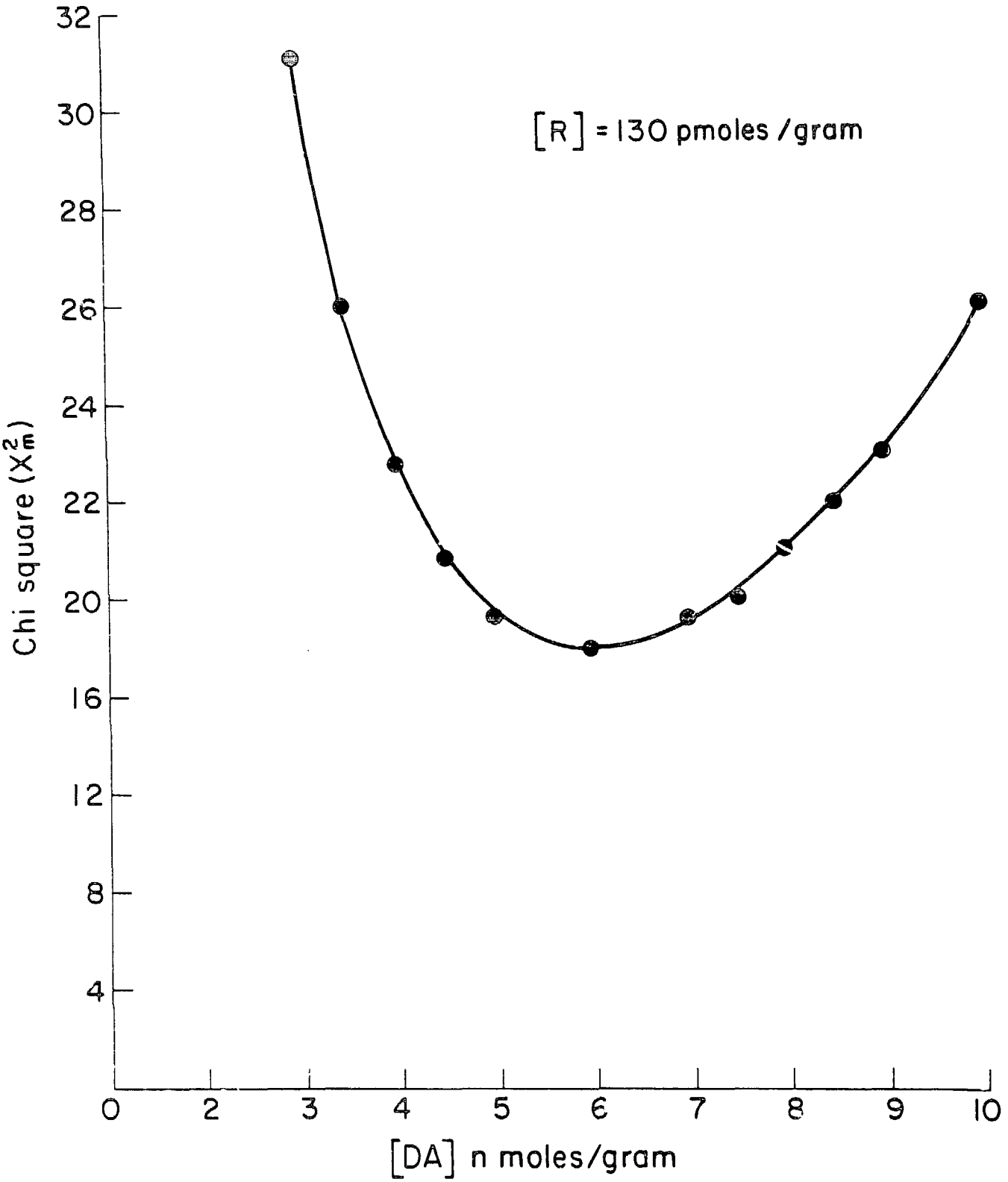

Frac. of Receptors Bound
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
[B] = 10^-10 - 10^-12
[B] = 4 X 10^-9
[B] = 10^-8
-12
-11
-10
-9
Log Conc [A]

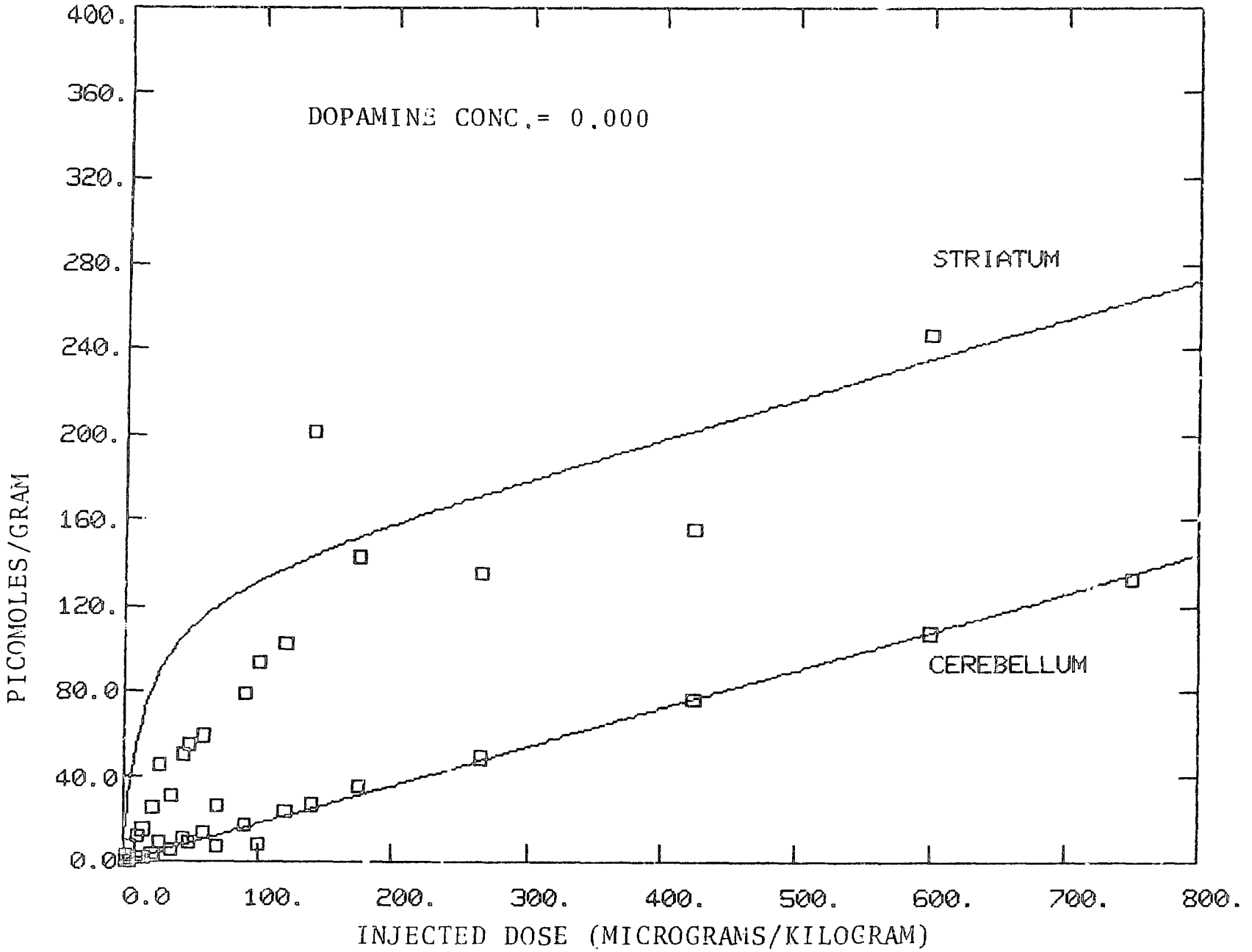
Fig. 3

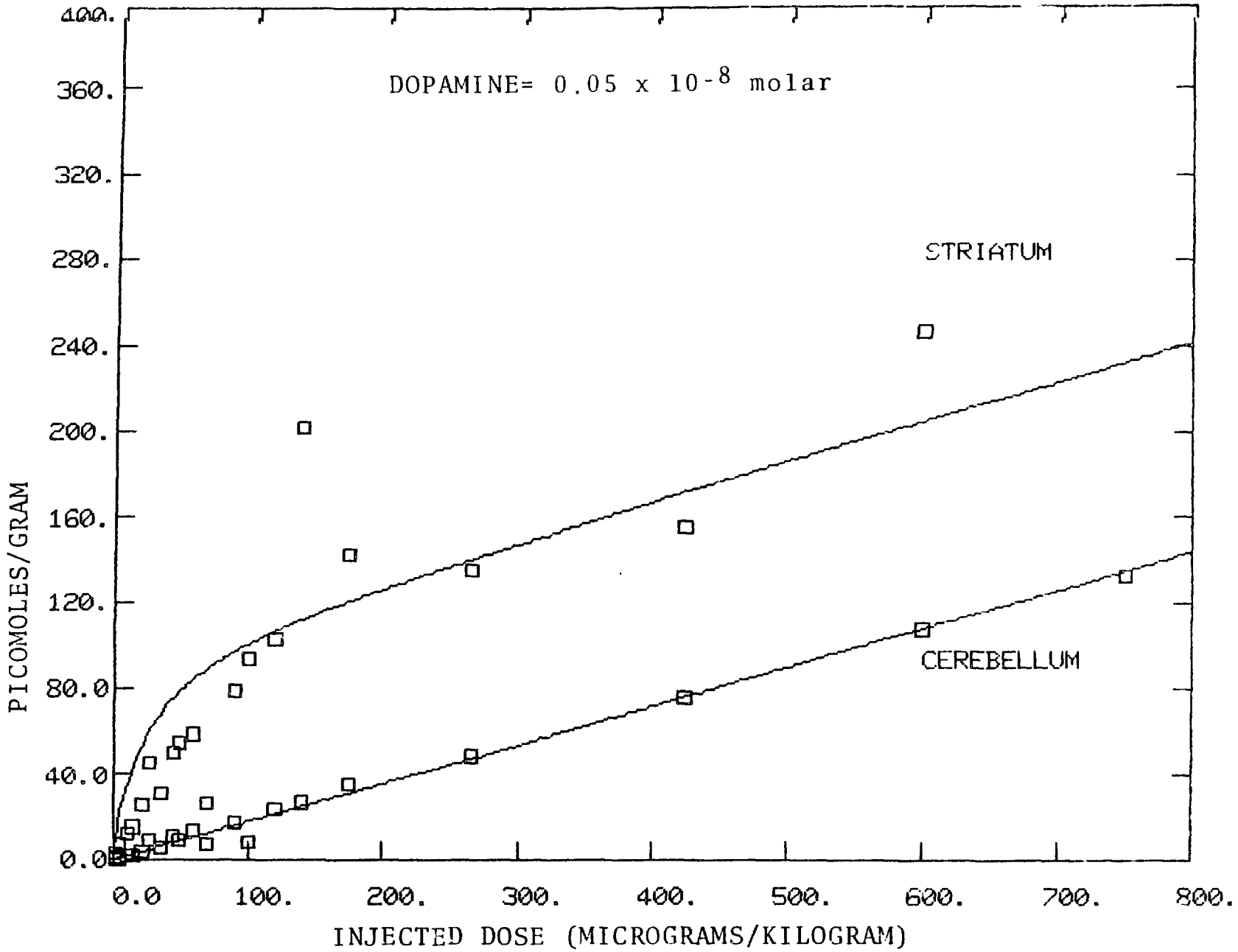


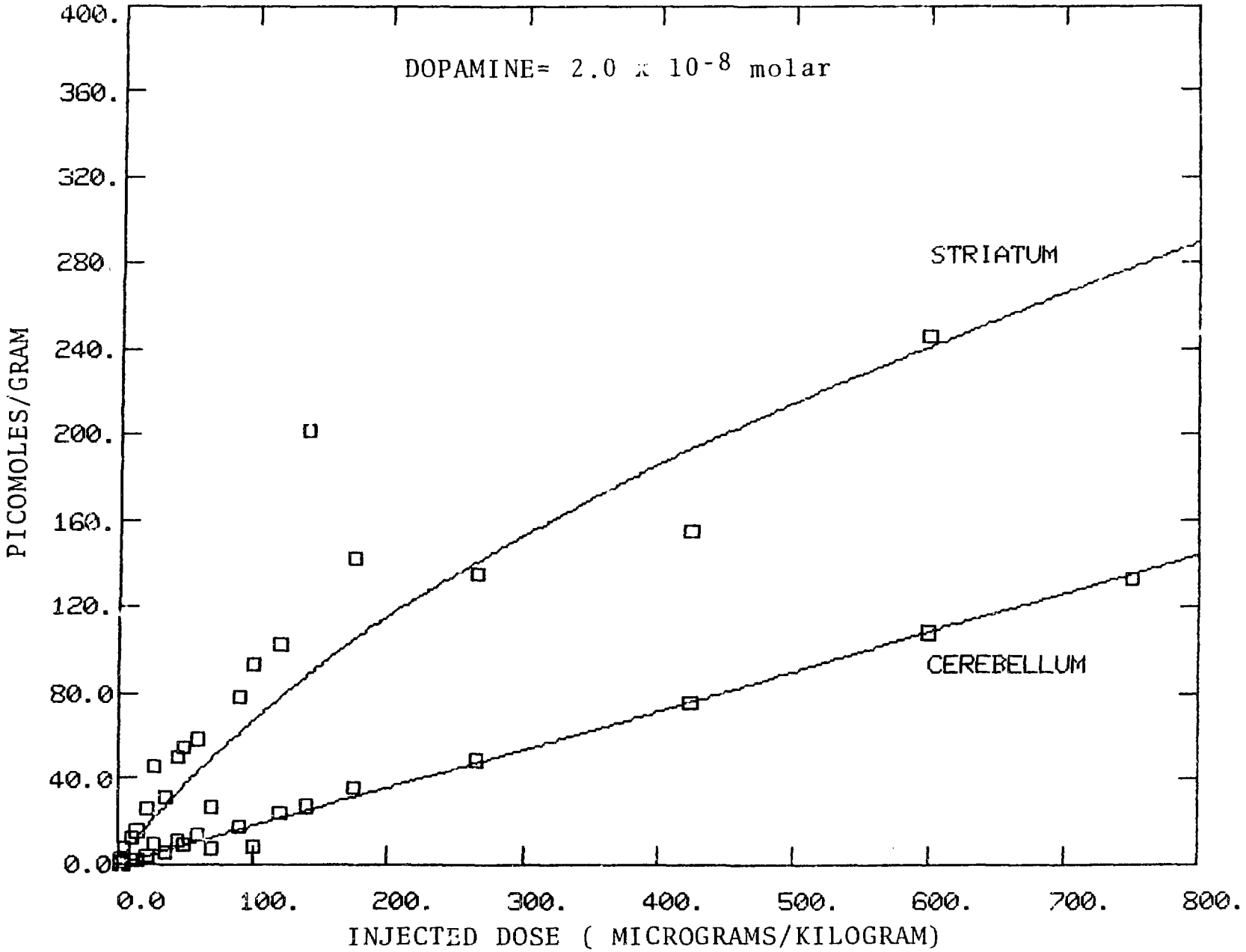

8
7
6
5
4
3
2
1
0
Bound / Free Ratio
SP
BrSP
● ^{3}HSP
△ *BrSP
-12
-11
-10
-9
Log [cs]

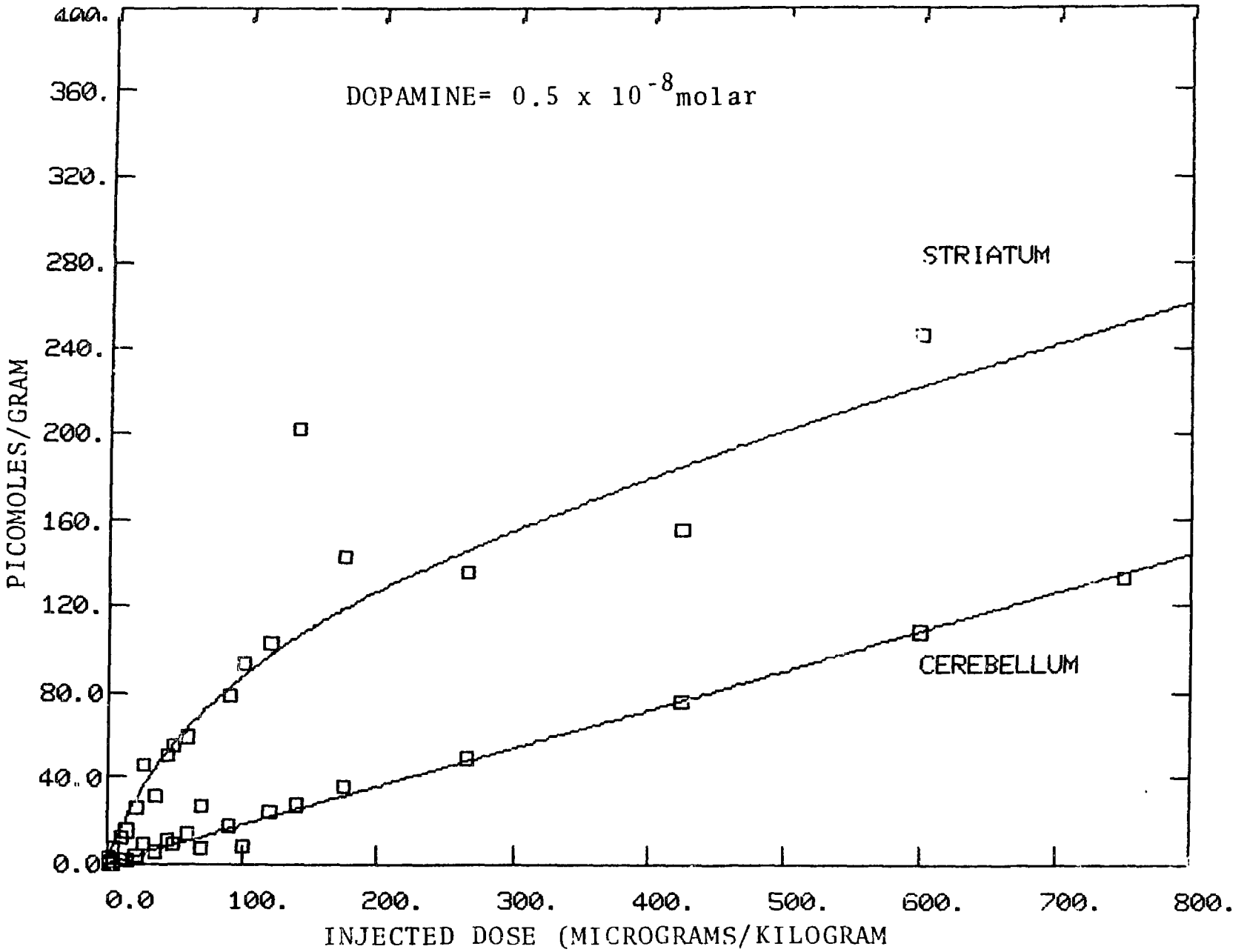

Minimum chi square (X^2_m)
50
40
30
20
10
0
60 80 100 120 140 160 180 200 220 240 260 280 300
[Receptor] μ moles / gram

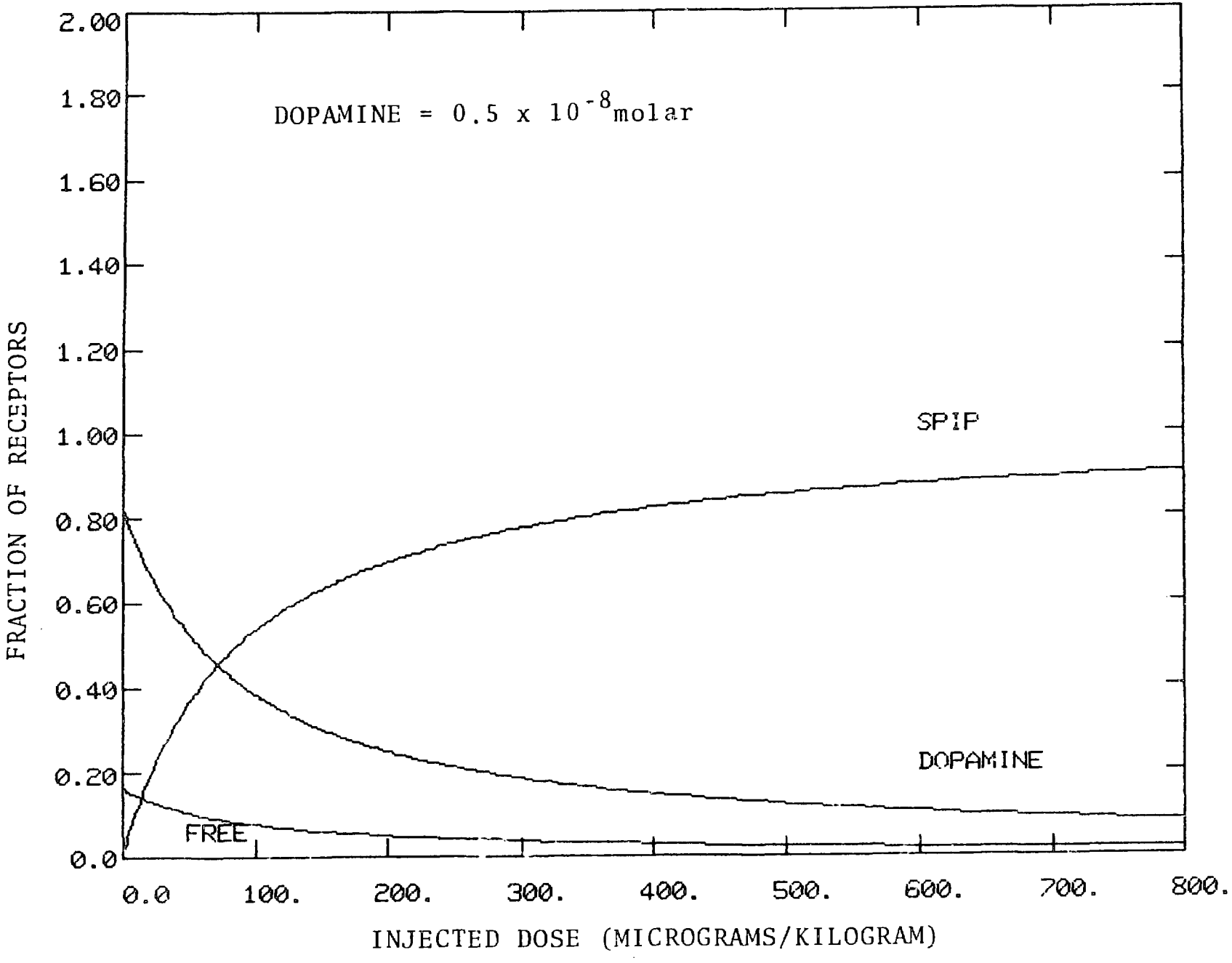

[R] = 130 pmoles /gram
Chi square (X^2_m)
32
30
28
26
24
22
20
16
12
8
4
0
2
3
4
5
6
7
8
9
10
[DA] n moles/gram


DOPAMINE CONC.= 0.000
STRIATUM
CEREBELLUM
PICOMOLES/GRAM
INJECTED DOSE (MICROGRAMS/KILOGRAM)
400.
360.
320.
280.
240.
200.
160.
120.
80.0
40.0
0.0
0.0
100.
200.
300.
400.
500.
600.
700.
800.


DOPAMINE= 0.05 x 10-8 molar
STRIATUM
CEREBELLUM
PICOMOLES/GRAM
INJECTED DOSE (MICROGRAMS/KILOGRAM)
400.
360.
320.
280.
240.
200.
160.
120.
80.0
40.0
0.0
0.0
100.
200.
300.
400.
500.
600.
700.
800.


DOPAMINE= 2.0 x 10-8 molar
STRIATUM
CEREBELLUM
PICOMOLES/GRAM
INJECTED DOSE (MICROGRAMS/KILOGRAM)
400.
360.
320.
280.
240.
200.
160.
120.
80.0
40.0
0.0
0.0
100.
200.
300.
400.
500.
600.
700.
800.


DOPAMINE= 0.5 x 10^{-8}molar
STRIATUM
CEREBELLUM
PICOMOLES/GRAM
INJECTED DOSE (MICROGRAMS/KILOGRAM
400.
360.
320.
280.
240.
200.
160.
120.
80.0
40.0
0.0
0.0
100.
200.
300.
400.
500.
600.
700.
800.


DOPAMINE = 0.5 x 10^-8 molar
SPIP
DOPAMINE
FREE
FRACTION OF RECEPTORS
INJECTED DOSE (MICROGRAMS/KILOGRAM)
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.0
0.0
100.
200.
300.
400.
500.
600.
700.
800.