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A Structure-Activity Study of the Transport Sites for the Hypothalamic and Striatal Catecholamine Uptake Systems

Similarities and Differences

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SUMMARY

A series of eight substrate molecules (substituted phenethylamines, guanethidine, and bretylium) had slightly less affinity for striatal than for hypothalamic synaptosomal uptake receptors as judged by ratios of striatal (s) to hypothalamic (h) IC_{50} values (s/h average = 3.9; range 2.1–6.0). Catecholamine uptake in striatum was very insensitive to tricyclic antidepressant inhibitors, whereas catecholamine uptake in hypothalamus was very sensitive to these agents (s/h average = 233; range 24–570). By way of contrast with both the substrates and the tricyclic inhibitors, the inhibitors with less rigidly fixed rings or analogous groups (deoxypipradrol, methylphenidate, cocaine) were potent in both brain preparations (s/h average = 1.2; range 0.6–2.3). It is concluded that the rings of nontricyclic inhibitors are able to bind to appropriate hydrophobic binding groups in both receptors, that these receptive groups have different topography in striatum and in hypothalamus, and that the topography in the striatum is incompatible with binding tricyclic systems. The data also indicate that there is great similarity, if not identity, in the receptive area for substrates in striatum and hypothalamus. Although the substrates and inhibitors bind to some groups in common in this substrate receptive area, it is the surrounding hydrophobic molecular environment that is clearly different and permits the phenomenon of selective blockade with drugs.

INTRODUCTION

Carlsson *et al.* (1) and Horn *et al.* (2) demonstrated with *in vivo* and *in vitro* experiments, respectively, that the catecholamine uptake mechanism (amine pump) present in nerve endings in hypothalamus and in cortex was very sensitive to inhibition by tricyclic antidepressants, whereas in striatum it was very insensitive. The uptake mechanism in peripheral adrenergic neurons, like that in hypothalamus and cortex, was very sensitive to tricyclic inhibitors (3, 4). Furthermore, in hypothalamic synaptosomes (2) and in peripheral adrenergic neurons (5–7) these compounds have been reported to be competitive inhibitors, but in striatal synaptosomes to be noncompetitive inhibitors (2). The data suggested that the amine pump present in central and peripheral noradrenergic neurons differed from the pump present in dopaminergic neurons.

It was considered of interest to determine whether an array of substrates for the amine pumps (Fig. 1) would also show marked differences in its affinities for the striatum and the hypothalamus, since substrate molecules are much less bulky than are the tricyclic inhibitors; i.e., they contain a lesser number of aromatic and/or saturated rings (compare Figs. 1 and 2). Such hydrophobic groups are known to be important for the binding of tricyclic inhibitors to uptake receptors in nonstriatal areas (5, 8, 9).

It was also considered of interest to determine whether deoxypipradrol, methylphenidate, and cocaine would exhibit sharp differences in affinities for the two uptake receptor sites. These compounds, like tricyclic agents, are potent, bulky inhibitors of uptake into cortex, hypothalamus, and peripheral adrenergic neurons (10, 11), but the rings they contain [and also the flat carbomethoxy groups in methylphenidate and cocaine (10)] are less rigidly fixed in relationship to one another than are the rings in tricyclic systems (Fig. 2). Therefore, these groups can assume positions when the inhibitors are on the uptake receptor which are not possible in tricyclic compounds and thereby permit additional possibilities for hydrophobic binding.

METHODS

Synaptosomal preparation. Male albino Sprague-Dawley rats weighing 200–250 g were used in these stud-

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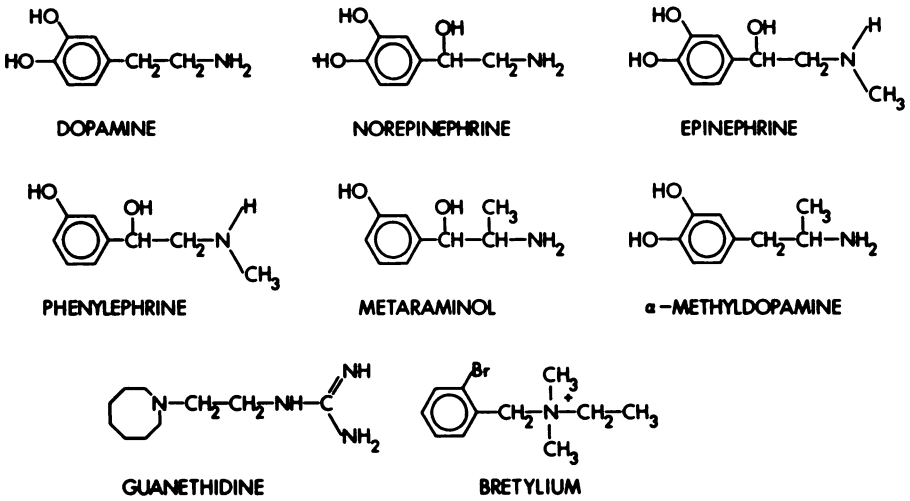

FIG. 1. *Structures of substrates for the amine pumps in peripheral and central adrenergic neurons*

ies. For each experiment two rats were killed by decapitation, their brains were rapidly removed, and the hypothalamus and the striatum were dissected from the rest of the brain on an ice-cold Lucite block. The pooled hypothalamus and striatum were immediately weighed and homogenized in 10 volumes of 0.32 M sucrose. The homogenization consisted of 10 up-and-down strokes with a pestal revolution of 840 rpm. The nuclei and cell debris were separated by centrifugation at 1000 × *g* for 10 min. The supernatant was gently separated from the pellet and thoroughly mixed to yield a uniform suspension of synaptosomes.

Uptake studies. The standard incubation medium consisted of Krebs bicarbonate; glucose, 11 mM; ascorbic acid, 1 μM; and iproniazid, 10 μM. Tritiated dopamine (0.02 ml of 3 × 10^{-6} M) (final concentration 3 × 10^{-8} M) plus 0.1 ml of the synaptosomal preparation were added to 2 ml of incubation medium. All incubations were conducted at 37° for 5 min in an atmosphere of 95% O_2-5% CO_2. The uptake at 0° was also studied for each experiment in order to determine the amount of tritiated dopamine accumulated by a temperature-insensitive uptake process. The amount of tritiated amine accumulated by a temperature-insensitive process (uptake at 0°) was subtracted from the total uptake at 37° to yield the uptake by the temperature-sensitive process only. All drugs were dissolved in 0.9% sodium chloride containing 1 μM ascorbic acid and preincubated with the synaptosomal preparation for 5 min at 37° in an atmosphere of 95% O_2-5% CO_2 before addition of the labeled substrate. The reaction was stopped by the addition of 2.0 ml of ice-cold 0.32 M sucrose, and the tubes were centrifuged at 49,600 × *g* for 10 min. The supernatant was discarded and the pellet was washed by resuspension in 5 ml of 0.9% sodium chloride. The suspension was again centrifuged at 49,600 × *g* for 10 min. The supernatant was discarded and the pellet was drained of all excess saline. The pellet was lysed by means of gentle resuspension

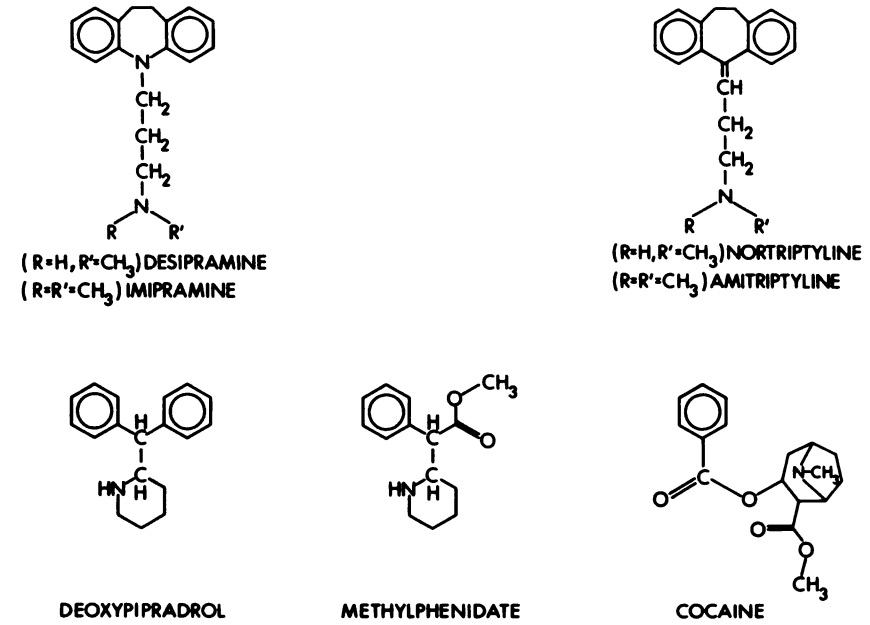

FIG. 2. *Structures of tricyclic and other inhibitors of the amine pumps in peripheral and central adrenergic neurons*

with a glass rod in 1 ml of absolute pure ethyl alcohol. The alcohol plus the synaptosomes were added to a scintillation vial. The tube was then rinsed with another 1 ml of ethyl alcohol, and this also was added to the scintillation vial. To this were added 10 ml of scintillation fluid (mixture of 1 gallon of toluene and 15 g of New England Nuclear Omnifluor) and counted. Through this procedure we were able to count the total activity in each pellet. The IC_{50} values were determined by incubating the synaptosomal preparations in the presence of 0.02 ml of tritiated dopamine and 0.02 ml of four different concentrations of the inhibitors producing 10–90% inhibition of dopamine uptake. The results were plotted on semilogarithmic paper and the line was determined by connecting the points on the graph.

The K_m values for norepinephrine and dopamine in the hypothalamus and striatum were determined by incubating 0.1 ml of synaptosomes with five different concentrations of tritiated dopamine or norepinephrine ranging from 10^{-8} to 10^{-7} M for 5 min. Exceptions are indicated in the legends. All other experimental conditions were the same as for uptake studies. The calculations of K_m values were performed with the aid of the Fortran programs written for enzyme kinetics as presented by Cleland (12).

[2-^{3}H]Dopamine (22.8 Ci/mmole) and L-[7-^{3}H]norepinephrine (4.50 Ci/mmole) were obtained from New England Nuclear Corporation (Boston, Mass.).

RESULTS

K_m values for [^{3}H]catecholamines in rat hypothalamic and striatal synaptosomal preparations. The uptake of (−)-[^{3}H]norepinephrine and [^{3}H]dopamine (from solutions ranging in concentrations from 0.01 to 0.1 μM) into hypothalamic and striatal synaptosomal preparations was determined. K_m values were calculated from double-reciprocal plots of concentration versus uptake and are presented in Table 1. An inspection of the data reveals that the K_m values for dopamine were not significantly different in striatal and hypothalamic synaptosomes (s/h[3] = 1.3), but that the K_m value for norepinephrine was significantly higher in the striatum than in the hypothalamus (s/h = 8.7). Thus, dopamine rather than norepinephrine was selected as the radiolabeled substrate against which to estimate the IC_{50} values for the other substrates and for the inhibitors.

Effects of substrates on the uptake of [^{3}H]dopamine. Figure 3 shows the results obtained with norepinephrine as an inhibitor of [^{3}H]dopamine uptake. The plots are typical of those obtained in the current study. Table 2 presents the IC_{50} values for eight substrates. The IC_{50} values in the striatal preparations generally were slightly greater than those in the hypothalamic preparations (s/h < 7). (+)-α-Methyldopamine was the most potent and bretylium the least potent inhibitor in both preparations.

Effects of tricyclic inhibitors on the uptake of [^{3}H]dopamine. Table 3 gives the IC_{50} values for the tertiary dimethylamine tricyclic antidepressants, imipramine and amitriptyline, as well as those for the secondary monomethyl amine inhibitors, desipramine and nortriptyline.

[3] The abbreviation used is: s/h, ratio of striatal to hypothalamic IC_{50} values.

TABLE 1

K_m values for dopamine and norepinephrine in hypothalamic and striatal synaptosomal preparations

[^{3}H] Catecholamine	Hypothalamic synaptosomes	Striatal synaptosomes	s/h	*p*
	M	*M*		
Dopamine	$1.3 \pm 0.2 \times 10^{-7}$	$1.7 \pm 0.1 \times 10^{-7}$	1.3	>0.1
Norepinephrine	$1.5 \pm 0.1 \times 10^{-7}$	$13.0 \pm 3.5 \times 10^{-7}$	8.7	<0.05

The secondary monomethyl amine inhibitors were the most potent compounds. The striatal preparation was very insensitive to all of the tricyclic agents (s/h range 24–570).

Figure 4 presents double-reciprocal plots obtained with desipramine and nortriptyline as inhibitors of the uptake of [^{3}H]dopamine into hypothalamic and striatal synaptosomes. Both compounds were competitive inhibitors in the hypothalamic preparation but were noncompetitive inhibitors in the striatal preparation.

Effect of the inhibitors deoxypipradrol, methylphenidate, and cocaine on uptake of [^{3}H]dopamine. Table 4 presents the IC_{50} values for deoxypipradrol isomers, methylphenidate isomers, and cocaine. All compounds were essentially equipotent in both preparations. (−)-Deoxypipradrol was the most potent compound in both preparations. Figure 5 demonstrates that the inhibition by (−)-deoxypipradrol of [^{3}H]dopamine uptake into the striatal synaptosomal preparation was competitive.

DISCUSSION

Substrates for the amine pump have in common a single aromatic or saturated ring separated by a short chain from a single basic group (amine, guanidine, or ammonium). At physiological pH the amino- and guanidine-containing molecules are predominantly in the positively charged form; the quaternary ammonium compounds are totally positively charged. Both the ring and

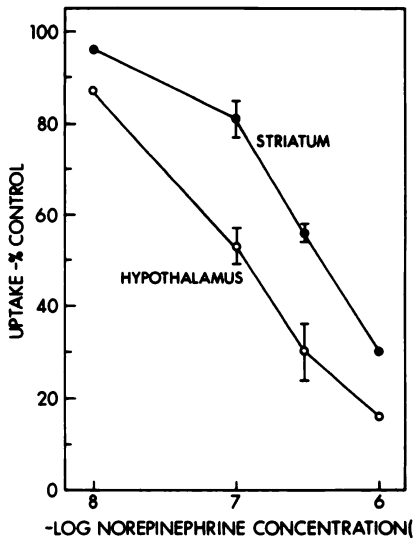

FIG. 3. *Effect of (−)-norepinephrine on the 5-min, temperature-dependent uptake of [^{3}H]dopamine into synaptosomal preparations of rat striatum and hypothalamus*

TABLE 2

Effect of small-molecule substrates on the capacity of rat synaptosomal preparations to take up [^{3}H]dopamine

Substrate	IC_{50}[a] ± SE		s/h	p
	Hypothalamic synaptosomes	Striatal synaptosomes		
	M	M		
(−)-Norepinephrine	$1.3 \pm 0.3 \times 10^{-7}$	$4.0 \pm 0.3 \times 10^{-7}$	3.1	<0.01
(−)-Epinephrine	$7.0 \pm 2.0 \times 10^{-7}$	$2.6 \pm 0.3 \times 10^{-6}$	3.7	<0.01
(−)-Phenylephrine	$3.8 \pm 0.9 \times 10^{-6}$	$2.3 \pm 0.1 \times 10^{-5}$	6.0	<0.001
(±)-Metaraminol	$2.2 \pm 0.6 \times 10^{-7}$	$6.3 \pm 2.4 \times 10^{-7}$	2.9	<0.2, >0.1
(+)-α-Methyldopamine	$2.7 \pm 0.6 \times 10^{-8}$	$1.2 \pm 0.3 \times 10^{-7}$	4.4	<0.01
(−)-α-Methyldopamine	$5.3 \pm 1.8 \times 10^{-8}$	$2.8 \pm 0.2 \times 10^{-7}$	5.3	<0.001
Guanethidine	$3.9 \pm 1.1 \times 10^{-6}$	$1.4 \pm 0.2 \times 10^{-5}$	3.6	<0.01
Bretylium	$5.3 \pm 2.3 \times 10^{-5}$	$1.1 \pm 0.4 \times 10^{-4}$	2.1	<0.3, >0.2

[a] n = four or more in all experiments.

the charged basic group of substrates (and of inhibitors as well) are important in binding to receptors (5, 7–11). The other substituents, e.g., hydroxyl and methyl groups, bromine, also strongly influence binding (either increasing or decreasing it) and account for the wide spectrum of potency that the substrates exhibit in hypothalamus (1962-fold) and striatum (917-fold). Horn (13) has studied the influence of such substituents on the potency of catecholamines as uptake inhibitors and found that generally the norepinephrine uptake process was more sensitive to changes than the dopamine uptake system.

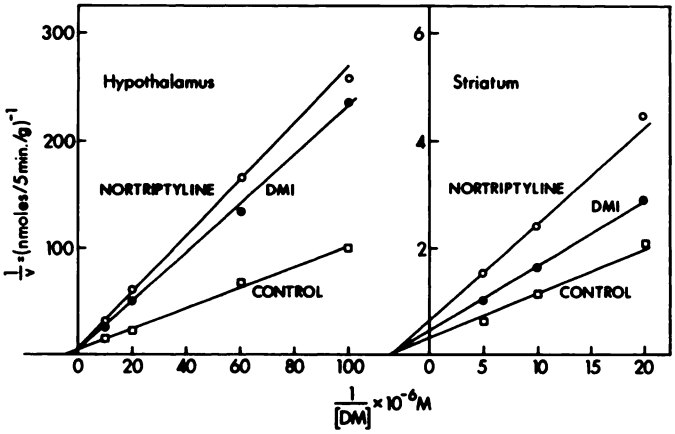

FIG. 4. *Double-reciprocal plots of the 5-min, temperature-dependent uptake of [^{3}H]dopamine into hypothalamic and striatal synaptosomal preparations in the absence and presence of tricyclic inhibitors*

Inhibitor concentrations were 10^{-6} M in hypothalamus and 10^{-5} M in striatum. The data obtained at 10^{-8} M dopamine have been omitted from the kinetic plots in striatum for ease of representation. Extrapolation of the lines will intercept with 10^{-8} M data points. All points have been used in the computation of the slopes of the lines. The slopes of all lines used in the kinetic studies were determined by least-squares fit using a computer. Values are the means of three separate experiments where each point was determined in duplicate. *DMI*, Desipramine.

The substrates generally had a slightly greater affinity for the hypothalamic receptors than for the striatal receptors as judged by IC_{50} values (s/h average = 3.9). Snyder and Coyle (14) found a 5-fold greater K_m value for both dopamine and norepinephrine uptake into striatal synaptosomes as compared with the K_m for their uptake into hypothalamic synaptosomes. We found no difference in K_m values for dopamine in the two areas but found an 8-fold greater K_m for norepinephrine in the striatum as compared with the hypothalamus. These relatively small differences in affinity suggested that the molecular characters of the receptive sites for substrates were similar in both areas. To go one step further, the fact that the range of differences in IC_{50} values for substrates was fairly narrow (2.1–6.0), despite the varied chemical structures and wide range of potencies among the substrates, argued that we might be dealing with chemically identical receptive sites but that the concentrations of the substrates at the uptake site in the striatal synaptosomal preparation were subject to some negative bias, e.g., a diffusion barrier, or a partial sequestering by nonspecific binding, or conceivably even a minor capacity to release dopamine differentially from the two synaptosomal preparations.

The differences in the IC_{50} values for tricyclic inhibitors in striatal and hypothalamic synaptosomes cannot be explained even in part by differential release of catecholamines, since these inhibitors do not cause release from either synaptosomal preparation (8). The very large discrepancies in the IC_{50} values (s/h average = 233; range

TABLE 3

Effect of tricyclic inhibitors on the capacity of rat synaptosomal preparations to take up [^{3}H]dopamine

Substrate	IC_{50}[a] ± SE		s/h	p
	Hypothalamic synaptosomes	Striatal synaptosomes		
	M	M		
Imipramine	$1.1 \pm 0.7 \times 10^{-7}$ (3)	$1.9 \pm 0.1 \times 10^{-5}$ (3)	172	<0.01
Amitriptyline	$2.7 \pm 0.9 \times 10^{-7}$ (4)	$6.5 \pm 1.0 \times 10^{-6}$ (5)	24	<0.01
Desipramine	$2.8 \pm 1.5 \times 10^{-8}$ (4)	$1.6 \pm 0.5 \times 10^{-5}$ (7)	570	<0.01
Nortriptyline	$4.4 \pm 1.0 \times 10^{-8}$ (4)	$7.4 \pm 0.7 \times 10^{-6}$ (5)	168	<0.01

[a] Numbers in parentheses are numbers of experiments.

TABLE 4

Effects of cocaine, methylphenidate, and deoxypipradrol on the capacity of rat synaptosomal preparations to take up [^{3}H]dopamine

Inhibitor	IC_{50}[a] ± SE		s/h	p
	Hypothalamic synaptosomes	Striatal synaptosomes		
	M	M		
Cocaine	$2.4 \pm 0.8 \times 10^{-7}$ (4)	$3.3 \pm 0.5 \times 10^{-7}$ (4)	1.4	>0.4
(±)-*threo*-Methylphenidate[b]	$3.3 \pm 1.0 \times 10^{-7}$ (8)	$2.1 \pm 0.6 \times 10^{-7}$ (8)	0.6	>0.3
(+)-*threo*-Methylphenidate	$1.5 \pm 0.5 \times 10^{-7}$ (3)	$3.3 \pm 0.9 \times 10^{-7}$ (3)	2.2	<0.2, >0.1
(−)-Deoxypipradrol	$5.5 \pm 1.1 \times 10^{-8}$ (3)	$4.0 \pm 1.2 \times 10^{-8}$ (3)	0.7	>0.4
(+)-Deoxypipradrol	$1.2 \pm 0.6 \times 10^{-6}$ (3)	$1.1 \pm 0.1 \times 10^{-6}$ (3)	0.9	>0.5

[a] Numbers in parentheses are numbers of experiments.
[b] Commercial Ritalin (Ciba-Geigy Company).

24–570) indicated that the hydrophobic groups of the receptor that were involved in binding the tricyclic ring system, or the location of these groups, were different in the two areas. The inhibition of uptake into hypothalamus by tricyclic inhibitors was both potent and competitive, indicating the occurrence of significant binding via the ring system. On the other hand, the inhibition of uptake by tricyclic inhibitors in striatal synaptosomes was weak and noncompetitive, indicating that no specific binding to a receptor was occurring. These data confirm the findings of Horn *et al.* (2).

By way of contrast with the tricyclic inhibitors, the inhibitors with less rigidly fixed rings (deoxypipradrol, methylphenidate, cocaine) and carbomethoxy groups (methylphenidate, cocaine) were essentially equipotent in both brain preparations (s/h average = 1.2; range 0.6–2.2; differences not significant). These compounds have only modest or no capacity to release catecholamines from either preparation (11). All three agents have been shown to be competitive inhibitors of catecholamine uptake into adrenergic neurons (10), and one, (−)-deoxypipradrol, was shown in the present communication to be a competitive inhibitor in the striatal synaptosomal preparation. It appears that the rings or the analogous carbomethoxy groups of deoxypipradrol and related inhibitors, because of their relative flexibility, are able to bind to appropriate hydrophobic groups in both receptors, that these hydrophobic groups have different topography in striatal and hypothalamic receptors, and that their topography in the striatum is incompatible with the binding of tricyclic ring systems.

In broadest terms, the data suggest that there is great similarity, if not identity, in the receptive area for the substrates (small molecules), the natural ones being dopamine and (−)-norepinephrine in striatum and hypothalamus, respectively. Although both the substrates and the inhibitors have common binding groups in this area, it is the hydrophobic molecular environment surrounding this receptive area for the substrates that is clearly different and permits the phenomenon of selective blockade with bulky inhibitor drugs.

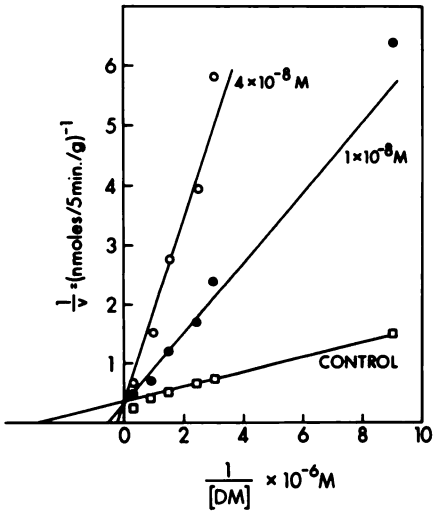

FIG. 5. *Double-reciprocal plots of the 5-min, temperature-dependent uptake of [^{3}H]-dopamine into the striatal synaptosomal preparation in the absence and presence of several concentrations of (−)-deoxypipradol*

The concentration of substrate ranged from 0.1 μM. The K_m for [^{3}H] dopamine uptake was $3.3 \pm 1.5 \times 10^{-7}$ M. Values are the means of three separate experiments where each point was determined in duplicate. The slopes of all lines used in the kinetic studies were determined by least-squares fit using a computer.

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