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[^{3}H]Mazindol Binding Associated with Neuronal Dopamine and Norepinephrine Uptake Sites

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SUMMARY

[^{3}H]Mazindol labels neuronal dopamine uptake sites in corpus striatum membranes (K_D = 18 nM) and neuronal norepinephrine uptake sites in cerebral cortex and submaxillary/sublingual gland membranes (K_D = 4 nM). The potencies of various inhibitors of biogenic amine uptake in reducing [^{3}H]mazindol binding in striatal membranes correlate with their potencies for inhibition of neuronal [^{3}H]dopamine accumulation, whereas their potencies in reducing [^{3}H]mazindol binding to cortical and salivary gland membranes correlate with their potencies for inhibition of neuronal [^{3}H]norepinephrine accumulation. Similar to the dopamine and norepinephrine uptake systems, [^{3}H]mazindol binding in all three tissues is dependent upon sodium (with potassium, lithium, rubidium, and Tris being ineffective substitutes) and chloride (with sulfate and phosphate being ineffective substitutes). In membranes of the cerebral cortex and salivary gland, half-maximal stimulation is observed at 50–80 mM NaCl, whereas in membranes of the corpus striatum half-maximal stimulation occurs at 240 mM NaCl. In striatal membranes NaCl increases the affinity of [^{3}H]mazindol binding with no effect on the maximal number of sites. The enhancement of affinity is due to a selective slowing of the dissociation of the ligand from its binding site. The association of [^{3}H]mazindol binding sites with neuronal dopamine uptake sites in the corpus striatum is further supported by the reduction of [^{3}H]mazindol binding sites in striatal membranes following destruction of dopaminergic neurons by 6-hydroxydopamine. Similarly, the association of [^{3}H]mazindol binding sites with neuronal norepinephrine uptake sites in cerebral cortex is supported by the reduction of [^{3}H]mazindol binding to cortical membranes following destruction of noradrenergic neurons by *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine.

INTRODUCTION

Synaptic inactivation of the catecholamines, norepinephrine and dopamine, is primarily achieved by high-affinity uptake into nerve terminals. A variety of evidence indicates substantial differences in the recognition site of the dopamine and norepinephrine neuronal uptake processes (1–3). Drugs such as benztropine have somewhat greater affinity for the dopamine uptake site (4), whereas the tricyclic antidepressants, such as desipramine, are much more potent in inhibiting norepinephrine uptake (5, 6). Most studies of catecholamine uptake have monitored the accumulation of radiolabeled catecholamines either into peripheral tissue slices or into nerve terminals in brain slices or synaptosomal preparations. Recently, [^{3}H]desipramine has been used to label binding sites associated with the neuronal norepinephrine uptake system in both brain and peripheral tissues (7–13). Furthermore, sodium-sensitive cocaine binding to striatal membranes may in part label dopamine uptake sites (14), although the relatively low affinity of cocaine for the binding site makes a detailed evaluation difficult.

Mazindol is a clinically utilized appetite suppressant which potently inhibits both norepinephrine and dopamine uptake (15–18). Recently we reported that [^{3}H]mazindol binds with high affinity to sites on rat corpus striatum membranes which are associated with neuronal dopamine uptake sites (19). In the present study we have characterized properties of [^{3}H]mazindol binding related to dopamine uptake sites in the corpus striatum and also report binding of [^{3}H]mazindol to sites associated with neuronal norepinephrine uptake in the cerebral cortex and salivary gland.

EXPERIMENTAL PROCEDURES

Materials. Unlabeled mazindol was supplied by Sandoz Pharmaceuticals (East Hanover, N. J.); nomifensine by Hoechst-Roussel (Somer-

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ville, N. J.); bupropion by Burroughs-Wellcome (Research Triangle Park, N. C.); Exp-561[2] by DuPont (Wilmington, Del.); DITA by R. Heikkila (Rutgers Medical School, Piscataway, N. J.); and DSP-4 by Astra Lakemedel A.B. (Sodertaalje, Sweden). Sources of other drugs used in the present study have been described previously (7, 8). [^{3}H] Mazindol (11 Ci/mmole) was obtained from Dr. T. C. Kung, of New England Nuclear Corporation (Boston, Mass.). (±)-[^{3}H]Norepinephrine (11.8 Ci/mmole) and [^{3}H]dopamine (27.4 Ci/mmole) were also obtained from New England Nuclear Corporation.

[^{3}H]Mazindol binding. The binding of [^{3}H]mazindol was assayed under conditions similar to those utilized for the labeling of norepinephrine uptake sites with [^{3}H]desipramine (8). Corpus striatum, cerebral cortex, and submaxillary and sublingual salivary glands from male Sprague-Dawley rats (150–250 g) were homogenized in 30 volumes of ice-cold assay buffer (50 mM Tris-HCl/120 mM NaCl/5 mM KCl, pH 7.9 at 4°) with a Brinkmann PT-10 Polytron at a setting of 5 for 10 sec and centrifuged at 50,000 × *g* for 10 min. The pellet was resuspended in 30 volumes of the assay buffer by a Polytron at setting 5 for 5 sec and centrifuged again. This washing procedure was repeated twice, and the final pellet was either suspended in 30 volumes (cerebral cortex and salivary gland) or 80 volumes (corpus striatum) of assay buffer.

To facilitate filtration of salivary gland homogenates, the initial homogenate was incubated with 300 mM KCl on ice for 10 min with intermittent mixing prior to the first centrifugation (20). The homogenate was then filtered through loosely woven gauze and the filtrate prepared as described above.

Unless otherwise mentioned, 200 μl of the membrane preparation was incubated in triplicate at 0° for 1 hr with 4 nM [^{3}H]mazindol in the presence or absence of various tested drugs and ions in a final volume of 250 μl. The incubation was terminated by the addition of 5 ml of ice-cold assay buffer to each tube and immediate filtration under reduced pressure through Whatman GF/F glass-fiber filters. Filters were washed twice with 5 ml of ice-cold buffer, and radioactivity was measured by liquid scintillation spectrometry. Nonspecific binding in both corpus striatum and salivary gland was defined as binding in the presence of 1 μM unlabeled mazindol. Nonspecific binding in cerebral cortex was defined as binding in the presence of 0.3 μM desipramine (see below). Specific binding was calculated by subtracting the respective nonspecific binding from total binding and usually expressed as picomoles per gram of tissue. Protein concentrations, where indicated, were determined according to the method of Lowry *et al.* (21).

To study the effect of various cations on [^{3}H]mazindol binding, tissue was prepared in ice-cold 50 mM Tris-HCl (pH 7.9 at 4°). Specific binding was measured in the presence of various concentrations of sodium, potassium, lithium, rubidium or Tris choloride. The effects of chloride on [^{3}H]mazindol binding were examined in tissue prepared in ice-cold 100 mM sodium phosphate buffer (pH 7.4). Chloride concentration was varied while maintaining a final sodium concentration of 300 mM by adding an appropriate mixture of sodium chloride and sodium sulfate.

Catecholamine uptake. The uptake of [^{3}H]dopamine [(±)-[^{3}H]norepinephrine] by striatal (cortical) synaptosomes was studied by a modification of the procedure of Koide and Uyemura (22). Corpus striatum (cerebral cortex) from male Sprague-Dawley rats was homogenized in 50 (10) volumes of ice-cold 0.3 M sucrose in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 1,000 × *g* for 10 min. The pellet was discarded and the supernatant was centrifuged at 17,000 × *g* for 20 min. The pellet was resuspended in 50 (10) volumes of 0.3 M sucrose in a glass homogenizer. Fifty microliters of the suspension were added to 0.4 ml of uptake buffer [40 mM sodium phosphate buffer (pH 7.4), 100 mM NaCl, 4 mM KCl, 11 mM glucose, and 0.2% ascorbic acid], and the mixtures were preincubated at 37° or 0° for 5 min. The uptake of [^{3}H]dopamine ([^{3}H]norepinephrine), in triplicate samples, was initiated by the addition of 50 μl of [^{3}H] dopamine ([^{3}H]norepinephrine), to give a final concentration of 1.5×10^{-8} (5×10^{-8}) M. After 3 (5) min, the incubation was terminated by filtration of the mixture, under reduced pressure, through Whatman GF/B glass-fiber filters. Each of the filters was washed twice with 4 ml of 0.3 M sucrose. Radioactivity was measured by liquid scintillation spectrometry. To correct for passive diffusion and adsorption to membranes and filters, control samples were incubated at 0°. Active uptake was calculated by subtracting the 0° control value from the 37° value and was expressed as femtomoles per milligram of tissue per 3 (5) min.

6-Hydroxydopamine and DSP-4 lesions. To destroy dopaminergic neurons, male rats (200 g) were anesthetized with pentobarbital, and 6-hydroxydopamine (200 μg in 15 μl; dissolved in 0.9% NaCl containing 1% ascorbic acid) was injected into the right lateral ventricle under stereotaxic control. Thirty minutes prior to the injection of 6-hydroxydopamine, rats received injections of desipramine (25 mg/kg, i.p.) to prevent destruction of noradrenergic neurons (23) and pargyline (50 mg/kg, i.p.) to increase destruction of dopaminergic neurons (23). Control animals received the same volume of vehicle. Animals were killed 10 days after treatment, and dopamine uptake into striatal synaptosomes was measured as described above. A portion of the homogenate was diluted with binding assay buffer and prepared for [^{3}H]mazindol binding as described above.

Noradrenergic neurons were destroyed by the injection of 0.5 ml of freshly prepared DSP-4 (50 mg/kg, i.p., in 0.9% NaCl) (24). Control animals received the same volume of vehicle. After 2 days, the rats were given a second injection of DSP-4 (25 mg/kg). Four days after the first injection, the rats were killed, and the cerebral cortex was prepared for norepinephrine uptake and [^{3}H]mazindol binding as described above.

RESULTS

General properties of [^{3}H]mazindol binding. In typical experiments utilizing 4 nM [^{3}H]mazindol, total binding to striatal membranes is about 2500 cpm with nonspecific binding measured in the presence of 1 μM unlabeled mazindol of about 500 cpm. In rat salivary gland membranes, total [^{3}H]mazindol binding is typically 1000 cpm with nonspecific binding measured in the presence of 1 μM unlabeled mazindol or 0.3 μM desipramine of about 500 cpm. In rat cerebral cortex membranes, total [^{3}H] mazindol binding is about 2500 cpm. This is reduced to about 1600 cpm in the presence of 1 μM mazindol. To examine selectively the component of [^{3}H]mazindol binding associated with norepinephrine uptake (see below), nonspecific binding was measured utilizing 0.3 μM desipramine, and in typical experiments is about 2000 cpm.

The amount of specific [^{3}H]mazindol binding varies with temperature. Maximal binding occurs at 0°, the temperature utilized for routine experiments. At 23° and 37°, specific binding is less than half that obtained at 0°. Specific [^{3}H]mazindol binding to striatal membranes is time-dependent, with half-maximal binding apparent at about 4 min at 0°. At 23° and 37°, binding occurs more rapidly, but fails to reach the levels seen at 0°.

[^{3}H]Mazindol binding to membranes of rat corpus striatum is saturable (Fig. 1). Specific binding begins to plateau at 60 nM [^{3}H]mazindol and half-maximal binding is apparent at about 18 nM [^{3}H]mazindol. Scatchard analysis indicates a single component of binding with an equilibrium dissociation constant (K_D) of 18.2 nM and

[2] The abbreviations used are: Exp-561, 1-amino-4-phenylbicyclo[2,2,2]octane; DITA, 3′4′-dichloro-2(2-imidazolin-2-yl-thio)acetophenone hydrobromide; DSP-4, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine.

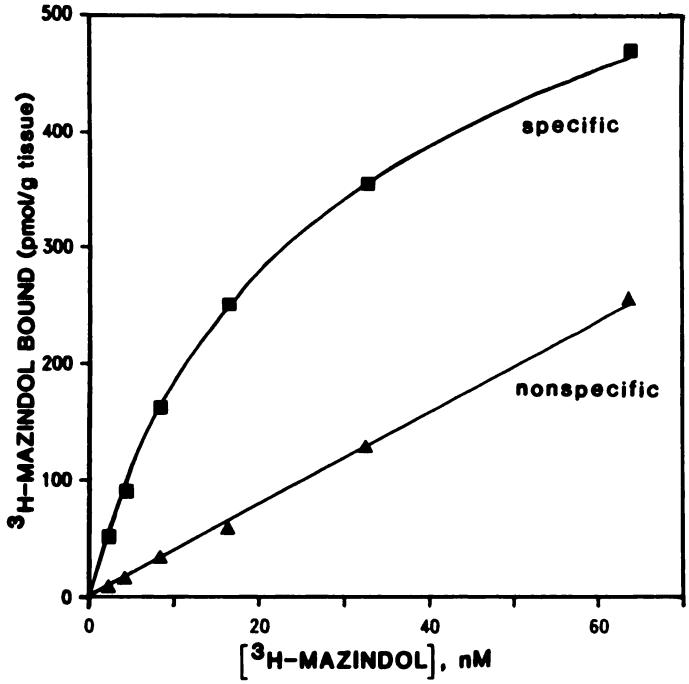

FIG. 1. *Saturation of specific [^{3}H]mazindol binding to striatal membranes with increasing concentrations of [^{3}H]mazindol*

Specific [^{3}H]mazindol binding was calculated by subtracting from total binding the nonspecific binding in the presence of 1 μM mazindol. Each point represents the mean of three separate experiments performed in triplicate. The standard error of the mean is less than 10% for each value.

maximal number of binding sites (B_{max}) of 570 pmoles/g of tissue (7.3 pmoles/mg of protein).

In salivary gland homogenates, [^{3}H]mazindol binding is saturable and displays a single component upon Scatchard analysis with a K_D of 4 nM and a B_{max} of about 10 pmoles/g of tissue (500 fmoles/mg of protein). In homogenates of the cerebral cortex, with 1 μM mazindol employed to define nonspecific binding, Scatchard analyis of specific [^{3}H]mazindol binding reveals a high-affinity and a low-affinity component. Under these conditions, inhibition of specific [^{3}H]mazindol binding by desipramine is markedly biphasic with a high-affinity inhibition component of about 10–15 nM and a lower-affinity component of about 10 μM. In addition, when 1 μM mazindol is used to define nonspecific binding, the potencies of various drugs in inhibiting specific [^{3}H]mazindol binding differ from drug potencies for inhibition of norepinephrine uptake. A concentration of 0.3 μM desipramine maximally inhibits the high-affinity component of [^{3}H] mazindol binding to cortical membranes without affecting the lower-affinity component. Accordingly, for routine studies, [^{3}H]mazindol binding to cerebral cortex was assayed utilizing 0.3 μM desipramine blanks. In the cerebral cortex, specific [^{3}H]mazindol binding, assessed using 0.3 μM desipramine to define nonspecific binding, is saturable and displays a single component with a K_D of about 4 nM and a B_{max} of 10 pmoles/g of tissue (150 fmoles/mg of protein).

Drug specificity of [^{3}H]mazindol binding sites. Specific [^{3}H]mazindol binding to striatal membranes is inhibited by mazindol, benztropine, desipramine, *d*-amphetamine,

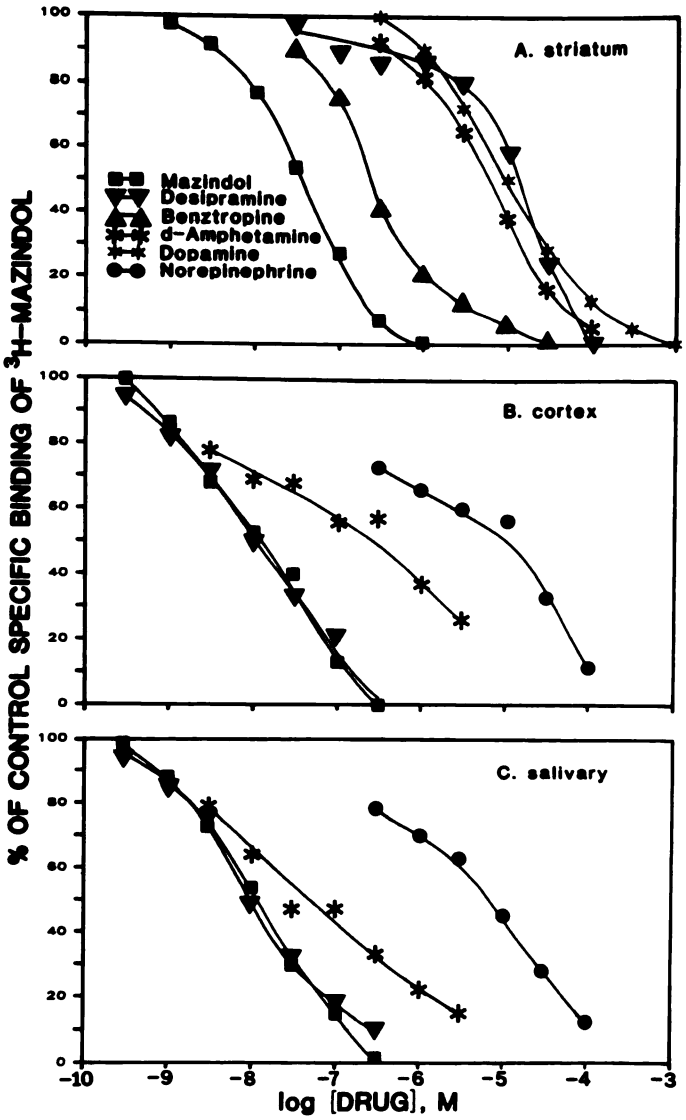

FIG. 2. *Inhibition of specific [^{3}H]mazindol (4 nM) binding to rat (A) striatal, (B) cerebral cortical, and (C) salivary gland membranes by mazindol, desipramine, benztropine, d-amphetamine, dopamine, and norepinephrine*

Results represent the mean of three or four experiments performed in triplicate, which varied less than 20%.

and dopamine in a monophasic fashion with pseudo-Hill coefficients not significantly different from unity (Fig. 2A). In cerebral cortex and salivary gland homogenates, similar patterns of competition are observed, with mazindol and desipramine displaying monophasic competition curves, while shallower curves are apparent with (−)-norepinephrine and *d*-amphetamine (Fig. 2B and C).

Evidence that [^{3}H]mazindol binding to striatal membranes is associated with dopamine uptake sites comes from the close correlation observed between potencies of drugs in competing for [^{3}H]mazindol binding and in inhibiting synaptosomal accumulation of dopamine in the corpus striatum (Table 1; Fig. 3A). In the corpus striatum, mazindol is the most potent agent, with apparent K_i values of 15–25 nM for inhibition of dopamine uptake and competition for binding. For antidepressants and other inhibitors of biogenic amine uptake, potencies

TABLE 1

Drug inhibitory potencies on [^{3}H]dopamine and [^{3}H]norepinephrine uptake and [^{3}H]mazindol binding in the rat corpus striatum, cerebral cortex, and salivary gland

Drug effects were examined on specific [^{3}H]mazindol binding as described under Experimental Procedures. Apparent K_i was determined by the Cheng and Prusoff equation (25) assuming competitive inhibition and using IC_{50} values obtained from linear regression of log-logit plots. The values are the means of two to four separate experiments performed in triplicate, which varied less than 20%.

Drugs	Apparent K_i				
	[^{3}H]Dopamine uptake[a]	[^{3}H]Mazindol binding			[^{3}H]Norepinephrine uptake[b]
	Striatum	Striatum	Cortex	Salivary	Cortex
			μM		
Uptake inhibitors					
1. Mazindol	0.016	0.023	0.007	0.008	0.002
2. Nomifensine	0.043	0.084	0.095	0.14	0.005[a]
3. DITA	0.076[c]	0.11	0.013	0.010	0.012
4. Benztropine	0.098	0.22	0.41	1.6	0.21
5. EXP-561	0.24[d]	0.18	0.013	0.008	0.015[d]
6. Cocaine	0.28	0.54	0.115	1.7	0.14[a]
7. Nisoxetine	0.36[e]	0.66	0.009	0.012	0.008
8. Bupropion	0.54	0.54	0.88	6.5	0.98[a]
9. *d*-Amphetamine	1.3[f]	4.7	0.18	0.10	0.066
10. Chlordesipramine	2.0	5.0	0.005	0.015	0.014
11. Protriptyline	2.9	5.1	0.038	0.032	0.010
12. Nortriptyline	3.2	5.4	0.043	0.038	0.017
13. Chlorimipramine	3.8	9.5	0.11	0.10	0.19
14. *l*-Amphetamine	5.1[f]	18.4	0.50	0.50	0.10
15. Desipramine	8.1	12.2	0.005	0.006	0.007
16. Doxepin	11.6	50.0	0.15	0.075	0.14
17. Iprindole	12.5	51.8	3.3	4.4	3.7
18. Imipramine	16.1	17.0	0.095	0.095	0.058
19. Trazodone	17.0	31.6	5.2	50.0	7.2[a]
20. Mianserin	35.7	10.1	0.43	0.30	0.30
Neurotransmitters					
Dopamine	0.11	8.3	2.3	2.3	0.10
(−)-Norepinephrine	0.18[g]	62.4	9.8	7.1	0.14

[a] Data from Hyttel (18) except where noted.
[b] Data from Lee *et al.* (8) except where noted.
[c] Data from Heikkila *et al.* (16). The value has been normalized using the potency of mazindol to account for differences in potencies obtained with tissue slices versus striatal synaptosomes.
[d] Data from Wong *et al.* (26).
[e] Data from Wong and Bymaster (27).
[f] Data from Koe (15).
[g] Data from Horn (5).

in inhibiting [^{3}H]mazindol binding and dopamine uptake are very closely correlated, with a correlation coefficient of 0.96 ($p < 0.001$). A considerable discrepancy in absolute potencies occurs for the neurotransmitter biogenic amines. Thus, dopamine is 80 times more potent in inhibiting [^{3}H]dopamine accumulation than [^{3}H]mazindol binding, and an even greater discrepancy occurs with norepinephrine.

Drug potencies in competing for [^{3}H]mazindol binding in homogenates of the cerebral cortex and salivary gland correlate closely with potencies in inhibiting cortical synaptosomal accumulation of [^{3}H]norepinephrine ($r = 0.91, p < 0.001$ and $r = 0.90, p < 0.001$, respectively) (Fig. 3D and F). However, drug potencies in inhibiting [^{3}H]mazindol binding in cerebral cortex and salivary gland do not correlate with potencies in inhibiting dopamine uptake into corpus striatal synaptosomes (Fig. 3C and E). The differentiation of [^{3}H]mazindol binding sites in corpus striatum and salivary gland or cerebral cortex is further underscored by the lack of correlation between drug potencies in inhibiting [^{3}H]mazindol binding in the corpus striatum and potencies in inhibiting norepinephrine uptake in cerebral cortical synaptosomes (Fig. 3B).

Drug potencies in inhibiting [^{3}H]mazindol binding in salivary gland and cerebral cortex are extremely similar (Table 1; Fig. 3C–F). Of the 20 drugs evaluated, only 4 display pronounced differences. For cocaine and the antidepressants bupropion and trazodone, apparent K_i values for inhibiting [^{3}H]mazindol binding are about 10 times higher in the salivary gland than in the cerebral cortex, whereas benztropine is 4 times more potent in the cortex.

Further evidence that [^{3}H]mazindol binding involves similar sites in the salivary gland and cerebral cortex which differ from the sites in the corpus striatum derives from the close correlation of drug potencies in competing for [^{3}H]mazindol binding in salivary gland and cerebral cortex ($r = 0.94, p < 0.001$) and the absence of correlation between competition for [^{3}H]mazindol binding in sali-

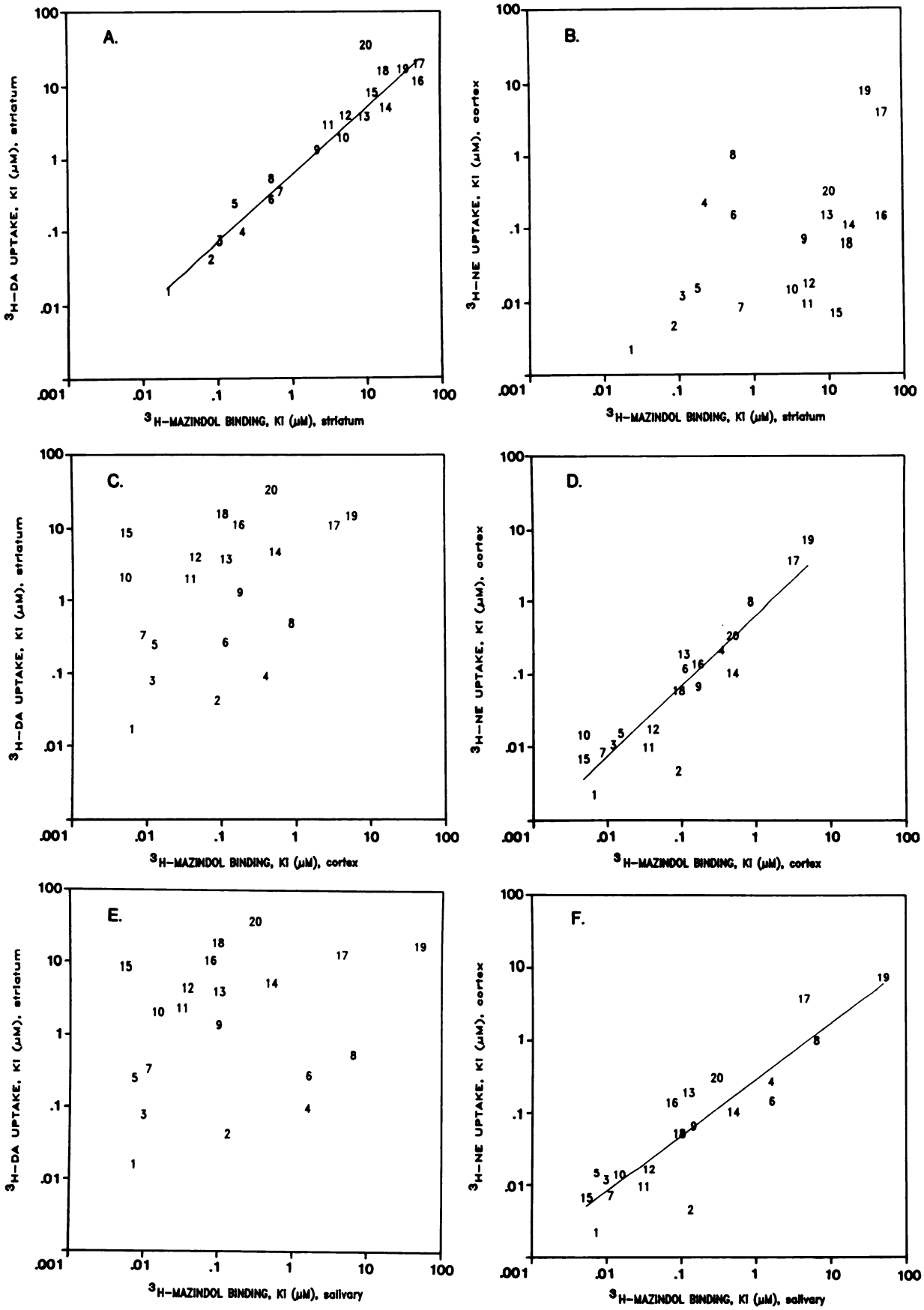

FIG. 3. *Correlation of the inhibition of specific [^{3}H]mazindol binding by uptake inhibitors to either corpus striatal, cerebral cortical, or salivary gland membranes with inhibition of synaptosomal uptake of either [^{3}H]dopamine or [^{3}H]norepinephrine*

The correlation coefficients are (A) 0.96, (B) 0.53, (C) 0.42, (D) 0.91, (E) 0.28, and (F) 0.90. Each number represents a drug and refers to the numbering of Table 1. See legend to Table 1 for experimental details.

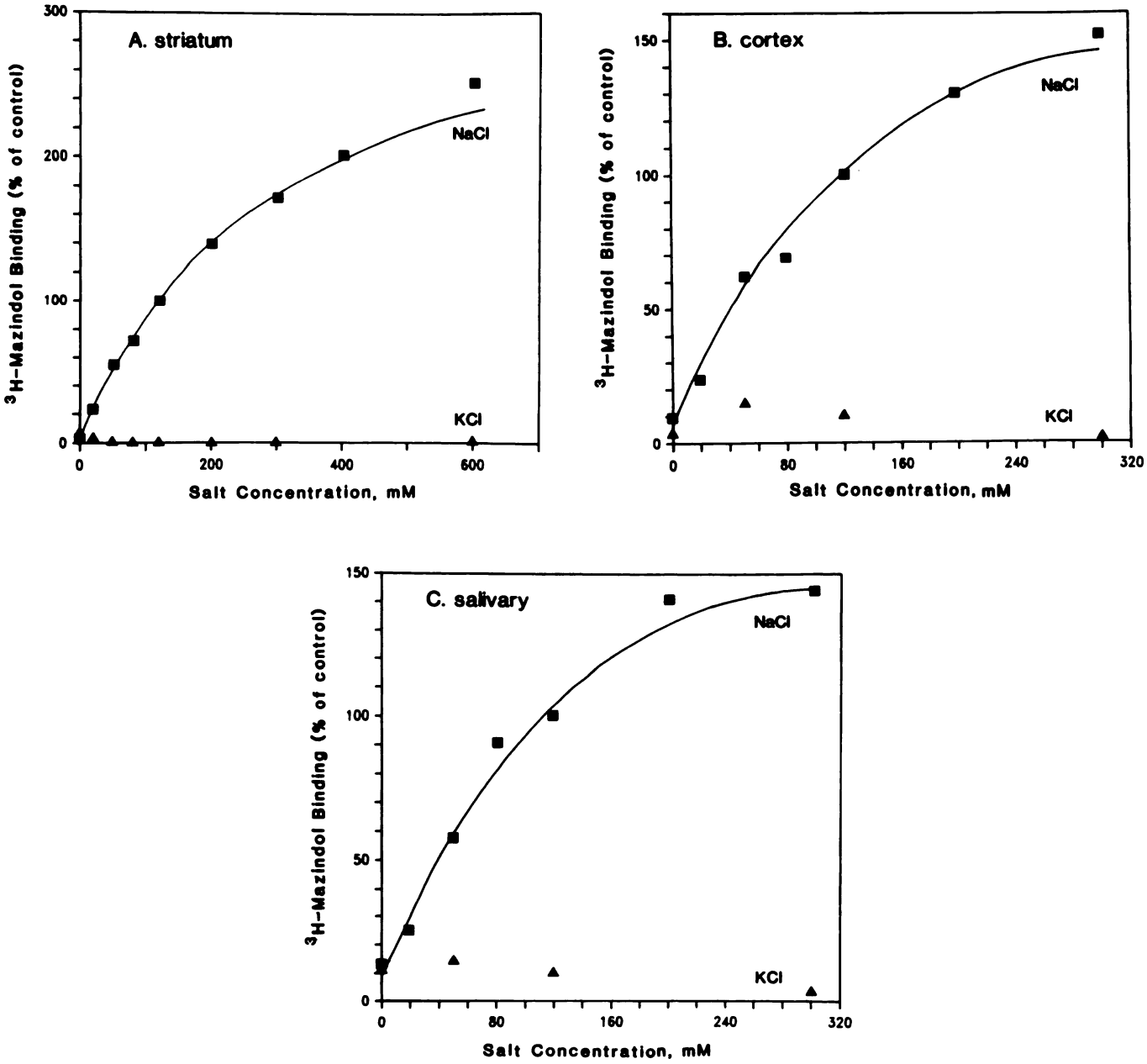

FIG. 4. *Sodium dependency of [^{3}H]mazindol (4 nM) binding to rat (A) striatal, (B) cerebral cortical and (C) salivary gland membranes*
Specific binding of [^{3}H]mazindol was measured in the presence of various concentrations of sodium or potassium chloride. The "% of control" values are expressed as the percentage of binding at a given ion concentration relative to 120 mM sodium chloride in each tissue and are the means of three experiments. The standard error of the mean is less than 10% for each value.

vary gland or cerebral cortex and corpus striatum (r = 0.30, r = 0.44 respectively). Interestingly, the absolute potencies of benztropine, cocaine, bupropion, and trazodone in inhibiting [^{3}H]mazindol binding in cerebral cortex are similar to their potencies in inhibiting norepinephrine uptake into cerebral cortical synaptosomes.

Effect of sodium on [^{3}H]mazindol binding. Previous reports indicate that [^{3}H]desipramine binding associated with norepinephrine uptake sites is dependent upon sodium (7, 13). In the present study a similar sodium dependence was observed for [^{3}H]mazindol binding both to norepinephrine uptake-related sites in the cerebral cortex and salivary gland and to dopamine uptake-related sites in the corpus striatum (Fig. 4). In all three tissues, binding is dependent upon the presence of sodium ion, with negligible specific binding apparent after incubation in the absence of sodium. Sodium is less potent in enhancing binding in the corpus striatum than in the cerebral cortex and salivary gland. Half-maximal binding occurs at 50–80 mM NaCl in salivary gland and cortex and at 240 mM NaCl in the corpus striatum. The requirement for sodium is highly specific, as no enhancement of binding is observed with potassium, lithium, rubidium, or Tris chloride.

The mechanism of sodium stimulation of [^{3}H]mazindol binding was examined in greater detail in striatal membranes. Increasing concentrations of sodium increase the affinity of [^{3}H]mazindol for its binding sites

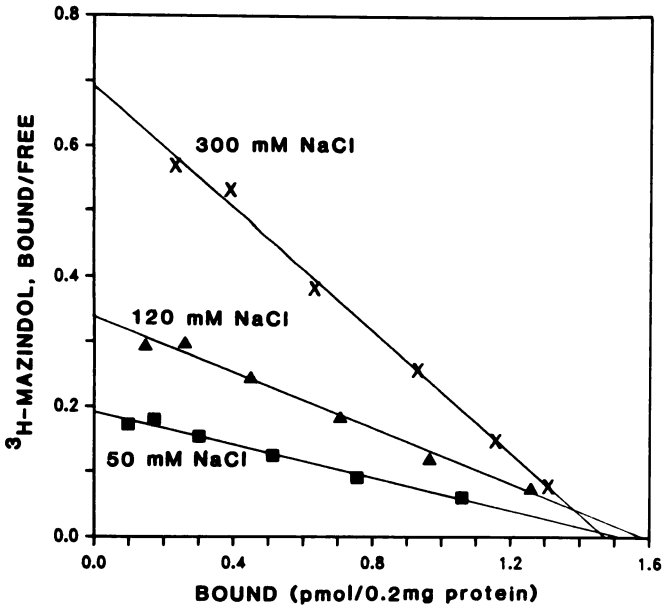

FIG. 5. *Scatchard analysis of the saturation of [3H]mazindol (2–60 nM) binding to rat striatal membranes in the presence of 50, 120, 300 mM sodium chloride*

Results are from a typical experiment performed in triplicate which was replicated three times.

with no change in the maximal number of sites (Fig. 5). Increasing the concentration of sodium chloride from 50 mM to 120 mM produces a 2-fold decrease in K_D. An effect of similar magnitude is seen by increasing the concentration from 120 to 300 mM (Fig. 5; Table 2).

The dissociation rate of [3H]mazindol from its binding sites was measured by determining the specific binding of [3H]mazindol (4 mM) to striatal membranes (previously incubated to equilibrium) at various times after the addition of 1 μM unlabeled mazindol. Sodium chloride slows the dissociation of [3H]mazindol from its binding site in a concentration-dependent manner (Fig. 6B). Thus, the half-time for dissociation in the presence of 300 mM NaCl is about 3 times greater than the half-time with 120 mM NaCl and almost 5 times greater than the half-time with 50 mM NaCl (Table 2). When dissociation is initiated with 1 mM dopamine in addition to 1 μM mazindol, no change in the dissociation rate is observed (Fig. 6B).

The apparent association rate (K_{ob}) of [3H]mazindol to striatal membranes is slower in the presence of increasing sodium concentrations (Fig. 6A). However, this change is primarily due to the change in dissociation rate (k_{-1}) (Fig. 6B), and no significant change is seen in the true association rate (k_1) (Table 2). Thus, the enhancement of [3H]mazindol affinity by sodium can be attributed almost exclusively to a slowing of the dissociation of the ligand. The K_D values calculated from the ratio of k_{-1}/k_1 are in good agreement with K_D values determined in equilibrium binding studies.

Effect of chloride on [3H]mazindol binding. [3H]Desipramine binding associated with norepinephrine uptake sites is dependent on chloride as well as sodium (8). Thus, specific [3H]desipramine binding is greater in the presence of sodium chloride than in the presence of the same concentration of various other sodium salts. In the present study, a similar augmentation of the specific binding of [3H]mazindol to corpus striatum, cerebral cortex, and salivary gland was observed with sodium chloride relative to sodium sulfate. To evaluate further the chloride requirement, the concentration of chloride in the incubation was varied by the addition of an appropriate mixture of sodium chloride and sodium sulfate to maintain the sodium concentration of 300 mM. In all three tissues, specific binding is significantly reduced in the absence of chloride, and less than 25 mM chloride is needed to attain levels of binding which are half those seen with 120 mM chloride (Table 3).

Effect of neuronal lesions on [3H]mazindol binding. The effects of selective destruction of dopaminergic and noradrenergic neurons on [3H]mazindol binding to striatal and cortical membranes were examined. Following administration of 6-hydroxydopamine (after pretreatment with desipramine and pargyline), [3H]dopamine uptake into striatal synaptosomes and specific [3H]mazindol binding to striatal membranes were decreased 89% and 72%, respectively (Table 4A). In addition, DSP-4, a toxin which causes selective destruction of noradrenergic neurons (24), reduced [3H]norepinephrine uptake into cortical synaptosomes and specific [3H]mazindol binding to cortical membranes by 63% and 76%, respectively (Table 4B).

DISCUSSION

The major finding of the present study is that [3H] mazindol labels sites related to dopamine uptake in the

TABLE 2

Effects of sodium chloride on [3H]mazindol binding to corpus striatal membranes

Saturation experiments were performed using six concentrations of [3H]mazindol (2–60 nM). K_D and B_{max} values were determined by linear regression analysis of Scatchard plots. Values for saturation experiments are the means ± standard error of the mean of three to five separate experiments performed in triplicate. See the legend to Fig. 6 for details of the kinetic experiments. k_{-1} and k_{ob} were determined by linear regression analysis of dissociation and association experiments, respectively. k_1 was determined by pseudo-first order reaction analysis. Values for kinetic experiments are the means of two to six separate experiments, each performed in triplicate.

Sodium chloride concentration	K_D	B_{max}	k_{-1}	k_{ob}	k_1	k_{-1}/k_1
mM	*nM*	*pmoles/g tissue*	sec^{-1}	sec^{-1}	$nM^{-1}\ sec^{-1}$	*nM*
50	33.5 ± 1.6	562 ± 31	3.5×10^{-3}	3.9×10^{-3}	0.9×10^{-4}	40.0
120	18.2 ± 0.6	570 ± 47	2.5×10^{-3}	3.0×10^{-3}	1.5×10^{-4}	16.4
300	8.7 ± 0.2	578 ± 31	0.8×10^{-3}	1.2×10^{-3}	1.2×10^{-4}	6.4

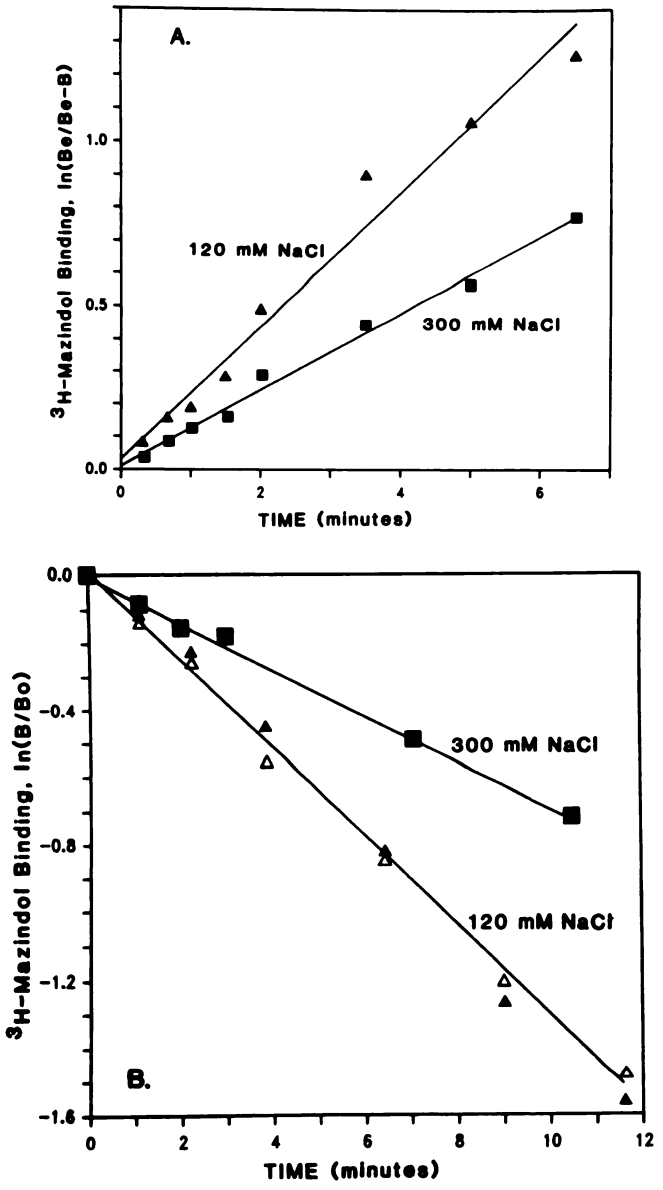

FIG. 6. *Kinetic analysis of the (A) association and (B) dissociation of specific [^{3}H]mazindol binding to rat striatal membranes in the presence of 120 and 300 mM NaCl*

Total and nonspecific binding (defined with 1 μM mazindol) were determined at the indicated time intervals to yield the specific binding values. The data were plotted according to a pseudo-first order reaction, and the slope (k_{ob}) was determined by linear regression analysis. For dissociation experiments, 4 nM [^{3}H]mazindol was incubated to equilibrium with striatal membranes at 0° for 1 hr. Dissociation was then initiated by adding 1 μM mazindol (■, ▲) or 1 μM mazindol plus 1 mM dopamine (△), and the residual binding was measured at indicated time points. Binding at time zero (B_0) was defined as the equilibrium level. The slope (k_{-1}) was determined by linear regression analysis. The results are from a typical experiment which was repeated two to five times in triplicate.

corpus striatum and to norepinephrine uptake in the cerebral cortex and salivary gland. The major evidence for this conclusion is the close correlation of drug potencies in inhibiting [^{3}H]mazindol binding and the respective catecholamine uptake. Additionally, lesions of the dopamine system with 6-hydroxydopamine reduced [^{3}H]

TABLE 3

Chloride dependence of [^{3}H]mazindol binding

[^{3}H]Mazindol binding was assayed as described under Experimental Procedures. Values are expressed as means ± standard deviation of two experiments performed in triplicate. The "% control" values are expressed as the percentage of binding at a given chloride concentration relative to 120 mM chloride.

Tissue	Chloride concentration	Specific [^{3}H]mazindol binding	% Control
	mM	*pmoles/g tissue*	
Corpus striatum	0	16.5 ± 6.5	28
	25	31.9 ± 1.7	55
	120	57.9 ± 1.0	100
Cerebral cortex	0	0.8 ± 0.5	13
	25	4.5 ± 1.2	73
	120	6.2 ± 1.2	100
Salivary gland	0	2.2 ± 1.9	27
	25	5.5 ± 0.5	66
	120	8.3 ± 1.2	100

mazindol binding and synaptosomal [^{3}H]dopamine accumulation in the corpus striatum, whereas lesions of the noradrenergic system with DSP-4 depleted [^{3}H]mazindol binding and synaptosomal [^{3}H]norepinephrine accumulation in the cerebral cortex. The B_{max} for [^{3}H]mazindol binding to cerebral cortical and salivary gland membranes is 10 pmoles/g of tissue. This is in close agreement with the B_{max} for high-affinity [^{3}H]desipramine binding to these tissues [7.5 pmoles/g of tissue (9)] and is consistent with the hypothesis that both of these ligands label norepinephrine uptake sites. Furthermore, preliminary autoradiographic studies using [^{3}H]mazindol reveal highest levels of binding in corpus striatum, nucleus accumbens, and olfactory tubercle, areas rich in dopaminergic nerve terminals.[3]

Although the potencies of most drugs in competing for [^{3}H]mazindol binding correlate with their potencies in inhibiting the respective catecholamine uptake, discrepancies exist for the catecholamines themselves. Norepinephrine and dopamine are substantially weaker in inhibiting [^{3}H]mazindol binding than [^{3}H]catecholamine uptake in the corpus striatum, the cerebral cortex, and the salivary gland. Additionally, in salivary gland and cerebral cortex, competition curves by the catecholamines are more shallow than competition curves by antidepressants and other drugs. Norepinephrine, dopamine, and serotonin are much weaker in inhibiting [^{3}H]desipramine binding associated with norepinephrine uptake sites than in inhibiting [^{3}H]norepinephrine accumulation into cortical synaptosomes (8). These findings suggest that the site labeled by [^{3}H]desipramine or [^{3}H]mazindol may not be identical with the site which recognizes the catecholamine. Mazindol and desipramine may bind to the amine uptake recognition site when it is in a conformation different from the one which optimally recognizes the amines themselves (28). Another possibility is that drugs which inhibit biogenic amine uptake

[3] J. A. Javitch, S. M. Strittmatter, and S. H. Snyder, manuscript in preparation.

TABLE 4

Effect of 6-hydroxydopamine and DSP-4 lesions on [³H]mazindol binding, [³H]dopamine uptake, and [³H]norepinephrine uptake in the rat corpus striatum and cerebral cortex

Uptake and binding were assayed as described under Experimental Procedures. [³H]Mazindol was used as a concentration of 5 nM for binding assays. Values are expressed as means ± standard error of the mean of *n* separate determinations, each performed in triplicate.

A. Lesions of the dopaminergic system—corpus striatum		
Animals	[³H]Dopamine uptake	Specific [³H]mazindol binding
	fmoles/mg tissue/3 min	*pmoles/g tissue*
Saline Control ($n = 3$)	297 ± 102 (100%)	82 ± 5 (100%)
6-Hydroxydopamine ($n = 4$)	33 ± 21 (11%)[a]	23 ± 6 (28%)[b]
B. Lesions of the noradrenergic system—cerebral cortex		
Animals	[³H]Norepinephrine uptake	Specific [³H]mazindol binding
	fmoles/mg tissue/5 min	*pmoles/g tissue*
Saline control ($n = 4$)	129 ± 7 (100%)	5.8 ± 0.4 (100%)
DSP-4 ($n = 4$)	48 ± 4 (37%)[b]	1.4 ± 0.5 (24%)[b]

[a] $p < 0.05$ compared with control.
[b] $p < 0.001$ compared with control.

may act at sites which are allosterically linked to the amine recognition sites. However, both our finding that dopamine does not alter the dissociation rate of [³H]mazindol from its binding site in striatal membranes and the earlier finding that norepinephrine does not change the dissociation rate of [³H]desipramine from its binding site (8) argue against an allosteric linkage. Nevertheless, an allosteric relationship of the serotonin uptake recognition site and the [³H]imipramine binding site has recently been suggested (29, 30).

Studies of neuronal norepinephrine accumulation have suggested that the norepinephrine uptake process is essentially the same in central and peripheral neurons (31). The relative potencies of most drugs are similar at [³H]mazindol binding sites in the salivary gland and cerebral cortex. However, bupropion, trazodone, and cocaine are only about one-tenth as potent in inhibiting [³H]mazindol binding in the salivary gland as in the cerebral cortex, and benztropine is one fourth as potent. This may indicate a difference in the central and peripheral uptake sites.

Interestingly, [³H]mazindol binding in the corpus striatum, salivary gland, and cerebral cortex has an absolute dependence upon sodium. A similar dependence on sodium has been reported for [³H]desipramine binding associated with norepinephrine uptake sites (7). [³H]Imipramine binding to serotonin uptake-associated sites is also dependent upon sodium (32, 33). Furthermore, norepinephrine uptake sites labeled with either [³H]desipramine or [³H]mazindol are also dependent upon chloride, as are the dopamine uptake-related sites in the corpus striatum labeled by [³H]mazindol. A similar chloride dependence of [³H]imipramine binding has also been reported (8). Biogenic amine accumulation by nerve endings is a sodium- and chloride-dependent process (34, 35), and it has been thought that the sodium requirement for catecholamine uptake reflects a relationship with the sodium-potassium ATPase pump (34). However, it seems unlikely that the effect of sodium on [³H]mazindol or [³H]desipramine binding reflects an influence upon the sodium-potassium ATPase, since ouabain (1–100 μM) has no effect on [³H]desipramine binding (8) or on [³H]mazindol binding in any of the three tissues studied.[4] These results suggest that a sodium recognition site distinct from the sodium-potassium ATPase as well as a chloride recognition site may form part of the macromolecular complex in the catecholamine and serotonin transport mechanisms.

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