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BINDING CHARACTERISTICS OF THE DOPAMINE UPTAKE INHIBITOR [^{3}H]NOMIFENSINE TO STRIATAL MEMBRANES*

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Abstract—Binding of the radiolabeled antidepressant [^{3}H]nomifensine to rat and rabbit striatal membranes has been characterized. The specific binding of [^{3}H]nomifensine to striatal membranes was stable, reversible and saturable. Saturation experiments indicated that [^{3}H]nomifensine labeled a single site with an affinity (K_d) of 80 nM and a total number of binding sites (B_{max}) of 6.5 pmoles/mg protein both in rat and rabbit striatal membranes. The affinity constants obtained from kinetic analyses and competition experiments were in fairly good agreement with those obtained in saturation experiments. Compounds known to inhibit [^{3}H]dopamine uptake *in vitro*, such as nomifensine, 4-hydroxy-nomifensine, mazindol, amfonelic acid and benztropine, were the most potent competitors of nomifensine binding. Additionally, the absolute potencies of various drugs in competing for [^{3}H]nomifensine binding to rat and rabbit striatal membranes correlated closely with their potencies in inhibiting [^{3}H]dopamine uptake into striatal synaptosomes. Specific [^{3}H]nomifensine binding was dependent on the presence of NaCl which is also consistent with its association with the dopamine uptake pump. The number, but not the affinity, of striatal [^{3}H]nomifensine binding sites was reduced significantly following *in vivo* lesions with 6-hydroxydopamine. The number of [^{3}H]nomifensine binding sites was found to be highest in areas rich in dopamine nerve terminals such as the striatum and olfactory tubercle. These results suggest that [^{3}H] nomifensine binds to a site on dopaminergic nerve terminals associated with the dopamine uptake pump.

The primary mechanism for inactivation of biogenic amines released into the synapse is high affinity uptake into nerve terminals. Tricyclic antidepressants are potent inhibitors of the neuronal uptake of both norepinephrine and serotonin, effects which are thought to contribute to their clinical efficacy as antidepressants [1–3]. Recent studies have described specific and saturable high affinity binding sites for [^{3}H]imipramine [4–9] and for [^{3}H]desipramine [10–13] in membranes isolated from both brain and platelets. The binding sites for [^{3}H]desipramine and [^{3}H]imipramine appear to be associated with the neuronal uptake sites for norepinephrine and serotonin respectively [4–7, 10–13]. Nomifensine, which also possesses antidepressant [14, 15], is more potent than any of the classical tricyclic antidepressants, such as desipramine or imipramine, at inhibiting the neuronal uptake of dopamine [2, 3]. In the present study we have characterized a binding site for [^{3}H]-nomifensine in rat and rabbit striatal membranes which appears to be associated primarily with the neuronal dopamine uptake site.

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MATERIALS AND METHODS

Tissue preparation. Male Sprague–Dawley rats (150–180 g) and albino rabbits (1.5–3 kg) were killed by decapitation. Striata and other brain regions were dissected and homogenized in ice-cold Tris–HCl tissue buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl; pH 7.4) with a Brinkmann Polytron PT-10 at setting 5 for 10 sec. The homogenate was centrifuged at 50,000 *g* for 10 min at 4°. The pellet was washed twice by resuspension and centrifugation in assay buffer. The washed striatal membranes were resuspended by homogenization at a concentration of 500 μg protein/ml for both rat and rabbit.

Binding assays. Radioligand and drugs were dissolved in 1 mM HCl. The binding of [^{3}H]nomifensine was routinely measured after incubation at 0° for 1 hr. Binding was initiated by addition of 210 μl aliquots of membranes resuspended in the Tris–HCl tissue buffer to tubes containing 20 μl of appropriate [^{3}H]nomifensine concentrations and 20 μl of drugs or vehicle. For both competition and saturation experiments, specific binding was defined as the difference in radioligand bound in the absence and presence of 100 μM benztropine. Reactions were terminated by addition of 5 ml of ice-cold Tris–HCl buffer, and the contents were immediately filtered through glass fiber filters (Schleicher and Schuell No. 30). Each filter was washed twice with 5 ml of the cold buffer. Radioactivity was determined by liquid scintillation spectrometry in 3 ml of 3a70 fluor (Research Products International Corp., Mt Prospect, IL). The counting efficiency was approximately 40%.

The final concentrations of [^{3}H]nomifensine (sp. act. 45 Ci/mmole) used in competition experiments were 2–6 nM. Between eleven and twenty concentrations of each drug (1 nM–100 μM) were examined for their ability to inhibit the total binding of [^{3}H]nomifensine. Total binding was measured in the presence of vehicle. Competition curves were analyzed by fitting the data to a two-site model using an iterative computer program (BIPHAS). The input in this program corresponded to specifically bound and free values. These values were used to find the equation of best fit to a two-site model of receptor binding. The equation of best fit is defined as the combination of values for which the sum of the differences between the observed and calculated values, squared, is least. The statistical significance between the equations of best fit for a two-site model and a one-site model was evaluated using F-test analysis.

Saturation curves were constructed using twelve concentrations of [^{3}H]nomifensine (2–250 nM). The saturation curves for specifically bound radioligand were transformed by the method of Scatchard [16]. Analyses of both competition and saturation curves were used to determine the density of binding sites (B_{max}) and the equilibrium-dissociation constants (Kd values).

Protein concentrations were determined by the dye-binding method of Bradford [17] using bovine serum albumin as the standard. Sodium hydroxide (0.1 N) was added to each sample to solubilize the proteins before assay.

6-Hydroxydopamine lesions. Rats were anesthetized with methoxyflurane. Control animals received bilateral infusions under stereotoxic control (L 1.2, V 3.5) of 10 μl vehicle (10 mM citrate, 150 mM NaCl, 10 mM glucose and 5 mM sodium ascorbate; pH 6.1) at a rate of 1 μl/min into each lateral ventricle. 6-Hydroxydopamine-treated animals received a treatment identical to that of the controls with the exception that the infusion contained 100 μg 6-hydroxydopamine (free base)/10 μl vehicle. Animals were killed 16 days after the injection.

Dopamine levels. The concentration of dopamine in the striatum was determined by liquid chromatography and electrochemical detection (LCEC Application Notes 12 and 14, Bioanalytical Systems, West Lafayette, IN, 1982). A known amount of striatal tissue (approximately 10 mg) was homogenized by sonication in 400 μl of 0.1 M perchloric acid (pH 1) containing 1.3 mM disodium EDTA, 7.9 mM sodium metabisulfite and 0.29 μM dihydroxybenzylamine (internal standard). Following centrifugation of the homogenate at 30,000 g and 4° for 10 min, catecholamines were isolated from the supernatant fraction onto acid-washed alumina by gentle shaking for 10 min in 1.5 vol. of 1.5 M Tris buffer (pH 8.6) containing 1.3 mM disodium EDTA and 7.9 mM sodium metabisulfite. The suspension was spun in a microfuge, and the supernatant fraction was aspirated off. The alumina was washed twice more by mixing with a solution containing 1.3 mM disodium EDTA and 7.9 mM sodium metabisulfite, centrifugation, and aspiration. The catecholamines were eluted from the alumina by gentle shaking in 480 μl of the original perchloric acid tissue buffer (pH 1). After filtering (filter pore size 0.2 μm) the perchloric acid solution containing the catecholamines, 100 μl of this solution was injected into the high performance liquid chromatograph. The mobile phase, 0.15 M monochloroacetate (pH 3) containing 2 mM disodium EDTA and 0.12 mM sodium octylsulfate, had a flow rate of 1.8 ml/min. The temperature of the Biophase ODS column (5 μM) was 30°, and the electrode potential was 0.65 V. The concentrations of dopamine in the samples were calculated from the peak height ratios and a standard curve of peak height ratios for known concentrations of dopamine. The recovery of dopamine in the samples was approximately 42%.

Drugs were obtained from commercial sources or the pharmaceutical company of origin. The sources of the drugs were: nomifensine, M-1 (4-OH-nomifensine), M-2 (3-methoxy-4-OH-nomifensine) and M-3 (3-OH-4-methoxy-nomifensine) from Hoechst-Roussel Pharmaceuticals (Somerville, NJ); benztropine from Merck Sharp & Dohme Research Laboratories (West Point, PA); amfonelic acid from Sterling-Winthrop Research Institute (Rensselaer, NY); mazindol from Sandoz Pharmaceuticals (East Hanover, NJ); and bupropion from Bourroughs Wellcome Laboratories (Research Triangle Park, NC). [^{3}H]Nomifensine (sp. act. 45 Ci/mmole) was custom synthesized by New England Nuclear (Boston, MA).

RESULTS

Characterization of [^{3}H]nomifensine binding. The binding of [^{3}H]nomifensine to rat and rabbit striatal membranes was stable and reversible. At 0° in striatal membranes from either species, the binding of a concentration of [^{3}H]nomifensine equal to half of the K_D value reached steady state by 30 min and was stable for at least 120 min. The association rate constant (k_1) determined in rabbit striatal membranes using the pseudo first-order equation was 3.0 μM $\times$ min^{-1} (inset, Fig. 1A). Specific [^{3}H]nomifensine binding was rapidly reversed ($t_{\frac{1}{2}} \sim 4$ min) by the addition of excess competing ligand (100 μM benztropine). The rate constant for dissociation (k_{-1}) was 0.17 min^{-1} (inset, Fig. 1B). The kinetic K_d value for [^{3}H]nomifensine calculated from the ratio k_{-1}/k_1 was 57 nM. Similar kinetic analysis (data not shown) in rat striatal membranes resulted in a K_d value of 30 nM.

The pharmacological characterization of [^{3}H]-nomifensine binding both in rat and rabbit striatal membranes was carried out with tracer concentrations of radioligand (2–6 nM). Compounds that are potent inhibitors of dopamine uptake [2, 3, 18], namely benztropine, mazindol, nomifensine and the nomifensine metabolite M-1, were very effective in competing for [^{3}H]nomifensine binding sites (Fig. 2). All four of these compounds also inhibited the binding of [^{3}H]nomifensine to approximately the same extent (Fig. 2). Subsequently, 100 μM benztropine was used to define specific binding; this constituted 80–90% of total binding to striatal membranes. Lee *et al.* [11] reported increases in the high affinity specific binding of [^{3}H]desipramine, but

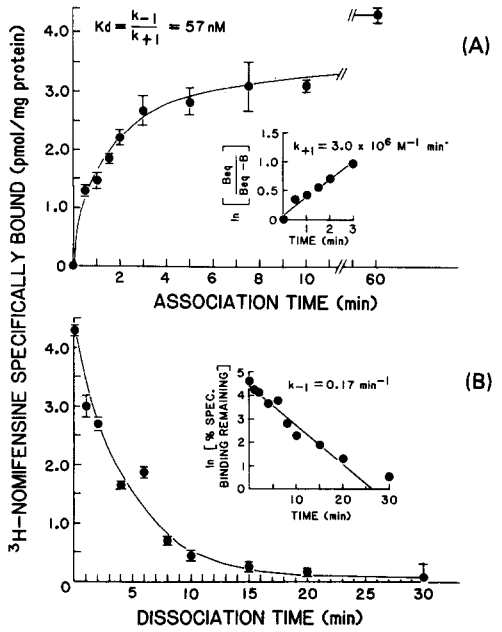

Fig. 1. Kinetic analysis of [³H]nomifensine binding to rabbit striatal membranes at 0°. Specific binding of [³H]nomifensine (35 nM) was defined as the difference between the binding in the absence and presence of 100 μM benztropine. Values shown are mean ± S.E.M. for N = 3. (A) Association time course. Inset: Pseudo first-order plot. B_{eq} represents the amount of specific binding at equilibrium (4.3 pmoles/mg protein), and B represents the amount of specific binding at each time. The k_{obs} (0.30 min^{-1}) is the slope of this plot. The association rate constant k_1 was calculated from the equation $k_1 = (k_{obs})\ (B_{eq})/([^3H]\text{nomifensine concn})\cdot(B_{max})$ and was equal to 3.0 μM min^{-1}. The B_{max} was derived from Scatchard analysis in the same tissue samples. (B) Dissociation time course. [³H]Nomifensine binding was brought to steady state (60 min) before the addition of 100 μM benztropine to all samples at dissociation time zero. Samples were filtered at various times thereafter, and the specific binding remaining was calculated. Inset: Semi-logarithmic plot of the decrease in specific binding with time. The dissociation rate constant (k_1), which is equal to the slope, was 0.17 min^{-1}.

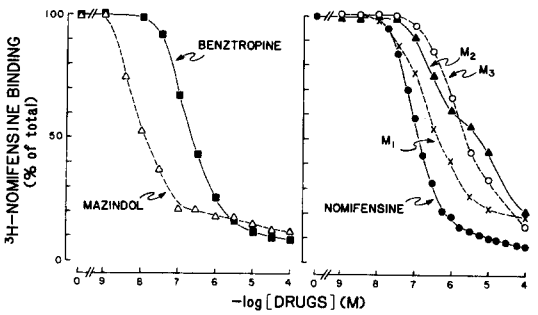

Fig. 2. Competition curves for the inhibition of [³H]nomifensine binding by various dopamine uptake inhibitors in rabbit striatal membranes. Washed rabbit striatal membranes were incubated with 2–6 nM [³H]nomifensine and the concentrations of drugs indicated. M-1, M-2 and M-3 are metabolites of nomifensine. The values shown are the means for three to six independent determinations.

Table 1. Pharmacological profile of [³H]nomifensine binding sites in rat and rabbit striatum

Drug	[³H]Nomifensine binding* K_i value (μM)	
	Rabbit striatum	Rat striatum
Mazindol	0.01	0.02
Nomifensine	0.07	0.05
Amfonelic acid	0.14	0.12
M-1	0.22	0.20
Benztropine	0.25	0.27
Cocaine	0.16	0.36
Phencyclidine	0.16	0.40
Bupropion	0.13	0.47
M-2	1.20	0.59
M-3	1.40	3.20
Nortriptyline	2.20	5.40
Spiperone	5.40	7.90
Desipramine	18.00	7.20
Phentolamine	7.40	30.00
Imipramine	63.00	45.00

* K_i values were calculated from the IC_{50} values obtained from competition curves using the method of Cheng and Prusoff [19]. The inhibition of specific binding of [³H] nomifensine (2–6 nM) to rat and rabbit striatal membranes was determined using eleven concentrations of competing drugs. Results are mean values, N = 3–9. S.E.M. values were less than 10% of the mean.

not [³H]imipramine, in membranes pretreated with 300 mM KCl. Under our experimental conditions, specific [³H]nomifensine binding defined with either benztropine or nomifensine was unaffected by pretreatment of rat or rabbit striatal membranes with KCl (data not shown). The IC_{50} values for the inhibition of [³H]dopamine uptake into striatal synaptosomes taken from the literature [2, 3, 18] are almost identical to the K_i values determined from [³H]nomifensine competition experiments for all compounds listed in Table 1. For most antidepressants (i.e. nortriptyline, desipramine, imipramine) and receptor antagonists (i.e. spiperone, phentolamine) evaluated, the potencies in decreasing [³H]dopamine uptake and [³H]nomifensine binding were quite similar; however, these compounds were effective only at micromolar concentrations.

In contrast, amphetamine was reported to be approximately 60-fold more potent at inhibiting [³H]dopamine uptake [2, 20] than inhibiting [³H]-nomifensine binding (Table 2). Likewise, the catecholamines dopamine and norepinephrine were much weaker in inhibiting [³H]nomifensine binding than their reported K_i values for uptake [21] into rat striatum (Table 2). The indirect acting agent tyramine was also 40-fold less potent at competing for [³H]nomifensine binding than for [³H]dopamine uptake [22] (Table 2).

Saturation studies of the specific binding of [³H]-nomifensine, over a concentration range of 2–250 μM, resulted in linear Scatchard plots both in rat (data not shown) and rabbit (Fig. 3) striatal membranes. It is interesting to note that the observed affinities, as well as the number of binding sites, were identical in both species (Table 3). In this range of concentrations the Scatchard plots were linear,

Table 2. Comparison between the potency to inhibit [^{3}H] nomifensine binding and [^{3}H]dopamine uptake of indirect releasing agents and biogenic amines

Drug	[^{3}H]Nomifensine binding* K_i value (μM) Rabbit striatum	[^{3}H]Nomifensine binding* K_i value (μM) Rat striatum	[^{3}H]Dopamine uptake† K_i value (μM) Rat striatum
Amphetamine	13	15	0.24‡
Dopamine	16	6.3	0.4§
Norepinephrine	86	40	1§
Tyramine	54	32	1‖
Serotonin	180	68	

* K_i values were calculated from the IC_{50} values obtained from competition curves using the method of Cheng and Prusoff [19]. The inhibition of specific binding of [^{3}H] nomifensine (2–6 nM) to rat and rabbit striatal membranes was determined using eleven concentrations of competing drugs. Results are mean values, N = 3–9. S.E.M. values were less than 10% of the mean.

† K_i values for inhibition of [^{3}H]dopamine uptake are from: ‡ Hyttel [3], § Snyder and Coyle [21], and ‖ Heikkila *et al.* [22].

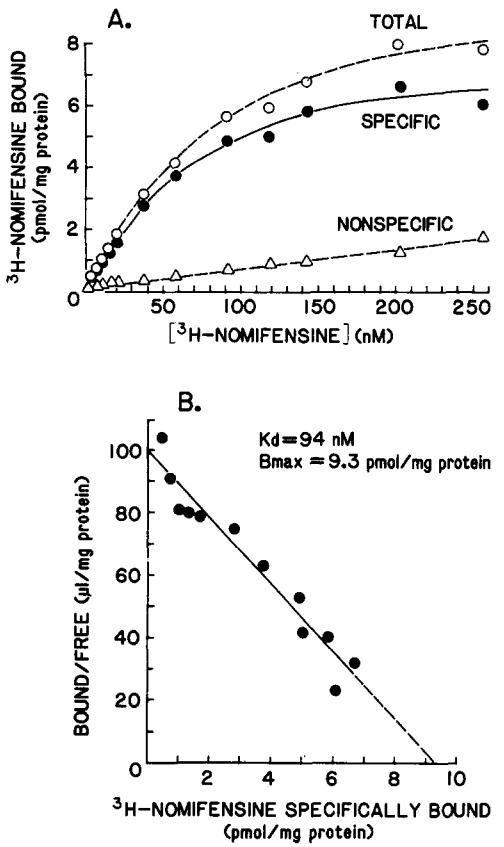

Fig. 3. Direct Scatchard analysis of [^{3}H]nomifensine binding to rabbit striatal membranes. Membranes were incubated with various concentrations of [^{3}H]nomifensine (2–250 mM) for 60 min at 0°. Nonspecific binding was measured in the presence of 100 μM benztropine. Values shown are from a representative experiment performed in duplicate. (A) Saturation curve. (B) Transformation of the saturation data by the method of Scatchard.

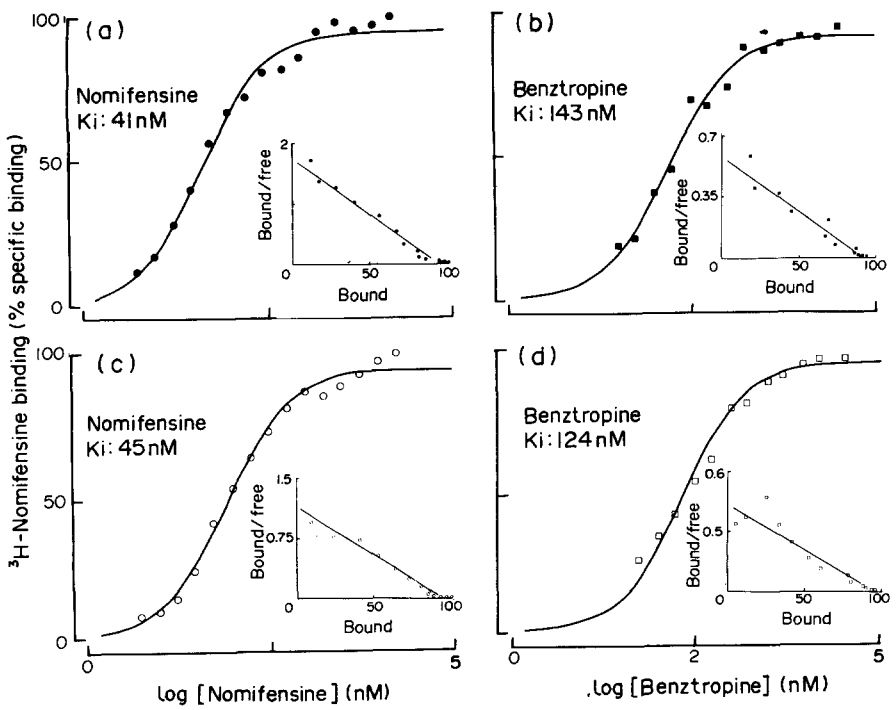

Fig. 4. [^{3}H]Nomifensine binding to a single site in rabbit striatal membranes. Rabbit striatal membranes were incubated with either 6 nM (a, b) or 60 nM (c, d) [^{3}H]nomifensine. Inhibition of the binding of [^{3}H]nomifensine was determined by 20 concentrations of either nomifensine (1 nM–100 μM; a, c) or benztropine (1 nM–100 μM; b, d). Points shown are from a single experiment, performed in triplicate. Insets: The same data plotted according to the method of Scatchard.

Table 3. Affinity and number of striatal [3H]nomifensine binding sites determined from Scatchard analysis*

Tissue	N	K_d (nM)	B_{max} (pmoles/mg protein)
Rabbit striatum	10	80 ± 5.3	6.4 ± 1.2
Rat striatum	10	82 ± 7.0	6.6 ± 0.6

* Values are means ± S.E.M.

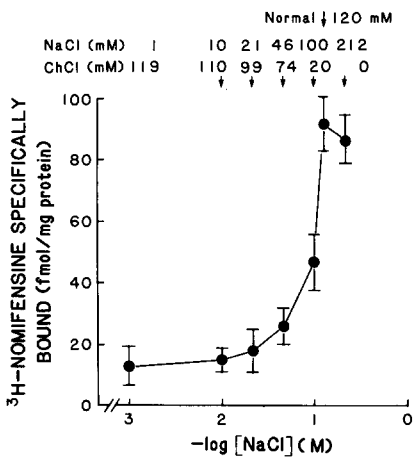

Fig. 5. Sodium chloride-dependency of specific [3H]nomifensine binding to rat striatal membranes. Specific binding of 2.5 nM [3H]nomifensine was measured as described in Materials and Methods in the presence of various concentrations of NaCl (0–212 mM). The osmolarity of the assay buffer was maintained by replacing NaCl by an equivalent concentration of choline chloride. Specific binding in the absence of NaCl was negligible. Results are the mean values ± S.E.M. for N = 3–4.

suggesting that [3H]nomifensine binds to a single site in striatal membranes.

To investigate further whether the binding of [3H]nomifensine is to a single site or multiple sites, more detailed competition curves were analyzed. At both low (6 nM; Fig. 4) and higher (60 nM; Fig. 4) fractional occupancy with [3H]nomifensine, either nomifensine or benztropine produced monophasic reductions in binding. Hill coefficients were not different from one. Transformation of these competition curves of [3H]nomifensine at both fractional occupancies gave linear Scatchard plots regardless of the concentration of radioligand used (insets, Fig. 4), again suggesting that binding of [3H]nomifensine is to a single site.

Comparison of several characteristics of the striatal dopamine uptake pump and [3H]nomifensine binding. The high affinity neuronal uptake of dopamine is largely dependent upon the presence of NaCl [23, 24]. In the present experiments we found that NaCl was also essential for the expression of specific [3H]nomifensine binding (Fig. 5). At 120 mM NaCl, specific [3H]nomifensine binding was maximal and was increased almost 9-fold, with respect to 1 mM NaCl. The EC_{50} for NaCl was 100 mM. The strict dependence of [3H]nomifensine binding on NaCl

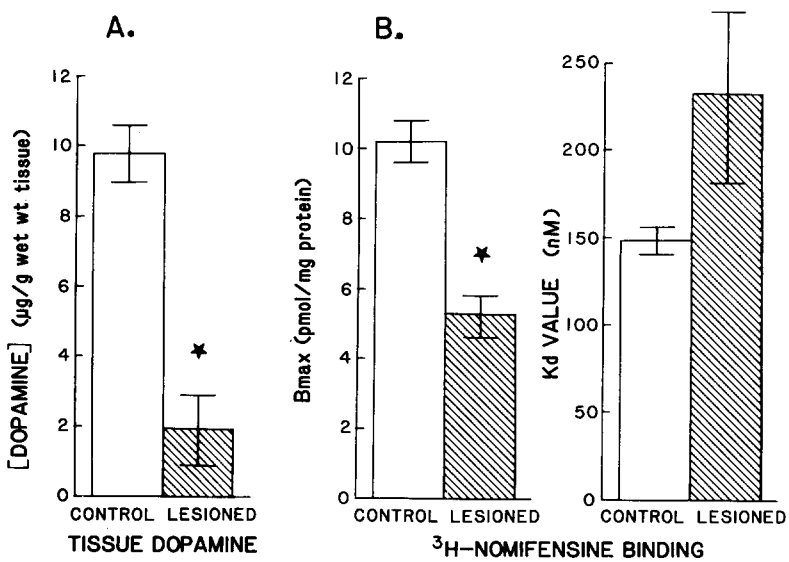

Fig. 6. Effect of 6-hydroxydopamine lesions of rat striatal dopaminergic nerve terminals on [3H] nomifensine binding. A 10 µl volume of 6-hydroxydopamine (100 µg free base) was infused at a rate of 1 µl/min into each lateral ventricle of an anesthetized rat. Control rats received an equivalent infusion of vehicle. Biochemical determinations were performed 16 days later. (A) The dopamine levels in the rat striatum were measured using HPLC with electrochemical detection. (B) The number (B_{max}) and affinity (K_D) of [3H]nomifensine binding sites were determined by Scatchard analysis. Results are the mean values S.E.M. for N = 4–6. An asterisk (*) indicates $P < 0.001$ when compared with controls.

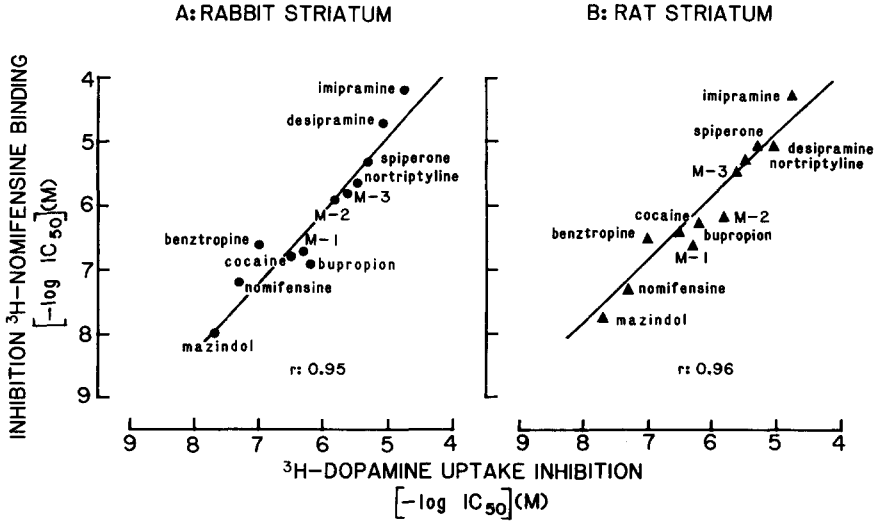

Fig. 7. Correlation between the affinities of compounds to inhibit [^{3}H]nomifensine binding and [^{3}H] dopamine uptake. The K_i values for inhibition of [^{3}H]nomifensine binding in rabbit (A) and rat (B) striatal membranes were taken from Table 1. The IC_{50} values for inhibition of [^{3}H]dopamine uptake into rat striatal synaptosomes were taken from Hyttel [3], with the exception of values for M-1, M-2 and M-3 which were calculated from Tuomisto [18]. Linear regression of the points gave slopes of 1.1 for the rabbit and 1.0 for the rat striatum.

suggests that these binding sites are associated with the dopamine uptake site in striatal membranes.

To study further the possible association between [^{3}H]nomifensine binding sites and the dopamine uptake pump, the number and affinity of [^{3}H]nomifensine binding sites were determined in striatal membranes from rats treated centrally with 6-hydroxydopamine. Sixteen days after a single bilateral intraventricular injection of 6-hydroxydopamine, the levels of dopamine in the striatum were decreased by 80% (Fig. 6). In the lesioned animals, there was a concomitant 48% reduction in the number of [^{3}H]nomifensine binding sites but no significant change in the K_d values (Fig. 6). It must be noted, however, that the higher K_d value and large S.E.M. associated with that value for the lesioned animals was due to one animal in which the apparent affinity was 360 nM.

In support of the possible association between the dopamine uptake pump and [^{3}H]nomifensine binding, highly significant correlations were found between the potency of the drugs shown in Table 1 for inhibition of [^{3}H]nomifensine binding in rabbit (Fig. 7A) or rat striatum (Fig. 7B) with the inhibition of [^{3}H]dopamine uptake into rat striatal synaptosomes as reported in the literature [3, 18].

Regional distribution of specific [^{3}H]nomifensine binding in rat brain. Nomifensine is a potent inhibitor of the high affinity uptake of both dopamine and norepinephrine [3, 14]. While competition curves of desipramine inhibition of [^{3}H]nomifensine binding in striatum showed an affinity of approximately 10 μM for desipramine, those in hippocampus indicated a binding site with an affinity of approximately 4 nM for desipramine. The percentage of total [^{3}H]-nomifensine binding inhibited in the presence of 3 μM desipramine in hippocampal membranes, however, was low. Therefore, specific binding defined as the difference in [^{3}H]nomifensine bound in the absence and presence of 3 μM desipramine constituted only 30% of total binding. To study a possible [^{3}H]nomifensine binding site associated with norepinephrine uptake, specific binding as defined with 3 μM desipramine was examined in a number of different regions. Figure 8 illustrates that, when 3 nM [^{3}H]nomifensine was used, 3 μM desipramine defined less than 40 fmoles/mg protein of specific binding sites in areas that are relatively rich in norepinephrine such as cortex, hippocampus and hypothalamus. Nonetheless, the contribution made

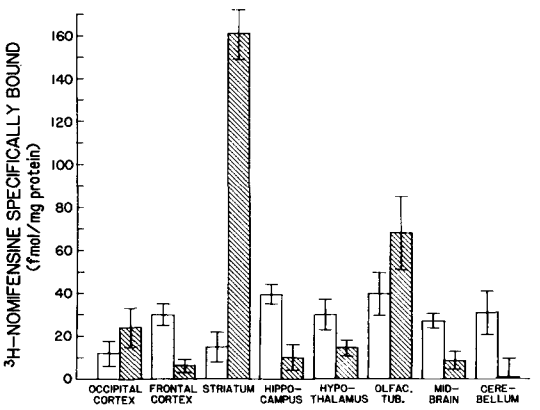

Fig. 8. Regional distribution of [^{3}H]nomifensine binding to membranes from rat brain. Nonspecific binding of [^{3}H]-nomifensine (3 nM) was defined in each area using either 100 μM benztropine or 3 μM desipramine. The open bars (□) represent the difference in binding in the absence and presence of desipramine. The hatched bars (▨) represent the difference in the specific binding defined by benztropine and that defined by desipramine. The values are the mean ± S.E.M. of four separate experiments performed in duplicate.

by these binding sites to those defined with 100 μM benztropine should be accounted for. It should also be noted that 3 μM desipramine will define sites associated with the serotonin uptake pump [25]; however, low concentrations of [^{3}H]nomifensine such as 3 nM used in this study are 300-fold lower than the IC_{50} value for nomifensine inhibition of [^{3}H]-serotonin uptake [3] and would not be expected to label these sites. Taking into consideration the above findings, the sites associated with dopamine uptake were those defined by 100 μM benztropine minus the sites associated with norepinephrine uptake defined by the low concentration of desipramine. The highest density of desipramine defined binding sites labeled by [^{3}H]nomifensine were found in rat hippocampus and olfactory tubercle, while they were significantly lower in other areas investigated (Fig. 8). By far the highest density of [^{3}H]nomifensine binding sites associated with the dopamine uptake pump was found in the striatum and olfactory tubercle of rat (Fig. 8) and rabbit (data not shown). In other areas, namely in occipital and frontal cortex, hippocampus, hypothalamus and midbrain, these binding sites were less than 15% of those in striatum (Fig. 8).

DISCUSSION

The results of the present investigation demonstrate that the antidepressant nomifensine [14, 15], known to inhibit the neuronal uptake of catecholamines, selectively labeled a site associated with the uptake pump for dopamine in brain areas rich in this neurotransmitter. The specific binding of the new radioligand [^{3}H]nomifensine was stable, reversible and saturable. Furthermore, it appeared to label a single site in membranes isolated from rat and rabbit striatum. The affinity constants derived from kinetic analyses (K_d: 30–57 nM) were in fairly good agreement with the K_i values obtained from competition and saturation experiments. The kinetics of binding were simple. Competition curves were monophasic and gave Hill coefficients of one. Scatchard plots from both competition and saturation studies were linear. The number of [^{3}H]nomifensine binding sites found in striatum in either species (B_{max} = 6.5 pmoles/mg protein) was almost identical with the number of sites reported for [^{3}H]mazindol, another radioligand which labels sites associated with the dopamine uptake pump [26].

The pharmacological characteristics of the site labeled by [^{3}H]nomifensine in the rat and rabbit striatum were studied using different agents and were found to be almost identical to the site labeled by [^{3}H] mazindol in rat striatum [26]. Compounds known to inhibit the uptake of [^{3}H]dopamine *in vitro*, such as nomifensine, 4-OH-nomifensine, mazindol, amfonelic acid, and benztropine [2, 3, 18], were the most potent competitors of [^{3}H]nomifensine binding. In contrast, the tricyclic antidepressants desipramine, imipramine and nortriptyline, known to be potent inhibitors of norepinephrine and/or serotonin neuronal uptake [3], were relatively weak inhibitors of both [^{3}H]dopamine uptake and [^{3}H]nomifensine binding. The excellent correlation between inhibition of [^{3}H]nomifensine binding and [^{3}H]dopamine uptake is shown in Fig. 7.

There were, however, certain interesting differences in the drug specificity to inhibit [^{3}H]nomifensine binding and [^{3}H]dopamine uptake (Table 2). Dopamine and other phenylethylamines, such as norepinephrine, amphetamine and tyramine, were substantially weaker in inhibiting [^{3}H]nomifensine binding than in inhibiting [^{3}H]dopamine uptake into the striatum. It is possible that the potent inhibitory effect of these compounds on [^{3}H]dopamine uptake in the striatum is only apparent [20, 22]. Heikkila and colleagues [20, 22] demonstrated that the apparent inhibition of [^{3}H]dopamine uptake by *d*-amphetamine and tyramine in the striatum results from the enhanced release of previously accumulated [^{3}H]-dopamine. Therefore, the apparent K_i values for inhibition of dopamine uptake by dopamine, amphetamine, norepinephrine and tyramine in the striatum appear to be artificially low and cannot be compared with the K_i values for inhibition of [^{3}H]-nomifensine binding. Other investigators have often reported that substrates for uptake systems are poor competitors at binding sites for [^{3}H]imipramine [4], [^{3}H]desipramine [10, 11, 13] and [^{3}H]cocaine [27], which are associated with the serotonin, norepinephrine and dopamine uptake pumps respectively. It can be concluded from these observations that competition studies of radioligand binding to specific uptake sites is a more valid method than accumulation studies to determine the affinities of the uptake pump for indirect releasing agents.

Nomifensine is a more potent inhibitor of the uptake of [^{3}H]norepinephrine than [^{3}H]dopamine into brain synaptosomes [3, 14]; however, the regional distribution of [^{3}H]nomifensine binding indicated preferential binding to brain areas rich in dopamine (i.e. striatum, olfactory tubercle). By contrast, the specific binding of [^{3}H]nomifensine defined with the norepinephrine uptake inhibitor desipramine in brain areas rich in norepinephrine, such as hypothalamus, hippocampus, cortex or midbrain, represented less than 30% of total binding. It is of interest that in the hippocampus the affinity (K_i = 23 nM) of [^{3}H]nomifensine for the binding site defined by desipramine (the putative norepinephrine uptake site) was higher than the affinity (K_i = 70 nM; Table 1) of this radioligand for the striatal dopamine uptake site, as could be expected by its potent effect on norepinephrine uptake [3, 14]. The low level of specific [^{3}H]nomifensine binding defined with desipramine in hypothalamus, hippocampus, cortex and midbrain may be related to the low density of noradrenergic innervation relative to the dopaminergic innervation of the striatum and the olfactory tubercle [28].

Several lines of evidence suggest that the dopamine uptake pump and the [^{3}H]nomifensine binding site might be associated. First, the numbers of [^{3}H]-nomifensine binding sites are highest in areas rich in dopamine nerve terminals, namely the striatum and olfactory tubercle [27]. Moreover, autoradiography of [^{3}H]nomifensine binding sites as defined by 100 μM benztropine in brain slices parallels the regional distribution found in isolated brain membranes [29]. Second, [^{3}H]nomifensine binding to membranes of rat striatum increased in a dose-dependent manner with the NaCl concentration;

little or no binding was observed in the absence of NaCl. The NaCl dependency of [^{3}H]nomifensine binding is consistent with its association with the dopamine uptake pump, since the active uptake of dopamine into nerve terminals requires the presence of NaCl [23, 24] and shows the same concentration dependency as the binding [23]. Third, the number but not the affinity of striatal [^{3}H]nomifensine binding sites was reduced significantly following *in vivo* lesions with 6-hydroxydopamine. The 80% reduction in striatal dopamine content observed in the treated animals suggests that the nigrostriatal dopamine-containing nerve terminals had been disrupted. Similarly, administration of 6-hydroxydopamine has been shown to cause a decrease in the apparent V_{max} for [^{3}H]dopamine uptake into striatal synaptosomes that correlates with the extent of dopamine depletion [30]. The decreased number of binding sites is consistent with but does not prove that [^{3}H]nomifensine binds to sites localized on dopaminergic nerve terminals. Fourth, the absolute potencies of various drugs in competing for [^{3}H]nomifensine binding to rat and rabbit striatal membranes correlate closely with their potencies in inhibiting [^{3}H]dopamine uptake into striatal synaptosomes (Fig. 7) [3, 18]. All these results taken together strongly suggest that [^{3}H]nomifensine binds to a site on dopaminergic nerve terminals associated with the dopamine uptake pump. Whether or not the dopamine uptake pump (transporter) and the [^{3}H]nomifensine binding site are the same or different sites remains to be investigated. It has, in fact, been suggested that [^{3}H]imipramine and [^{3}H]cocaine bind to components of the uptake pumps which are different from the serotonin and dopamine recognition transport sites respectively [25, 27]; however, there are data from [^{3}H]imipramine studies in platelets which support the opposite conclusion [6].

This new radioligand, [^{3}H]nomifensine, may prove to be a useful biochemical tool to label sites associated with the dopamine uptake pump and to study the regulation of these sites (a) after treatment with centrally acting drugs and (b) in postmortem brains from disease states that affect dopaminergic systems. Autoradiographic experiments with [^{3}H]nomifensine indicate binding of this radioligand to presynaptic dopamine uptake sites in postmortem human caudate nucleaus [29]. Moreover, we found that the density of [^{3}H]nomifensine binding sites to the dopamine uptake pump was reduced in postmortem human caudate nucleus obtained from a Parkinsonian patient. Therefore, [^{3}H]nomifensine appears to be a useful radioligand to label sites associated with the dopamine uptake pump both in isolated membranes and slices from the brain.

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