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Structural Characteristics for Inhibition of [^{3}H] Norepinephrine Uptake into Rat Brain Synaptic Vesicles by Beta-Carboline, Indolealkylamine, Phenethylamine and *n*-Alkylamine Derivatives

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SUMMARY

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The abilities of four different classes of monoamines to inhibit uptake of [^{3}H]norepinephrine into rat brain synaptic vesicles were evaluated. Compounds from all four produced competitive inhibition with the following general order of potency: β-carbolines> indolealkylamines> phenalkylamines> *n*-alkylamines. The minimal structural features required for uptake inhibition consisted of an amino nitrogen separated from a hydrophobic moiety by at least a 2-carbon bridge. The most important substituent group was the 5-hydroxyl group for indolealkylamines, the p-hydroxyl group for the phenalkylamines or the 7-methoxyl group for the β-carbolines. Linear alkylamines produced a dual effect, with competitive inhibition of uptake at low concentrations and detergent-like actions predominating with increasing chain length or with high concentrations. Analysis of the details of structure-activity relationships indicates that the characteristic of the amine transport site of brain catecholamine vesicles differs substantially from that of adrenomedullary vesicles.

INTRODUCTION

The catecholamine storage vesicle plays key roles in the synthesis, storage, and secretion of neurotransmitters (1–5). Central to vesicle function is the ability to take up amines, a process which requires ATP and Mg^{2+} and which is inhibited by reserpine (6–8). Because of methodologic difficulties, the properties of the uptake system in vesicles of the central system have been difficult to study directly, and much of the information concerning brain vesicles has been inferred from indirect studies in tissue slices or synaptosomal preparations (9, 10). Historically, the vesicles of the adrenal medulla have served as a model for evaluating the vesicular uptake system, since the organelles can be readily harvested from that tissue (3). Detailed analysis of nucleotide and ionic cofactors for uptake (1, 5) and of structural requirements for uptake (11, 12) or inhibition of uptake (13–15) have appeared for adrenomedullary vesicles. Based on these studies, the effects of a number of drugs that act on or through vesicular catecholamines via the uptake system have been determined. In general, the vesicular uptake system appears to differ substan-

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tially from the catecholamine uptake system of synaptic membranes in cofactor requirements and in substrate specificity (9, 10, 16); adrenomedullary vesicles appear to be very poorly selective for catecholamines (12, 15), while synaptosomal uptake systems have some ability to differentiate among norepinephrine, dopamine and 5-hydroxytryptamine (11, 12).

Methodologic advances over the past decade have made direct studies of the properties of brain vesicles feasible (8, 17–25). In these procedures, while a pure vesicle preparation containing only one transmitter has not been isolated, under the proper incubation conditions uptake of [^{3}H]norepinephrine occurs almost exclusively into the synaptic vesicles present in the preparation; furthermore, although the mixed population of vesicles contains various transmitters, the preparations display a single K_m and behave as though only one vesicle population were present (8, 23, 24). Studies utilizing chemical brain lesions suggest that [^{3}H]norepinephrine uptake occurs almost exclusively into the catecholamine vesicles in the preparation, and that the uptake system is identical in dopaminergic and noradrenergic vesicles (23, 24).

Utilizing the rat brain vesicle preparation, investigations have established similarities in ionic and nucleotide requirements for vesicular uptake, as well as for a number of *in vivo* and *in vitro* drug effects, as compared with adrenomedullary vesicle study results (8, 25–27). These have partially validated the historical use of the adrenal medulla as a model for central catecholamine tissues. In the present study, agreement between the two tissues has been found in establishing four groups of compounds that exhibit affinity for the amine transport site of the vesicular uptake system: β-carbolines, indolealkylamines, phenethylamines and *n*-alkylamines. Despite these agreements between the two tissue models, important differences were found between brain and adrenal in the specific structural characteristics required for affinity within these groups.

METHODS

Preparation of synaptic vesicles. The subcellular fraction containing brain synaptic vesicles was prepared by the differential centrifugation method of Philippu and Beyer (19), as modified by Seidler *et al.* (8). Male Sprague-Dawley rats (Zivic-Miller) weighing 250–350 g were decapitated and brains removed, pooled and homogenized in 4 volumes of 300 mM sucrose containing 25 mM Tris (pH 7.4) and 10 μM iproniazid (irreversible monoamine oxidase inhibitor), using 5 up-down strokes in a Duall ground-glass homogenizer. After discarding the 1000 × *g* and 20,000 × *g* pellets, the 100,000 × *g* pellet was resuspended in 130 mM potassium phosphate (pH 7.4) and used for uptake determinations as described previously (8). Although the microsomal fraction utilized here contains many particles and organelles, uptake of amines *in vitro* occurs primarily into the catecholamine synaptic vesicles present in the preparation (23–25).

Determinations of norepinephrine uptake. For measurements of norepinephrine uptake, incubations contained 0.67 ml of the vesicle preparation (corresponding to 133 mg of original wet weight of brain tissue) and final concentrations of 1 mM ATP-Mg^{2+}, 0.1 μM [^{3}H]norepinephrine, 2 μM ascorbic acid and 1.25 μM iproniazid in phosphate buffer in a final volume of 1.7 ml. Incubations lasted 5 min at 30°, with duplicates kept on ice to serve as blanks. Labeled vesicles were trapped by vacuum filtration on cellulose acetate filters, washed and radioactivity counted as described in detail previously (8).

Inhibition of uptake by various drugs was evaluated by inclusion of the inhibitors in the incubation medium. Dose-effect comparisons were made at a single norepinephrine concentration (0.1 μM) with varying inhibitor concentrations, and also by Lineweaver-Burk plots in which norepinephrine concentrations ranged from 0.06–0.8 μM in the presence or absence of a fixed concentration of inhibitor.

In studies in which NEM[2] was used, the vesicles were first preincubated with NEM alone for 3 min at 30° prior to addition of ATP-Mg^{2+} and [^{3}H]norepinephrine; uptake was allowed to proceed for 5 min after the

[2] The abbreviations used are: NEM, *n*-ethylmaleimide;

latter additions. In these cases, control preparations also were preincubated, but NEM was not added. The preincubation period was necessary because ATP interferes with the chemical reaction between NEM and transport ATPases (25, 28–30). The preincubation period itself alters neither the affinity nor capacity of the vesicular uptake system (25).

Determinations of norepinephrine release. In some cases, to evaluate whether an inhibitor could cause release of norepinephrine from the vesicles, drugs were added to the uptake incubation after 3 min; the incubation was continued for an additional 2 or 4 min. This enabled differentiation of the uptake inhibition caused by interference with inward norepinephrine transport from that caused by release of norepinephrine; in the former case, uptake ceases at the 3 min point without loss of the label already incorporated, while drug-induced release of norepinephrine produces loss of the label already incorporated (8, 27). It should be noted that the [^{3}H]norepinephrine is present in the medium during the release determination; since efflux from intact vesicles will occur passively, the values obtained in this fashion represent minimum rates of release. However, this technique can readily distinguish release due to damage or lysis of the vesicles (detergent-like actions), which is not affected by norepinephrine in the medium (8, 27).

Statistics. Results are reported as means ± standard errors with comparisons of the effects of inhibitors done by the two-tailed Student's *t*-test. Straight lines in the Lineweaver-Burk plots are drawn by least-squares analysis and intercepts compared by the *t*-test.

Materials. *l*-7-[^{3}H]Norepinephrine (2.20 Ci/mmol) was obtained from New England Nuclear Corp. Reserpine phosphate was provided by Ciba Pharmaceuticals. The following were obtained from Sigma Chemical Corp: *N*-ethylmaleimide, *l*-norepinephrine HCl, 5-hydroxytryptamine creatinine sulfate complex, *β*-phenethylamine, 5-methoxytyramine HCl, 3,4-dimethoxyphenethylamine, tryptamine HCl, 5-methyltryptamine HCl, 6-hydroxytryptamine creatinine sulfate complex, *N*-methyltryptamine, 5,6-dihydroxytryptamine creatinine sulfate complex, 5-hydroxytryptophan, tyramine HCl, 5-hydroxydopamine HCl, 3-methoxytyramine HCl, *dl*-normetanephrine HCl, *dl*-metanephrine HCl, *d*-amphetamine sulfate, *dl*-dopa, *dl*-octopamine HCl, *l*-isoproterenol HCl, cyclohexylamine, harmine HCl, harman HCl, 6-methoxyharman, yohimbine HCl and iproniazid phosphate. Aldrich Chemicals was the source for: 6-hydroxydopamine HBr, 2-(p-chlorophenyl)-ethylamine, *p*-aminophenol, 3-chloro-1propylamine HCl, 4-phenylbutylamine, 3-phenylpropylamine, salsolinol HBr, benzylamine, norharman, gramine, harmol HCl, harmaline HCl, 6-methoxyindole, *n*-butanol, ethylamine, *n*-propylamine, *n*-butylamine, *n*-amylamine, *n*-hexylamine, *n*-heptylamine, *n*-octylamine, *n*-nonylamine and *n*-decylamine. 2-Methylharmine and *dl*-norepinephrine were purchased from Regis Chemical Co., dopamine HCl from Calbiochem, absolute ethanol from U.S. Industrial Chemicals, *l*-epinephrine bitartrate from Winthrop Laboratories, *l*-metaraminol bitartrate from Merck Sharp & Dohme, pyridine from Matheson Coleman & Bell Chemicals, aniline from J.T. Baker Chemicals, ATP from P-L Biochemicals and apomorphine from S.B. Penick and Co.

RESULTS

Compounds from all four classes were able to inhibit uptake of norepinephrine into the brain vesicle preparation, and a wide range of relative activity could be obtained within each group (Fig. 1). Reserpine was by far the most potent inhibitor, with an IC_{50} of about 3 nM. Other *β*-carboline derivatives gave dose-response curves parallel to that of reserpine, with IC_{50}'s in the submicromolar range. Next in potency were the indolealkylamines; the most effective was 5-hydroxytryptamine (IC_{50} = 0.1 μM), with compounds such as gramine or 5-methoxytryptamine also giving parallel curves displaced several orders of magnitude toward higher concentrations. Dopamine and norepinephrine, the natural substrates for the catecholamine synaptic vesicles, were less potent than 5-hydroxytryptamine (IC_{50}'s of approximately 0.5 μM) but were more potent than their parent com-

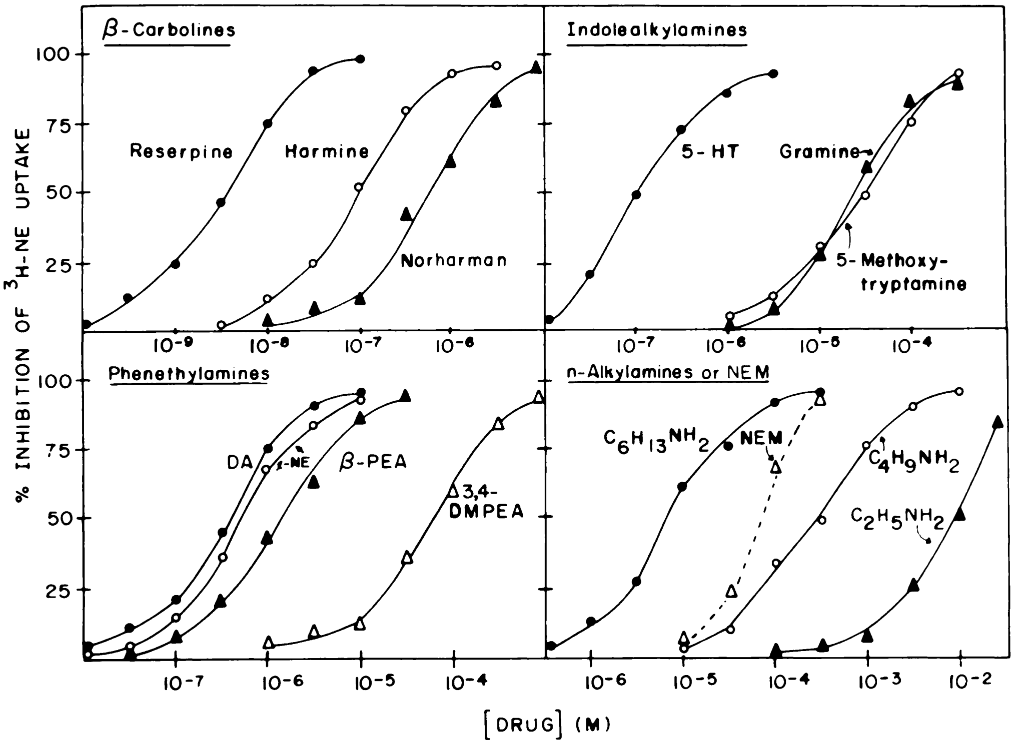


FIG. 1. *Inhibition of vesicular norepinephrine uptake by compounds from different classes of monoamines*
Points represent means of 4–12 individual determinations; standard errors were generally less than 5%. [^{3}H]Norepinephrine in the incubation medium was 0.1 μM, and uptake in the absence of inhibitors averaged 11.2 pmols/g original wet weight of tissue. The abscissa is logarithmic. Abbreviations: NE = norepinephrine; 5-HT = 5-hydroxytryptamine; DA = dopamine; β-PEA = β-phenethylamine; 3,4-DMPEA = 3,4-dimethoxyphenethylamine; NEM = N-ethylmaleimide.

pound, β-phenethylamine, or than the methoxy-substituted derivative, 3,4-dimethoxyphenethylamine. Inhibition could be obtained even with such simple primary amine structures as *n*-alkylamines, although these were less potent than the other classes of compounds. Within the alkylamine group, *n*-hexylamine was more potent than *n*-butylamine, and ethylamine was the least potent. In general, the dose-response curves for the β-carbolines, indolealkylamines, phenethylamines and *n*-alkylamines were all parallel to each other, requiring approximately one order of magnitude increase in drug concentration to go from 25% to 75% inhibition. On the other hand, the curve for inhibition by NEM was much steeper, with only a 4-fold difference in concentration between 25% and 75% inhibition.

Kinetic determinations of selected high-potency and low-potency compounds within each class indicated a competitive mechanism of action, with a shift in the K_m for norepinephrine without alterations in maximal uptake (Fig. 2 and Table 1). Again, the notable exception was NEM, which yielded noncompetitive kinetics.

Structure-activity relationships for β-carbolines. The structures of the β-carboline derivatives used in this study appear in Fig. 3. Of the compounds with minor substitution on the β-carboline nucleus, 2-methylharmine, harmaline and harmine were the most effective inhibitors of norepinephrine uptake (Table 2), with K_i's for the uptake system well below the K_m for norepinephrine (Table 1). Replacement of the 7-methoxyl group with an hydroxyl group (harmol) or with hydrogen (harman)

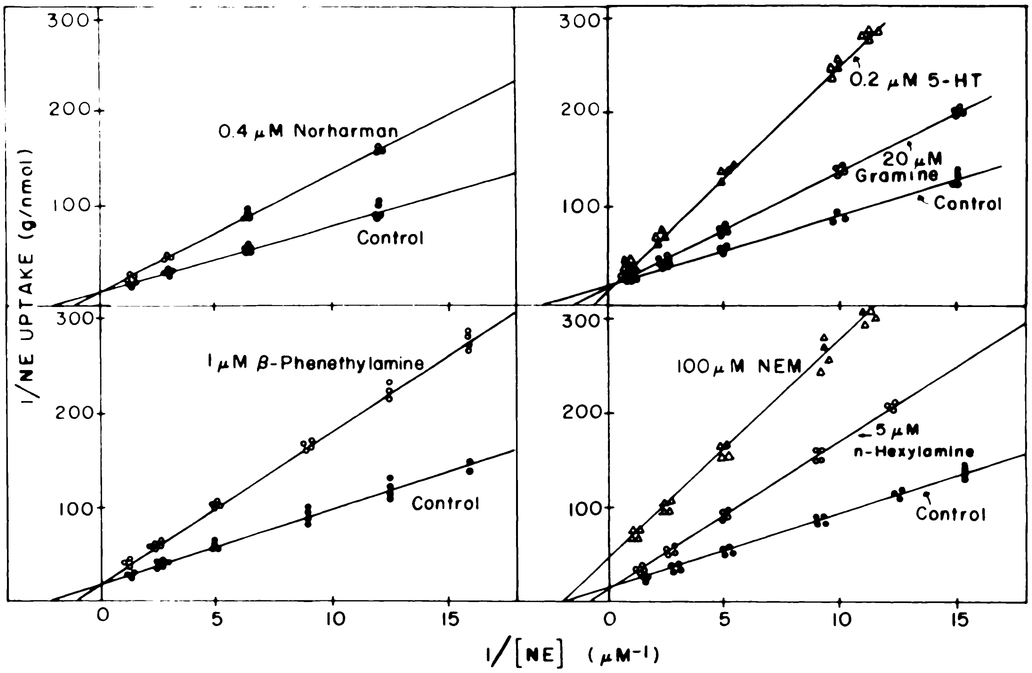


FIG. 2. *Examples of Lineweaver-Burk plots of inhibition of uptake by selected amines*

Complete data for all inhibitors appear in Table 1. Points represent individual determinations. With the exception of *N*-ethylmaleimide (NEM) all intercepts on the abscissa are significantly different from controls ($p < 0.005$ or $p < 0.001$) while intercepts on the ordinate are not significantly different. For NEM, intercept on the ordinate is significantly different from control and intercept on the abscissa is not.

reduced the inhibitory effect and an even greater loss of activity was produced by shifting the methoxyl to the 6-position (6-methoxyharman) (Table 2). The unsubstituted β-carboline nucleus (norharman), although one order of magnitude lower in potency than harmine, was still a competitive inhibitor with an affinity for the uptake system similar to that of the natural catecholamine substrates (Table 1). Elimination of the pyrido-ring (6-methoxyindole) destroyed the ability to inhibit uptake, while reserpine, with its extensive substitution on the pyrido-ring, demonstrated the highest potency of all (Table 2). However, yohimbine, which also has large substituent groups on the pyrido-ring, was totally ineffective.

Structure-activity relationships for indolealkylamine derivatives. Tryptamine was able to inhibit vesicular norepinephrine uptake, but was far less potent than 5-hydroxytryptamine (Table 3). There was a loss of effect when the 5-hydroxyl group was replaced by a methoxyl or methyl-substituent, or when the hydroxyl group was moved to the 6-position. Dihydroxyl substitution at the 5 and 6-positions resulted in inhibition equivalent to that obtained with just the 5-hydroxyl group. Alterations in the side chain also influenced activity of the indolealkylamines: *N*-methyltryptamine was slightly better than tryptamine as an inhibitor but 5-hydroxytryptophan, with an α-carboxyl substituent, was totally ineffective (Table 3); only slight inhibition could be obtained with 5-hydroxtryptophan, even at a concentration of 1 mM (data not shown). Although shortening the side chain by one carbon (gramine) reduced the potency of inhibition, gramine still possessed competitive inhibitory characteristics (Fig. 2, Table 1).

Structure-activity relationship for phenethylamine derivatives. The unsubstituted β-phenethylamine nucleus was sufficient to produce inhibition of norepinephrine uptake, and the inhibition was competitive

(Figs. 1 and 2). Activity was enhanced by substitution of either an hydroxyl group (tyramine) or chlorine (*p*-chlorophenethylamine) in the para-position (Table 4). Hydroxyl substitution in the meta-position also increased inhibitory capabilities, but not as dramatically as did para-substitution (dopamine *vs.* tyramine, metaraminol *vs.* amphetamine). Insertion of a third ring hydroxyl group in either the meta- or ortho-positions (5-hydroxydopamine, 6-hydroxydopamine) appeared to reduce inhibition relative to dopamine, but partial oxidation of these compounds during the incubation may have contributed to the loss of activity, particularly for 6-hydroxydopamine (which discolored even in the presence of ascorbic acid). Replacement of the ring hydroxyl groups with methoxyl groups markedly reduced the inhibitory potency: at equivalent concentrations, 3-methoxytyramine, normetanephrine and metanephrine were all poorer inhibitors than were dopamine, norepinephrine and epinephrine, respectively. Replacement of both hydroxyls with methoxyls resulted in further loss of potency (3,4-dimethoxyphenethylamine).

The hydroxyl group in the β-position on the side chain appeared to play a lesser role than did the ring hydroxyls (Table 4). Octopamine produced only slightly less inhibition than equivalent concentrations of tyramine (correcting for 50% *d*-isomer for octopamine) and norepinephrine was somewhat less than dopamine (Fig. 1, Table 2, Table 4). The stereochemistry at the β-position did play a critical role in the activity, however; *l*-norepinephrine produced greater inhibition than *dl*-norepinephrine (Table 4), and calculations of the K_i indicate that all the inhibition caused by *dl*-norepinephrine could be accounted for by

TABLE 1

Kinetics of inhibition of [^{3}H]NE uptake by other amines

Kinetic parameters are calculated from least squares determinations of double-reciprocal plots. K_i (competitive) is calculated from the equation $K_i = K_m I/(K'_m - K_m)$, where K_i is the Michaelis constant for the inhibitor, K_m is the Michaelis constant for *l*-norepinephrine, K'_m is the Michaelis constant for *l*-norepinephrine in the presence of inhibitor and I is the inhibitor concentration. For noncompetitive inhibition, $K_i = U_{max} I/(U_{max} - U'_{max})$ where U'_{max} and U_{max} are maximal uptake in the presence and absence of inhibitor, respectively. With NEM only, samples were preincubated for 3 min at 30° with NEM prior to addition of ATP-Mg^{2+} and [^{3}H]norepinephrine. Asterisks denote significant differences from controls ($p < 0.005$ or $p < 0.001$).

Amine	K_m or K'_m for NE	U_{max} or U'_{max} for NE	# of uptake points in determination	Type of inhibition	K_i for inhibitor
	(nM)	*(pmols/g)*			*(nM)*
Control	568 ± 34	81 ± 5	66	——	——
Norharman (0.4 μM)	1005 ± 79*	79 ± 9	13	Competitive	520 ± 42
Harmine (0.07 μM)	1243 ± 211*	80 ± 13	24	Competitive	59 ± 9
Reserpine (0.0041 μM)	1335 ± 80*	77 ± 5	25	Competitive	3.0 ± 0.4
Control	442 ± 21	55 ± 8	102	——	——
β-Phenethylamine (1 μM)	940 ± 94*	59 ± 6	22	Competitive	888 ± 90
Tyramine (0.8 μM)	1636 ± 115*	66 ± 4	19	Competitive	296 ± 79
Dopamine (0.3 μM)	1034 ± 120*	65 ± 8	17	Competitive	224 ± 25
l-Epinephrine (0.3 μM)	1026 ± 140*	65 ± 7	24	Competitive	227 ± 30
l-Metaraminol (0.3 μM)	651 ± 65*	57 ± 6	16	Competitive	634 ± 64
Control	499 ± 40	63 ± 5	92	——	——
n-Hexylamine (5 μM)	1065 ± 70*	67 ± 5	19	Competitive	4410 ± 310
5-Hydroxytryptamine (0.2 μM)	1800 ± 360*	73 ± 12	20	Competitive	77 ± 12
Gramine (20 μM)	1199 ± 216*	79 ± 16	24	Competitive	14,300 ± 2900
N-ethylmaleimide (100 μM)	447 ± 97	19 ± 4*	24	Non-competitive	140,000 ± 30,000

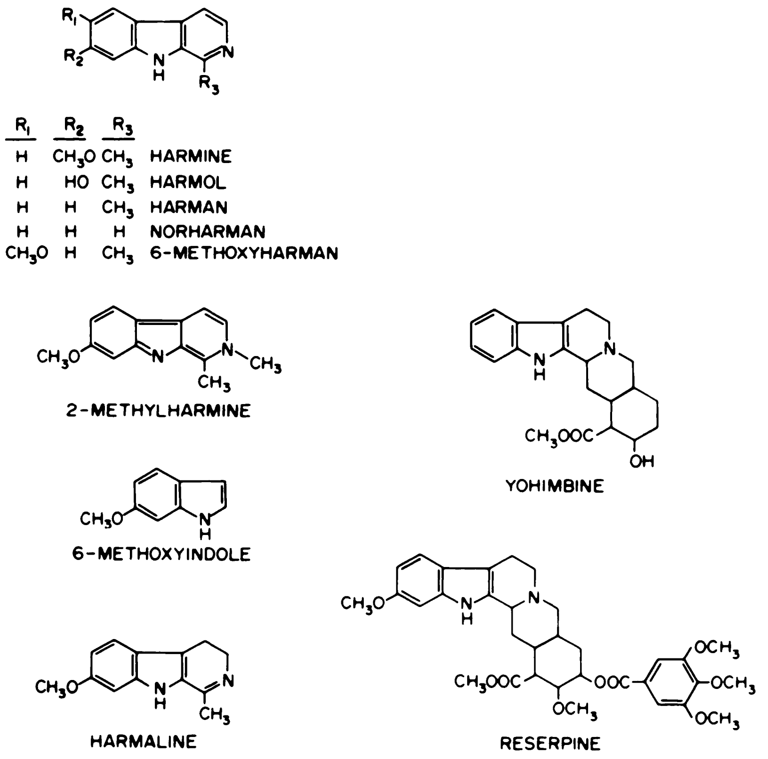


FIG. 3. *Structures of β-carboline derivatives*

TABLE 2

Inhibition of [^3H]NE uptake by β-carboline derivatives

Control uptake was 9.27 ± 0.20 pmols/g (30 determinations). Values represent means ± standard errors of the number of determinations in parentheses.

Drug	Percentage inhibition	
	0.1 μM	*1 μM*
Norharman	15 ± 1 (6)	61 ± 2 (6)
Harman	13 ± 3 (6)	72 ± 1 (6)
Harmol	20 ± 5 (6)	73 ± 1 (6)
Harmine	55 ± 3 (18)	91 ± 1 (12)
Harmaline	70 ± 5 (6)	—
6-Methoxyharman	3 ± 5 (6)	31 ± 3 (6)
6-Methoxyindole	−1 ± 1 (6)	6 ± 2 (6)
2-Methylharmine	83 ± 1 (6)	—
Reserpine	95 ± 2 (6)	—
Yohimbine	−6 ± 6 (6)	5 ± 3 (6)

the *l*-isomer. In the α-position on the side chain, substitution of a small, neutral group (methyl) did not influence activity of the compound, as amphetamine and β-phenethylamine were equipotent. However, addition of an α-carboxyl-group (dopa) resulted in total loss of activity. On the side-chain nitrogen, methyl substitution may have enhanced potency slightly (epinephrine vs. norepinephrine, Tables 2 and 4), but an isopropyl group decreased the inhibitory effect (isoproterenol, Table 4). More extensive changes in side-chain substitution or conformation also resulted in loss of activity (apomorphine, salsolinol).

Shortening the 2-carbon side chain resulted in complete loss of activity (Fig. 4), but lengthening by 1 or 2 additional carbons produced only a partial loss.

Structure-activity relationships for n-alkylamines. To determine the minimal structure required for inhibition of norepinephrine uptake, studies were undertaken with simple unsubstituted *n*-alkylamines. All the *n*-alkylamines tested could reduce norepinephrine uptake, and even ethylamine, although of very low potency, was effective (Fig. 1). The relationship of effect to chain length was biphasic (Fig. 5); *n*-hexylamine produced greater inhibition than either *n*-amylamine or *n*-heptylamine, but *n*-octylamine, *n*-nonylamine and *n*-decylamine were more effective than *n*-hexylamine. Addition of a chlorine to the ω-carbon (3-chloro-n-propylamine) did not affect the inhibition, and cyclization of the side chain reduced the inhibition (cyclohexylamine). Replacement of the amino-nitrogen with oxygen (to make corresponding alcohols) resulted in loss of activity (Table 5). Because alkylamines possess detergent-like properties (due to the combination of a polar nitrogen and a hydrophobic chain) it was important to establish whether the observed inhibition of norepinephrine uptake did indeed represent an effect on the transport system, or instead reflected outward leakage of label or lysis of the vesicles. To test these possibilities, norepinephrine uptake was allowed to proceed in the absence of inhibitors for 3 min and then the inhibitors were added and subsequent release of label evaluated. Previous studies with "true" uptake competitors such as the β-carbolines, indolealkylamines and β-phenethylamines have shown that addition of these agents prevents any further uptake from occurring without causing loss of the norepinephrine already taken up (8, 27), while detergents cause a loss of the amine already incorporated (8). The results obtained with the *n*-alkylamines indicated both inhibitor and detergent capabilities (Fig. 6). Low concentrations tended to cause inhibition of uptake without loss of incorporated label, while higher concentrations produced a definite reduction relative to the 3 min point. At low concentrations, inhibition of uptake by *n*-hexylamine appeared to be purely competitive (Fig. 2, Table 1), a kinetic characteristic that was lost at higher concentrations (data not shown).

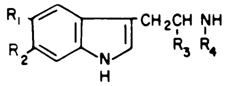
DISCUSSION

In broad terms, the rank order of activity of the four classes of uptake inhibitors in brain synaptic vesicles is comparable to results for adrenomedullary catecholamine vesicles reported previously (13–15). In both, the most potent compounds are from the β-carbolines followed in order by the indolealkylamines, phenethylamines and *n*-alkylamines, and all these agents are competitive inhibitors. Additionally, NEM pro-

TABLE 3

Inhibition of [^3H]NE uptake by indolealkylamine derivatives

Control uptake was 11.9 ± 0.3 pmols/g (18 determinations). Values represent means ± standard errors of the number of determinations in parentheses. For 5,6-dihydroxytryptamine, 1 mM ascorbate (which does not itself influence norepinephrine uptake) was added to retard oxidation of the drug.



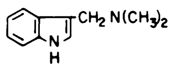
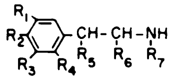
R₁	R₂	R₃	R₄	Drug	Percentage inhibition		
					(0.2 μM)	(2 μM)	(20 μM)
a) *Ring substituents*							
H	H	H	H	Tryptamine	11 ± 3 (6)	34 ± 1 (12)	——
OH	H	H	H	5-Hydroxytryptamine	59 ± 3 (18)	87 ± 1 (12)	——
OCH_3	H	H	H	5-Methoxytryptamine	5 ± 1 (6)	8 ± 3 (6)	40 ± 4 (4)
CH_3	H	H	H	5-Methyltryptamine	5 ± 1 (6)	16 ± 1 (6)	——
H	OH	H	H	6-Hydroxytryptamine	21 ± 2 (6)	——	——
OH	OH	H	H	5,6-Dihydroxytryptamine	61 ± 2 (12)	86 ± 1 (6)	——
b) *Side chain:*							
H	H	H	CH_3	*N*-Methyltryptamine	12 ± 1 (6)	47 ± 1 (6)	——
OH	H	COOH	H	5-Hydroxytryptophan	——	−1 ± 1 (6)	−3 ± 3 (6)
 CH2 N(CH3)2 N H				Gramine	——	10 ± 3 (6)	55 ± 3 (10)

TABLE 4

Inhibition of $[^3H]$NE uptake by phenethylamine derivatives

Control uptake was 8.27 ± 0.09 pmols/g (144 determinations). Values represent means ± standard errors of the number of determinations in parentheses. For 5-hydroxydopamine and 6-hydroxydopamine, 1 mM ascorbate was added to retard oxidation of the drug.



R_1	R_2	R_3	R_4	R_5	R_6	R_7	Drug	Percentage inhibition	
								(0.3 μM)	(0.6 μM)
a) *Ring substituents:*									
H	H	H	H	H	H	H	*β*-phenethylamine	18 ± 3 (6)	29 ± 4 (18)
H	OH	H	H	H	H	H	Tyramine	28 ± 2 (6)	50 ± 3 (19)
OH	OH	H	H	H	H	H	Dopamine	49 ± 1 (6)	60 ± 4 (20)
OH	OH	OH	H	H	H	H	5-Hydroxydopamine	8 ± 2 (6)	40 ± 2 (6)
OH	OH	H	OH	H	H	H	6-Hydroxydopamine	−5 ± 3 (6)	11 ± 1 (6)
H	Cl	H	H	H	H	H	*p*-Chlorophenethylamine	35 ± 2 (12)	—
								(2 μM)	(20 μM)
OH	OH	H	H	H	H	H	Dopamine	82 ± 1 (4)	98 ± 2 (4)
OCH_3	OH	H	H	H	H	H	3-Methoxytyramine	9 ± 4 (4)	49 ± 3 (6)
OCH_3	OCH_3	H	H	H	H	H	3,4-Dimethoxyphenethylamine	5 ± 3 (5)	28 ± 3 (5)
								(4 μM)	(40 μM)
OH	OH	H	H	OH	H	H	*dl*-Norepinephrine	80 ± 4 (5)	89 ± 2 (6)
OCH_3	OH	H	H	OH	H	H	*dl*-Normetanephrine	7 ± 3 (4)	43 ± 1 (6)
OCH_3	OH	H	H	OH	H	CH_3	*dl*-Metanephrine	7 ± 3 (4)	42 ± 2 (6)
								(4 μM)	(8 μM)
b) *Side Chain substituents:*									
H	H	H	H	H	H	H	*β*-Phenethylamine	58 ± 1 (6)	—
H	H	H	H	H	CH_3	H	*d*-Amphetamine	55 ± 2 (5)	—
OH	OH	H	H	H	H	H	Dopamine	85 ± 1 (6)	—
OH	OH	H	H	H	COOH	H	*dl*-Dopa	—	6 ± 7 (6)
								(0.6 μM)	(1.2 μM)
OH	OH	H	H	OH	H	H	*l*-Norepinephrine	47 ± 3 (11)	74 ± 3 (4)
OH	OH	H	H	OH	H	H	*dl*-Norepinephrine	26 ± 5 (11)	50 ± 1 (4)
OH	OH	H	H	H	H	H	Dopamine	60 ± 3 (6)	79 ± 2 (4)
H	OH	H	H	H	H	H	Tyramine	47 ± 2 (6)	—
H	OH	H	H	OH	H	H	*dl*-Octopamine	—	38 ± 1 (6)
OH	OH	H	H	OH	H	CH_3	*l*-Epinephrine	53 ± 3 (5)	—
OH	OH	H	H	OH	H	C_3H_7	*l*-Isoproterenol	5 ± 3 (5)	—
c) Other:									
Apomorphine								0 ± 2 (5)	—
Salsolinol								7 ± 2 (6)	—

vides noncompetitive inhibition through interference with ATP utilization for brain as well as adrenal vesicles (15, 25). However, details of the structure-activity relationships within each class of inhibitors, particularly for the indolealkylamines and phenethylamines, indicate that there are important differences in the characteristics of the transport system in vesicles between brain and adrenal medulla.

The indolealkylamines displayed a generally higher affinity for the vesicular up-

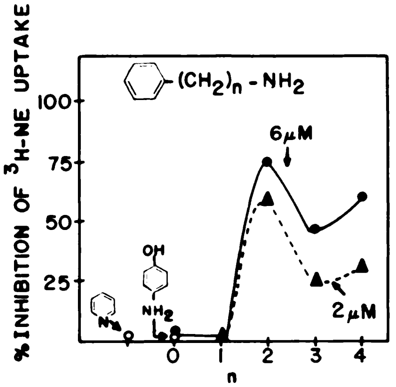


FIG. 4. *Effect of side chain length on inhibition of vesicular norepinephrine uptake by phenalkylamines*

Points represent means of 5–12 determinations at inhibitor concentrations of 2 μM (triangles) or 6 μM (circles). Standard errors were generally less than 5%. [^{3}H]Norepinephrine in the incubation medium was 0.1 μM. Control uptake averaged 10.8 pmols/g.

take site than the corresponding phenethylamines, a property typical of catecholamine vesicles from brain, heart, and adrenal medulla (8, 11, 12, 15, 24, 27). In the present study with rat brain synaptic vesicles, the structure-activity relationships for this series of compounds closely paralleled that of the phenethylamines: 1) affinity for the uptake system was markedly enhanced by hydroxylation at the 5-position (analogous to para-hydroxylation for the phenethylamines); 2) affinity was not enhanced by hydroxylation at the 6-position (meta-hydroxylation for phenethylamines); and 3) replacement of ring hydroxyls with methoxyls reduced the potency in both the indole and phenyl series. In contrast, the structure-activity relationships for these two classes of compounds in adrenomedullary vesicles are so dissimilar that it appears that the indolealkylamines and phenethylamines share only the binding site for the amino-nitrogen (15).

For the phenyl derivatives, unsubstituted β-phenethylamine provides the minimal structure for inhibition of norepinephrine uptake in both adrenomedullary vesicles (12, 15) and brain vesicles, as shortening the side-chain by one carbon results in marked loss of activity. This result, coupled with earlier reports (13–15) and with the competitive inhibition caused by low concentrations on *n*-hexylamine, suggests that the brain and adrenal vesicular transport systems possess an amino-nitrogen binding site and a hydrophobic site separated by the distance of a two carbon bridge. Beyond this point, there is little resemblance between the vesicles from the two tissues (1, 12, 15): 1. While in both preparations, potency is enhanced by para-hydroxylation, replacement of either meta- or para-hydroxyls with methoxyls results in marked loss of potency in the brain vesicles but not in the adrenal vesicles. 2. Proper stereochemistry of the β-position of the side chain is critical for activity in the brain vesicles, but is less so in the adrenal. 3. α-Methylation enhances the capability for inhibition

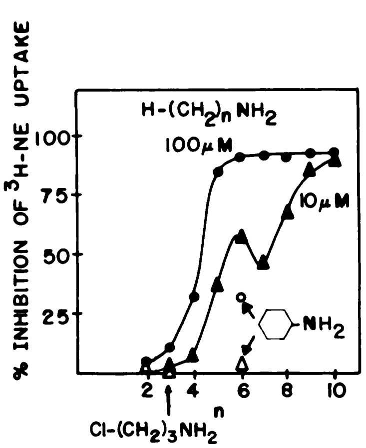


FIG. 5. *Effect of chain length on inhibition of vesicular norepinephrine uptake by n-alkylamines*

Points represent means of 5–12 determinations at inhibitor concentration of 10 μM (triangles) or 100 μM (circles). Standard errors were generally less than 5%. [^{3}H]Norepinephrine in the incubation medium was 0.1 μM. Control uptake averaged 8.6 pmols/g.

TABLE 5

Comparison of effects of alkylamines and alcohols on [^{3}H]NE uptake

Control uptake was 12.1 ± 0.6 pmols/g (11 determinations). Values represent means ± standard errors of the number of determinations in parentheses.

Drug	Concentration	Percentage inhibition
Ethylamine	30 mM	83 ± 5 (6)
Ethanol	30 mM	12 ± 6 (6)
n-Butylamine	3 mM	88 ± 4 (5)
n-Butanol	3 mM	10 ± 5 (5)

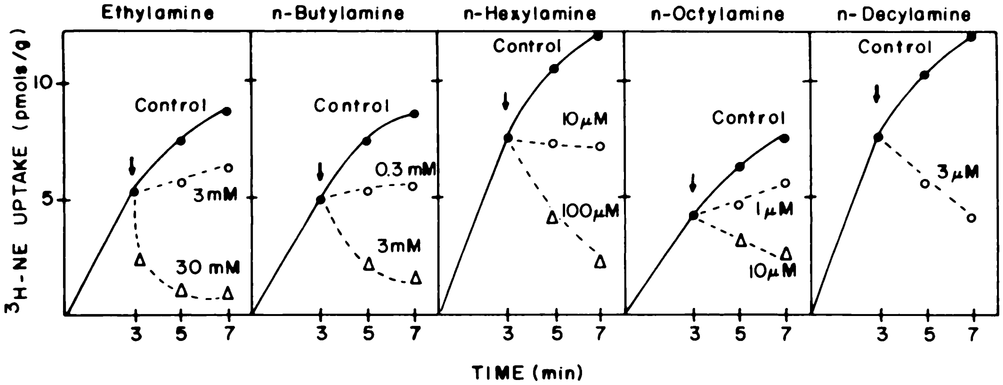


FIG. 6. *Termination of [^{3}H]norepinephrine uptake and release of [^{3}H]norepinephrine by subsequent addition of n-alkylamines*

Points represent means of 3–6 determinations; standard errors were generally less than 5%. [^{3}H]Norepinephrine in the incubation medium was 0.1 µM. Alkylamines were added after 3 min of incubation (arrow).

in the adrenal but not in the brain. 4. Substitution of an isopropyl group on the nitrogen does not influence activity in the adrenal but markedly hinders the inhibitory effect in the brain. 5. Extensive substitutions involving the side chain (apomorphine) or condensation of the side chain (salsolinol) both markedly reduce inhibition in the brain but do not have a major influence in the adrenal. (Apomorphine in higher concentrations does appear to inhibit vesicular uptake in synaptosomal preparations [31]). 6. Lengthening the ethylamine side chain reduces inhibition in brain vesicles but enhances inhibition in adrenal vesicles.

Thus, for phenethylamines, the brain synaptic vesicle uptake system requires more strict adherence to structures resembling the natural catecholamine substrates than does the uptake system of adrenomedullary vesicles.

The inhibitory activity of *n*-alkylamines appears to be a composite of two features. The competitive effect of these compounds is probably explained by their possessing the minimal criteria for inhibition discussed above, *viz.* an amino-nitrogen separated from a hydrophobic moiety by at least two carbons. While the competitive uptake inhibition can be observed at low concentrations of alkylamines, concentrations that totally inhibit norepinephrine uptake produce a detergent-like effect. This becomes more prevalent as the alkyl chain is lengthened: 30 mM ethylamine is required to release the norepinephrine already taken up, while a similar effect can be seen at 100 µM *n*-hexylamine or 3 µM *n*-decylamine. The dual effect probably accounts for the biphasic relationship between chain length and inhibition seen in Figure 5.

In contrast to the indolealkylamines and phenalkylamines, there is excellent agreement between data for effects of β-carbolines in adrenomedullary (13, 14) and brain vesicles. In both, the most important substituent is the methoxyl group in the 7-position, which cannot be shifted or replaced without substantial loss of potency. The lack of correspondence between effects of substituents on the indolealkylamines and the β-carbolines has been noted previously (14, 15). The relationship between reserpine and yohimbine is also the same in brain and adrenal; yohimbine, which has a different steric configuration from reserpine in the noncarboline portion of the molecule, is ineffective as an uptake inhibitor, while reserpine is the most potent inhibitor of all. These and earlier data (13, 14) indicate that an additional binding site may be involved for the noncarboline portion of reserpine.

In conclusion, the transport site of catecholamine vesicles from rat brain appears to be more primitive than that of synaptic membranes in that it is far less selective and displays affinity for a wide variety of compounds of different structure. The minimal structural features required for inhi-

bition of norepinephrine uptake consist of an amino-nitrogen with a two-carbon bridge to an hydrophobic group; the most important substituent is the para-hydroxyl group. While brain vesicles and adrenomedullary vesicles share a number of features pertaining to uptake cofactors and classes of inhibitory agents, the characteristics of the catecholamine transport site are different, with the brain preparation requiring a greater degree of resemblance to the natural catecholamine substrates.

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