Biochemical Pharmacology, Vol. 20, pp. 1769–1780. Pergamon Press, 1971. Printed in Great Britain

COMPOUNDS ANTAGONISTIC TO NOREPINEPHRINE RETENTION BY RAT BRAIN HOMOGENATES*

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(*Received* 7 *August* 1970; *accepted* 9 *October* 1970)

Abstract—Several compounds, either catechols, or amines with a phenyl-alkyl configuration or an approximation of it, diminished the ability of rat brain homogenates to retain *in vitro*, [^{3}H]norepinephrine accumulated in brain tissues *in vivo* following intracisternal injection. Among the active compounds were several phenethylamine derivatives, including β-phenethylamine, β-phenylthanolamine, amphetamine, *p*-tyramine, and octopamine: compounds which are indirectly acting amines or putative false transmitters. Presence of a β-hydroxyl group may increase activity somewhat. An aliphatic side chain of 2 or 3 carbons was important for activity of aromatic amines. Also active were serotonin, several catecholamines, and 6-hydroxy-dopamine, as well as the catechol amino acid, L-DOPA. Deaminated catechols, including dihydroxymandelic acid and dihydroxyphenylacetic acid were active at higher concentrations only. Catechols were inactivated by *O*-methylation. Certain non-aromatic cyclic amines, including amantadine, were active. Aliphatic amines and Ω-hydroxyl-amines of appropriate carbon chain length had some activity. Acetylcholine (with eserine) was inactive. That the active compounds may have significant central nervous system actions based partially on their ability to interfere with catecholamine re-uptake or storage, is suggested.

A RELATIVELY large amount of work has been done to define structural characteristics of accumulation, binding and retention of catecholamines in tissues innervated by the peripheral sympathetic nervous system.[1,2] The pharmacology of many compounds which interfere with this retention has also been well studied.[3,4] Furthermore, it has been demonstrated that certain endogenous and exogenous amines may act as false neurotransmitters by competing for noradrenergic binding sites, being released by nerve stimulation and having weak post-synaptic effects.[5] There has been less investigation of molecular requirements for catecholamine binding, or of structure-activity relationships of agents which interfere with catecholamine retention in the central nervous sytem. In fact, some work has suggested that the molecular requirements for retention may be less specific in the brain.[1,6] In the present study, a survey was made of a number of compounds for their ability to enhance the efflux *in vitro* of [^{3}H]norepinephrine (^{3}H-NE) accumulated by rat brain tissues *in vivo*. An attempt is made to find certain generalizations regarding the molecular-structural characteristics of central catecholamine "retention", a phenomenon which includes the processes of neuronal re-uptake and binding.

* Supported by U.S. Public Health Service (N.I.M.H.) Grant MH-16674-01.

† Recipient of Research Scientist Development Award, Type II, National Institute of Mental Health (1-KO2-MH-74370).

MATERIALS AND METHODS

Animals. Normal young adult (190–210 g) male albino rats (Charles River Co., Wilmington, Mass.) were maintained *ad lib.* on Charles River Laboratory Chow and water.

Radioisotopes. DL-[7-^{3}H]NE (New England Nuclear Corp., Boston) (6–8 c/m-mole) or L-[7-^{3}H]NE (2–3 c/m-mole) (Amersham/Searle Corp., Arlington Heights, Ill.) were used.

Chemicals and drugs. Compounds were obtained at the highest available purity from manufacturers indicated by superscripts in Tables 1–7, as follows: ([a]), Aldrich Chemical Co., Milwaukee, Wisconsin; ([b]), J. T. Baker Chemical Co., Phillipsburg, N.J.; ([c]), Cal-Biochem Co., Los Angeles, Calif.; ([d]), DuPont Co., Wilmington, Del.; ([h]), Hoffman-LaRoche, Co., Nutley, N. J.; ([k]), K and K Laboratories, Plainview, N.Y.; ([m]), Mattheson, Coleman and Bell Co., E. Rutherford, N.J.; ([σ]), Sigma Biochemicals, St. Louis, Mo.; ([s]), Sterling-Winthrop Research Institute, Rensselaer, N.Y.; ([w]), Walker Chemical Co., Mt. Vernon, N.Y.

Tissue preparation. Rats were given 10–12 μc of [^{3}H]NE in 25–30 μl of a physiologic medium intracisternally[7] under light ether anaesthesia and were sacrificed at 30 min or 3 hr by decapitation. Brains were removed and homogenized[8–10] in 7 vol. of iced 0·32 M sucrose by 12 strokes in a motor driven Teflon and glass Potter–Elvehjem type homogenizer specially modified to provide 0·25 mm clearance (Kontes Glass Co., Vineland, N.J.). Homogenates were centrifuged in a refrigerated Sorvall RC-2B centrifuge (0–4°) at 1000 *g* for 15 min to sediment debris and nuclei. Aliquots (1 ml) of supernatants containing synaptosomes and other cellular components[8–10] were added to 9 ml of a glucose-and amino acid-supplemented Krebs bicarbonate-buffered medium[11] at pH 7·2, modified to obtain cation concentrations similar to normal cerebrospinal fluid.[12] Compounds to be tested were dissolved in the medium immediately prior to use, and pH was readjusted to 7·2. Preparations were incubated for various times in 16 ml polypropylene centrifuge tubes (Sorvall Co.) in a Dubnoff-type water bath in air at 37° with shaking. Control and treated preparations were incubated and then transferred to ice in a randomized pattern and immediately centrifuged at 48,000 *g* for 20 min in the refrigerated centrifuge. Tissue pellets were quantitatively taken up and extracted with iced 0·4 N perchloric acid, with a Teflon and glass homogenizer, and recentrifuged at 10,000 *g* for 15 min. The clear supernatant extract was saved for assay of [^{3}H]NE or total tritium. [^{3}H]NE was isolated by alumina column chromatography.[13] Radioactivity was assayed by scintillation spectrometry using a toluene solution containing 2,5-diphenyloxazole (0·4%, w/v) and *p*-bis-2(5-phenyloxazolyl) benzene (0·01%, w/v) (Packard Instrument Co. Downers Grove, Ill.) and diluted 5:2 with absolute ethanol.[14] Counting efficiency and quenching were monitored by internal and external radioactivity standards. Unmetabolized [^{3}H]NE accounted for about 75 per cent of total tissue tritium. Aliquots of complete control incubation media were taken each day for protein determination by the method of Lowry *et al.*,[15] and were found to contain consistently a mean of 1·05 mg protein per ml of homogenate.

Studies of the effects of drugs on the ability of brain tissue to retain [^{3}H]NE are presented in tabular form as per cent of control ^{3}H remaining after 30 min of incubation with or without drugs present. Data were first calculated as nc ^{3}H per aliquot of homogenate (or per 1·05 mg protein) and compared with simultaneously determined

control values. Compounds were surveyed at a high concentration to seek differences among structural analogs, and active substances were also examined at lower concentrations.

RESULTS

The rate of efflux of bound [^{3}H]NE over time was studied (Fig. 1). Initially, tissue was found to contain 4·8 nc tritium (or 3·4 nc[^{3}H]NE) per mg protein or per ml of homogenate. This material escaped rapidly into the incubation medium for 10–20 min, and then efflux became slower and nearly linear on a logarithmic scale over time up to 90 min. The amount of residual tissue-bound tritium or [^{3}H]NE sensitively demonstrated the effects of drugs upon the rate of disappearance of tissue-bound

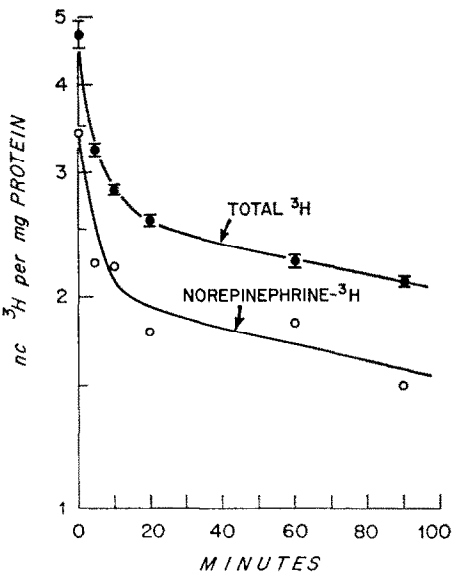


FIG. 1. The rate of efflux of total tritium or [^{3}H]NE from brain homogenates from rats exposed to intracisternal [^{3}H]NE for 30 min. Tissues were incubated in a Krebs' medium.

amine. Several classes of compounds were tested for their ability to affect NE retention.

Catecholamines and other biogenic amines. Several phenethylamines produced large and significant increases in the rate of disappearance of rat brain tissue-bound[^{3}H]NE. Of the compounds tested at a concentration of 10^{-3} M (Table 1), dopamine appeared to be the most effective in displacing bound [^{3}H]NE (to 48 per cent of control, or a 52 per cent enhancement of disappearance). The presence of a ring 3-*O*-methyl substituent decreased this effect of dopamine (to 62 per cent of control retention), suggesting that the presence of a catechol structure may account for part, but not all of the effect. Pure L-NE was only slightly more effective than D-NE, suggesting that competition for retention may not require stereo-specificity. L-Epinephrine, with an *N*-methyl substituent, but also a catechol group, appeared to have a somewhat greater

TABLE 1. DISPLACEMENT OF [^{3}H]NOREPINEPHRINE BOUND TO RAT BRAIN TISSUE

Compound	Control retention (%)	± S.E.M. (*n*)
(a) *Catecholamines*		
Dopamine[c]	48·0†	± 0·8 (6)
6-Hydroxy dopamine[b]	73·9†	± 1·8 (6)
3-*O*-Methyl-dopamine[a]	62·3†	± 3·1 (4)
L-Norepinephrine[c]	70·1†	± 5·7 (6)
D-Norepinephrine[a]	75·0*	± 1·9 (4)
L-Epinephrine[a]	50·8†	± 0·5 (6)
(b) *Other amines*		
Histamine[c]	74·6†	± 3·9 (8)
Serotonin[c]	55·3†	± 1·5 (6)
Acetylcholine[m] and eserine[c]	94·6	± 2·9 (6)

* $P < 0{\cdot}02$.
† $P < 0{\cdot}001$.
Tissue was incubated in normal medium vs. medium with added test substance (10^{-3} M) for 30 min. Data presented as percentage of control ± standard error of the mean (*n* = number of determinations). Superscripts refer to sources of compounds listed in Materials and Methods section.

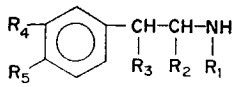
displacement effect than NE. Epinephrine, NE, and dopamine were also active in low concentrations (Table 8).

It was found that the indoleamine, serotonin, produced marked interference with NE retention, while the structurally less similar imidazoleamine, histamine, produced a lesser, but significant, effect. Serotonin was very effective at least to 10^{-5} M concentrations and slightly active at 10^{-6} M (Table 8). Acetylcholine alone at 10^{-3} M, or even with the protection of the cholinesterase inhibitor eserine (10^{-4} M), did not displace bound NE.

Substituted phenethylamines. A series of compounds, some of which are indirectly acting amines or putative false neurotransmitters and which compete for NE binding sites in the peripheral sympathetic nervous system[3–5] were tested (Table 2). All of the compounds tested were effective in displacing bound [^{3}H]NE. Tyramine, octopamine and phenethylamine produced significant decreases of retention even at 10^{-6} or 10^{-7} M and D-amphetamine was active at least to 10^{-5} M (Table 8).

The presence of a β-hydroxyl group appears to have enhanced the effectiveness of tyramine slightly (octopamine), but not phenethylamine (β-hydroxy-phenethylamine) (Tables 2 and 8). The presence of an *N*-methyl group (*N*-methylphenethylamine) resulted in one case in somewhat less effective displacement of bound NE, and this may be due to steric interference at the terminal amino group. The dimethoxy analog of phenethylamine was nearly as effective as the unsubstituted amine or high concentrations. Thus, the effectiveness of these compounds appears to result from a phenylethyl configuration, with the presence of a relatively unobstructed terminal amino group.

TABLE 2. DISPLACEMENT OF [^{3}H]NOREPINEPHRINE BOUND TO RAT BRAIN TISSUE: SUBSTITUTED PHENETHYLAMINES

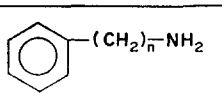


Compound	R_1	R_2	R_3	R_4	R_5	Control retention (%)	± S.E.M. (*n*)
β-Phenethylamine[σ]	H	H	H	H	H	49·7†	± 1·2 (10)
DL-β-Hydroxy-β-phenethylamine[k]	H	H	OH	H	H	71·1†	± 0·5 (4)
p-Tyramine[c]	H	H	H	H	OH	50·9†	± 2·2 (12)
DL-Octopamine[c]	H	H	OH	H	OH	45·5†	± 0·8 (12)
D-Amphetamine[w]	H	CH_3	H	H	H	74·7*	± 0·9 (6
N-Methyl-phenethylamine[a]	CH_3	H	H	H	H	71·9†	± 1·4 (4)
Dimethoxyphenethylamine[c]	H	H	H	OCH_3	OCH_3	58·4†	± 0·6 (6)

* $P < 0{\cdot}01$.
† $P < 0{\cdot}001$.
See footnote to Table 1.

Cyclic amines. The effectiveness of phenethylamines in interfering with NE retention led to an investigation of other structurally related compounds (Table 3). In a series of phenyl-alkyl-amines tested, the side chain length appeared to be important

TABLE 3. DISPLACEMENT OF [^{3}H]NOREPINEPHRINE BOUND TO RAT BRAIN TISSUE: CYCLIC AMINES

Compound	Control retention	± S.E.M. (*n*)
		
(a) *Unsubstituted aromatic amines*		
Aniline [k] $n = 0$	93·9	± 2·7 (5)
Benzylamine[k] $n = 1$	69·5†	± 2·6 (5)
β-Phenethylamine[σ] $n = 2$	49·7†	± 1·2 (10)
4-Phenylbutylamine[k] $n = 4$	62·1†	± 2·1 (5)
(b) *Non-aromatic cyclic amines*		
Cyclohexylamine[k]	71·3†	± 1·8 (5)
Amantadine[d]	66·9†	± 1·5 (4)

† $P < 0{\cdot}001$.
See footnote to Table 1.

in determining activity. Thus, aniline, benzylamine, and phenylbutylamine were less active than phenethylamine, suggesting that a side chain of 2 or 3 carbons may be optimal. The presence of a benzene ring does not seem to be essential for NE displacement, as cyclohexylamine and amantadine were also moderately active. Amantadine was also active at lower concentrations (Table 8).

Aliphatic amines. Further evidence that a benzene ring is not essential for catecholamine displacement, at least at higher concentrations, is presented in a series of aliphatic amines of increasing chain length (Table 4). Activity at 10^{-3} M increased with increasing chain length, although 4-, 5-, 6- and 7-carbon amines were about

TABLE 4. DISPLACEMENT OF [^{3}H]NOREPINEPHRINE BOUND TO RAT BRAIN TISSUE: ALIPHATIC AMINES
$CH_3(CH_2)_n NH_2$

Compound	n	Control retention (%)	± S.E.M. (n)
Ethylamine[k]	1	93·3*	± 1·9 (5)
Propylamine[k]	2	80·3†	± 0·1 (3)
Butylamine[k]	3	64·8†	± 0·4 (3)
Amylamine[k]	4	70·3†	± 0·7 (5)
Hexylamine[k]	5	71·5†	± 1·8 (5)
Heptylamine[k]	6	70·8†	± 2·0 (4)
Octylamine[k] (insoluble)	7	—	—

* $P < 0{\cdot}01$.
† $P < 0{\cdot}001$.
See footnote to Table 1.

equally active. None were as active as the phenyl-ethyl-amines, and activity decreased rapidly with decreasing concentrations of butylamine (Table 8).

Ω-hydroxy-aliphatic amines. For comparison with aliphatic amines, a series of terminal-hydroxy-aliphatic amines was tested (Table 5). These were found to displace bound [^{3}H]NE at 10^{-3} M concentrations when carbon chain length exceeded 2, and approached a maximum activity at a sidechain length of 5- or 6-carbon atoms. The

TABLE 5. DISPLACEMENT OF NOREPINEPHRINE[^{3}H] BOUND TO RAT BRAIN TISSUE: Ω-HYDROXY-ALIPHATIC AMINES
$HO{-}(CH_2)_n{-}NH_2$

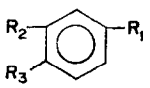
Compound	n	Control retention (%)	± S.E.M. (n)
2-NH_2-Ethanol[c]	2	99·8	± 5·5 (6)
3-NH_2-Propanol[k]	3	95·9	± 3·3 (4)
4-NH_2-Butanol[k]	4	82·1*	± 4·0 (5)
5-NH_2-Pentanol[k]	5	66·5†	± 1·8 (5)
6-NH_2-Hexanol[k]	6	57·8†	± 2·0 (5)

* $P < 0{\cdot}005$.
† $P < 0{\cdot}001$.
See footnote to Table 1.

compound, 6-amino-hexanol, appeared to be slightly more active than 5-, 6- or 7-carbon aliphatic amines lacking the terminal alcohol group (Table 4), and approached the aromatic amines (Tables 1 and 2) in effectiveness at higher concentrations, but not at lower concentrations (Table 8).

Non-amine catechols. The importance of the catechol structure for brain catecholamine retention was examined in another series of compounds (Table 6). Catechol itself was found to be readily oxidized (solutions turned red) when used alone at a

TABLE 6. DISPLACEMENT OF [^{3}H]NOREPINEPHRINE BOUND TO RAT BRAIN TISSUE: NON-AMINE CATECHOLS



Compound	R_1	R_2	R_3	Control retention (%)	± S.E.M. (*n*)
Catechols					
Pyrocatechol[b]	H	OH	OH	92·7*	± 0·3 (4)
O-Methyl catechol[k]	H	OCH_3	OH	80·2†	± 2·0 (4)
O,O-Dimethyl catechol[k]	H	OCH_3	OCH_3	110·7†	± 0·8 (4)
Deaminated metabolites					
Dihydroxyphenyl acetic acid[c] (DOPAC)	CH_2COOH	OH	OH	76·9†	± 2·0 (6)
DL-Dihydroxy mandelic acid[c] (DHMA)	CHOHCOOH	OH	OH	64·8†	± 1·6 (6)
Homovanillic acid[c] (HVA)	CH_2COOH	OCH_3	OH	98·4	± 1·6 (6)
DL-Vanillyl-mandelic acid[c] (VMA)	CHOHCOOH	OCH_3	OH	95·9	± 0·9 (6)

* $P < 0{\cdot}01$.
† $P < 0{\cdot}001$.
See footnote to Table 1.

concentration of 10^{-3} M in an unmodified incubation medium, and produced no effect on NE retention. Catechol increased [^{3}H]NE disappearance by 24 per cent at 10^{-2} M, in the presence of ascorbate, EDTA, and meta-bisulfite to inhibit oxidation. Catechol (10^{-3} M) in the presence of 2 mM ascorbate produced a small displacement (less than 10 per cent) of bound catecholamine. In each case, controls of identical composition, save catechol, were used. *O*-methyl catechol appeared to be moderately effective in displacing bound catecholamine. *O,O*-dimethyl-catechol was completely ineffective, and possibly even enhanced [^{3}H]NE retention to a small extent.

Other non-amine catechol compounds were tested (Table 6). These were deaminated-acid metabolites of endogenous catecholamines. They (dihydroxymandelate and dihydroxyphenylacetate) were found to be moderately active in displacing [^{3}H]NE though less active than phenylethylamines and inactive at lower concentrations (Table 8). The 3-*O*-methylated analogs of these compounds (vanillylmandelate, and homovanillate) were inactive at 10^{-3} M. This loss of activity by *O*-methylation suggests that the catechol structure is required for activity.

Aromatic amino acids. Several hydroxylated, or *O*-methylated, or other aromatic amino acids were tested (Table 7). Phenylalanine, neither a catechol nor an amine was not active in displacing bound [^{3}H]NE. The phenolic amino acid, tyrosine, was slightly active, and the catechol-amino acid, L-dihydroxy-phenylalanine (L-DOPA) was very active even at low concentrations (Table 8). The D-enantiomer of DOPA was

TABLE 7. DISPLACEMENT OF [^{3}H]NOREPINEPHRINE BOUND TO RAT BRAIN TISSUE: AROMATIC AMINO ACIDS

Compound	Control retention (%)	± S.E.M. (*n*)
L-Phenylalanine[c]	109·2	± 1·3 (6)
L-*p*-Tyrosine[c]	89·0*	± 1·2 (4)
L-DOPA[c]	59·2†	± 0·9 (6)
D-DOPA[σ]	79·2†	± 1·2 (7)
L-3-*O*-Methyl-DOPA[s]	100·6	± 1·0 (4)

* $P < 0{\cdot}01$.
† $P < 0{\cdot}001$.
See footnote to Table 1.

somewhat less active at 10^{-3} M. The presence of a 3-*O*-methyl substituent inactivated L-DOPA.

DISCUSSION

The observed interference with NE retention by a variety of substances could be due either to interference with uptake of the catecholamine at the neuronal membrane or with intraneuronal retention in a bound, complexed or otherwise sequestered form, or both. In either case, the result would be a net decreased retention or increased efflux of catecholamines. In peripheral tissues, there is evidence that certain indirectly acting amines, such as tyramine, may act both by inhibiting NE uptake and, after being taken up, by displacing endogenous NE from neuronal storage sites.[16] The tissue preparations utilized in the present work have been shown to contain pinched-off nerve endings or synaptosomes, which contain granular vesicles, mitochondria, and cytoplasm within portions of nerve cell membrane.[8] Thus, processes of both membrane transport and intracellular binding should occur. Experiments were done which demonstrated that cation-deficient media, or the addition of 10^{-3} or 10^{-5} M ouabain or imipramine to normal media result in a diminution of [^{3}H]NE retention by the present tissue preparations. Both conditions are known to impair the ability of synaptosomes to take up exogenous catecholamines.[17] Thus, conditions which interfered with neuronal uptake mechanisms were capable of producing net losses of NE from brain synaptosomal preparations.

Other important questions are whether the displacement phenomena observed are specific anatomically and chemically. The whole-brain preparations used were not made from anatomically discrete regions, and further work, seeking specific regional differences in the antagonism of amine retention, is needed. Furthermore, the tissue preparations were relatively crude nuclei- and debris-free, 1000 *g* supernatant fractions of homogenates prepared so as to preserve synaptosomal integrity. Such preparations were chosen for convenience and to avoid artefacts which may result from the prolonged and relatively unphysiologic procedures ordinarily used to isolate synaptosomes. Homogenate preparations have been demonstrated to retain labelled catecholamines,[10,18,19] or other phenethylamines,[20,21] in a fraction identifiable as synaptosomal by sedimentation characteristics,[10,18–21] electron microscopy,[10] and the coincidental distribution of exogenous amines with endogenous catecholamines

and lactic dehydrogenase.[10,18] Furthermore, the preparations yield particles which when isolated by ultrafiltration, are metabolically active and accumulate catecholamines.[19] Several experiments were carried out using purified synaptosomal preparations.[19] They demonstrated activities of several compounds at low concentrations similar to or slightly greater than those obtained with crude homogenates (cf. Table 8).

TABLE 8. DISPLACEMENT OF [^{3}H]NOREPINEPHRINE BOUND TO RAT BRAIN TISSUE

Compound	Molarity	Control retention %	± S.E.M. (*n*)
L-Norepinephrine	10^{-6}	64·0†	± 0·9 (6)
L-Epinephrine	10^{-6}	78·9†	± 1·3 (6)
Dopamine	10^{-6}	73·5†	± 0·9 (6)
L-DOPA	10^{-5}	58·8†	± 0·8 (6)
	10^{-6}	83·0†	± 0·9 (6)
Octopamine	10^{-5}	52·7†	± 0·1 (4)
	10^{-6}	74·1*	± 2·3 (4)
	10^{-7}	89·1	± 1·8 (4)
p-Tyramine	10^{-5}	64·5†	± 4·2 (4)
	10^{-6}	80·2†	± 1·8 (4)
β-Phenethylamine	10^{-5}	70·7†	± 2·7 (6)
	10^{-7}	82·5*	± 1·4 (4)
D-Amphetamine	10^{-5}	69·9†	± 1·0 (6)
Serotonin	10^{-5}	76·9†	± 2·1 (4)
	10^{-6}	91·3*	± 2·0 (6)
6-NH_2-Hexanol	5×10^{-5}	83·2†	± 1·2 (6)
Butylamine	5×10^{-5}	88·6†	± 1·1 (6)
Amantadine	5×10^{-5}	82·0†	± 0·8 (5)
DL-Dihydroxy-mandelic acid (DHMA)	5×10^{-5}	97·7	± 1·2 (6)

* $P < 0{\cdot}01$.
† $P < 0{\cdot}001$.
Tissue was incubated in normal medium *v.* medium with added test substance at various concentrations for 30 min. Data presented as percentage of control ± standard error of the mean. (*n* = Number of determinations.)

Thus, at 10^{-6} M dopamine, L-epinephrine, L-DOPA, and serotonin reduced [^{3}H]NE retention to 56, 68, 79 and 84 per cent of control, respectively.

Certain other aspects of the question of specificity have also been considered. For example, the material displaced is largely [^{3}H]NE itself and not its metabolites. The comparison of total tritium with chromatographically isolated [^{3}H]NE, which was done for many of the compounds tested, shows very similar or somewhat greater displacement of [^{3}H]NE than of total tritium (e.g. Table 9). Furthermore, since all compounds were tested with DL-[^{3}H]NE, a comparison was made with effects on L-[^{3}H]NE. Phenethylamine produced equal displacements in either case (Table 9). Increasing the time between intracisternal injection of [^{3}H]NE and tissue preparation, from 30 min to 3 hr also failed to produce a significant change in the per cent alteration of retention of total tritium or [^{3}H]NE produced by exposure to 10^{-5} M phenethylamine. This increased time should have allowed for greater removal and metabolism of exogenous catecholamine introduced into non-specific brain sites.

In order to reduce the likelihood of non-physiologic distributions of tracer material an *in vivo* method of labelling the central noradrenergic stores was chosen. Evidence in

TABLE 9. PERCENTAGE ^3H RETAINED IN RAT BRAIN TISSUE TREATED WITH PHENETHYLAMINE

	Total ^3H		[^3H]Norepinephrine	
	30 min	3 hr	30 min	3 hr
L-	78·0 ± 1·7	75·7 ± 0·9	55·0 ± 2·2	61·3 ± 3·8
DL-	72·6 ± 1·2	78·0 ± 1·2	55·0 ± 2·4	60·0 ± 3·2

[^3H]NE injected intracisternally in L or DL form, 30 min or 3 hr before *in vitro* incubation with 10^{-5} M phenethylamine. Data expressed as percentage of control ± S.E.M. ($n = 4$) for total tritium or chromatographically isolated [^3H]NE.

support of the validity of radioactive catecholamine introduced into the cerebrospinal fluid as a label of central catecholaminergic neurons has been reviewed elsewhere,[7,22] and it includes gross,[23] and subcellular[2] distribution data, autoradiographic findings[25] combined with electron microscopy,[26] and pharmacologic evidence.[27] Nevertheless, it is quite possible that some labelled catecholamine is displaced from unphysiologic sites of accumulation, for example in dopaminergic or serotonergic neurons or even in non-specific extraneuronal sites. In general, the problem of the specificity of tracer localization in brain tissues has not yet been satisfactorily resolved for either *in vivo* or *in vitro* approaches.

Several of the compounds found to be active might have pharmacological or even physiological importance. Acetylcholine at high concentrations has been reported to displace bound [^{14}C]NE *in vivo*[28] in superfused cat hypothalamus preparations. This did not occur in the present *in vitro* experiments and so the *in vivo* effect may be mediated by the stimulation of intact neurons capable of conducting impulses. The phenethylamine analogs actively interfere with NE retention in even low concentrations, and are known to have a variety of central excitatory effects,[21,22,29,30] and to participate in false transmitter formation in sympathetic nerve endings.[5] Low *in vitro* activity in low concentration, however, may not necessarily correlate with low biological activity. For example, the deaminated catechols, active at higher concentrations in the present experiments, might have biological activity at synapses if they accumulate locally.

The catecholamine-displacing activity of L-DOPA is probably of limited physiological significance since endogenous concentrations of this catechol-amino acid are normally extremely low.[31] Furthermore, the activity of L-DOPA in even low concentrations at which deaminated catechols are nearly inactive, suggests that this amino acid precursor may be active partly by decarboxylation to dopamine, or conversion to other active products. Pharmacologically, while even large doses of exogenous L-DOPA as administered in the treatment of Parkinsonism are largely detoxified by COMT,[32] it has been found that L-DOPA increases the rate of disappearance of intracisternally administered [^3H]NE and increases the turnover of brain NE.[33] Part of this *in vivo* effect may be due to interference with brain NE retention by L-DOPA (Table 7) or one of its metabolites, such as *O*-methyl-dopamine (Table 1). Amantadine, a 10-carbon primary amine with a symmetrical cyclic structure has also been found to be clinically effective in Parkinson's disease[34] and to raise

blood pressure in the dog.[35] It may act by displacing bound catecholamines or otherwise interfering with their normal metabolism. Non-aromatic cyclic amines may also interfere with NE uptake in peripheral tissues.[3]

The data presented suggest several tentative generalizations concerning structure–activity relationships. The compounds which actively increased the rate of disappearance of [^{3}H]NE bound by rat brain tissue have quite varied molecular characteristics. Aromatic compounds with alkyl-amine side-chains of 2 or 3 carbons were very active. The addition of a β-hydroxyl group may, but does not necessarily increase activity (Tables 2 and 8), and the L-entantiomer of NE was slightly more active than the D-configuration at the β-carbon (Table 1). *N*-methylation of a non-catechol aromatic amine, phenethylamine, reduced activity (Table 2), possibly by steric interference at the amino group. Aliphatic amines were only mildly active, at relatively high concentrations, and activity appears to correlate with carbon-chain length (Table 4). The aliphatic α-amino-Ω-alcohols with 5 or 6 carbon atoms were somewhat more active, possibly by pseudo-ring structure formation by electrostatic affinity of the terminal groups to facilitate a partial structural imitation of cyclic or aromatic amines. The activity of all aliphatic compounds decreased rapidly with decreasing amine concentration (Table 8).

The catechol compounds with a phenyl–ethyl configuration were moderately active at higher concentrations. *O*-methylation consistently inactivated the non-amine phenyl–ethyl catechols (Tables 6 and 7) and the activity of dopamine was reduced (Table 1). Catechol itself, lacking an ethyl side chain, had only limited activity at high concentrations. *O*-methyl catechol appeared to be moderately effective in displacing bound NE. These effects of catechol and methyl-catechol (Table 6) may be due to non-specific tissue toxicity of these phenolic compounds, and methyl catechol may appear to be more active if *O*-methyl substitution merely decreases oxidation of the compound. *O*,*O*-dimethyl catechol was completely ineffective, and possibly even enhanced [3H]NE retention to a small extent. The latter finding could conceivably be due to catechol-*O*-methyl transferase (COMT) inhibition, since certain methylated polyphenols are COMT inhibitors.[36]

Among the active compounds, phenethylamines, catecholamines and even non-aromatic amines clearly remained much more active at low concentrations than non-amine catechols (Table 8). The phenethyl-catechols may have less interaction with NE retention sites or may themselves be less actively taken up by neurons. These findings, and the observation that Nature has provided two quite separate and independent systems (oxidative deamination and *O*-methylation) to inactivate amine and catechol loci suggest that there may be at least two types of normal physiologic interaction between catecholamines and transport systems, binding sites, or post-synaptic effector sites. One possibility is that the phenyl–ethyl-amine structure is more important for uptake and intraneuronal binding, and that excesses are dealt with by intraneuronal monamineoxidase, and further, that the catechol group (and perhaps a β-hydroxyl group) is important for post-synaptic effects,[37] and is dealt with by COMT following release. Consistent with this view are the facts that most directly acting amines are catechols (or β-hydroxyl-phenols[4]) and most presently proposed sympathetic false neurotransmitters are taken up, stored, and released (phenethylamines, preferably β-hydroxylated), but relatively inactive post-synaptically (non-catechols).[5] An alternative hypothesis is that uptake, binding and post-synaptic interactions require

simultaneous participation of both ends of catecholamine molecules, the interference with either of which is adequate to diminish tissue retention.

Acknowledgement—The excellent technical assistance of Mrs. Marcella Vogt is gratefully acknowledged. We wish to thank the DuPont Company, the Hoffman-LaRoche Company, and the Sterling–Winthrop Research Institute for their kind donations of drug substances used in this work.

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