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On the Topology of the Norepinephrine Transport Carrier in Rat Hypothalamus

The Site of Action of Tricyclic Uptake Inhibitors

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SUMMARY

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The conformations of 31 rigid and semirigid compounds structurally related to the tricyclic antidepressant agents have been correlated with their inhibitory activities toward the uptake of norepinephrine in synaptosomes of rat hypothalamus. A relationship was found, in terms of four intramolecular distances, between the potencies of these compounds and their structural similarities to the most potent compound, *N,N*-dimethylspiro[5*H*-dibenzo[*a,d*]cycloheptene-5,1′-cyclohexane]-4-amine (A 1866). The stereochemical parameters were selected to describe the position of the nitrogen atom in relation to the centers of the two aromatic nuclei. It is suggested that the competitive antagonistic behavior of the tricyclic compounds is independent of their structural similarities to either the *gauche* or the *anti* conformation of norepinephrine. It is concluded that the norepinephrine transport carrier is equipped with active sites displaying two different topologies. One is complementary to the structure of A 1866, for which the tricyclic antidepressant agents have affinity; the other has a topology complementary to the structure of norepinephrine, to which the catecholamines and some phenylalkylamine derivatives fit.

INTRODUCTION

The hypothesis that the transport of norepinephrine through the neuron membrane is effected by a specific carrier mechanism is widely accepted (1). It is generally assumed that norepinephrine binds to a mobile carrier and that this complex is translocated across the membrane. Several other hydroxyphenethylamines appear to be transported by the same norepinephrine carrier mechanism (2), indicating that the structural requirements for uptake are not restricted solely to the norepinephrine configuration. A large number of compounds competitively inhibit the norepinephrine carrier, probably without being translocated (3). The relative potencies of these inhibitors should give some insight into the structural requirements for inhibition and thereby also provide information on the topology of the active site of the norepinephrine transport carrier. However, one

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limitation of this approach is that most of these compounds in solution have several possible conformations with small energy differences. The preferred conformations at the receptor site are therefore difficult to predict, and the structure of the drug-receptor complex is unknown at present. In order to overcome these obstacles and to obtain further information on the structural requirements for inhibition of the amine transport system in the brain, a series of rigid tricyclic spirocycloalkylamine derivatives related to the tricyclic antidepressants were synthesized and tested as inhibitors of neuronal norepinephrine and 5-hydroxytryptamine accumulation in mouse brain slices (4). In this series, some of the spiro compounds were potent inhibitors of norepinephrine uptake while others had a 100-fold lesser effect. A further analysis of the structure-activity relationships of these compounds and some other rigid tricyclic analogues may provide more detailed information about the structural requirements for inhibition of norepinephrine uptake and give some insight into the nature and topology of the drug-receptor complex.

By utilizing the structures of 31 rigid analogues of amitriptyline, we now report an attempt to elucidate the topology of the active site of the noradrenergic nerve terminals at which the tricyclic antidepressant agent inhibits the uptake of norepinephrine.

MATERIALS AND METHODS

Compounds

Rigid compounds (*Table 1*). The amino-substituted spiro[5*H*-dibenzo[*a,d*]cycloheptene-5,1′-cycloalkanes], **1–9**, and the 10,11-dihydro derivative, **10**, were synthesized as previously described (4). *N*-Methyl- and *N,N*-dimethylspiro[cyclohexane-1,9′-fluorene]-4-amine, **11** and **12**, were synthesized according to Stauffer and Fancher (5). 5-(3-Dimethylaminocyclohexylidene)-10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene, **14**, was synthesized from 5-hydroxy-5-(3-dimethylaminophenyl)-10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene by hydrogenation followed by dehydration (6). The following compounds were donated by the suppliers: cyproheptadine HCl, **15**, by Merck Sharp & Dohme; piroheptine HCl, **16**, by Fujisawa, Japan; and mianserine HCl, **17**, by Organon.

Semirigid compounds (*Table 2*). *N*-methyl- and *N,N*-dimethylspiro[5*H*-dibenzo[*a,d*] cycloheptene-5,1′-cyclopentane]-3′-methylamine, **18** and **19**, were prepared as previously described (4). *N*-Methyl-10,11-dihydrospiro[5*H*-dibenzo[*a,d*]cycloheptene-5,4′-tetrahydrofurfurylamine] HCl, **20**, was obtained from Bristol Laboratories.

Spiro[5*H*-dibenzo[*a,d*]cycloheptene-5,3′-azepine], **21**, and the *N*-methyl derivative, **22**, were prepared by a Schmidt rearrangement of the corresponding cyclohexanone, followed by reduction (7).

The *cis* and *trans* isomers of 5-(4-dimethylaminocyclohexyl)-10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene, **23** and **24**, were synthesized according to Villani *et al.* (8). The corresponding 3-dimethylaminocyclohexyl derivatives, **25** and **26**, were prepared by analogy with Villani's method from *N,N*-dimethyl-3-iodoaniline and 10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptenone. Physical characteristics of these compounds and the unsaturated analogue, **14**, will be reported elsewhere (6).

The following compounds were donated by the suppliers: 10,11-dihydro-5-[(1′-methyl-3′-pyrrolidyl)methylidene]-5*H*-dibenzo[*a,d*]cycloheptene HCl, **27**, by Mead Johnson; 10,11-dihydro-5-[(1′-methyl-3′-piperidyl)methylene]-5*H*-dibenzo[*a,d*]cycloheptene HCl, **28**, by Ayerst Laboratories; the corresponding 10,11-unsaturated analogue, **29**, and 5-[(1′-methyl-2′-piperidyl)-2″-ethylidene]-5*H*-dibenzo[*a,d*]cycloheptene HBr, **30**, by Sandoz; and 2′-(*N,N*-diemthylaminoethyl)-10,11-dihydrospiro-[cyclopropane-1′,5-5*H*-dibenzo[*a,d*]-cycloheptene] HCl, **31**, and *trans*-2-[5-(10,11-dihydro)-5*H*-dibenzo[*a,d*]cycloheptenyl]-*N,N*-dimethylaminomethylcyclopropane, **32**, by Smith Kline & French.

Reference compounds (*Table 3*). The following compounds were donated by the suppliers: didesmethylimipramine HCl, **34**, desipramine HCl, **35**, and imipramine HCl, **36**, by Ciba-Geigy; nortriptyline HCl, **37**, and amitriptyline HCl, **38**, by Lundbeck, Denmark; protriptyline HCl, **39**, by Merck Sharp & Dohme; *N*-methylprotriptyline

maleate, 40, by Lakeside Laboratories, Milwaukee; and nialamide HCl, by Pfizer. [−]-[7-^{3}H]Norepinephrine (3.2 Ci/mmole) was purchased from NEN Chemicals, West Germany.

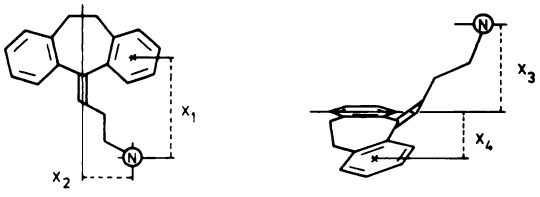
Structural Parameters

Four different parameters, X_1–X_4, were chosen to describe the essential structural features of the rigid compounds. Three of these define the position of the nitrogen atom in relation to one of the aromatic rings, and the fourth parameter is related to the planarity of the tricyclic nucleus in terms of intramolecular distances. These stereochemical parameters are outlined in the formula for amitriptyline, viewed perpendicular to the tricyclic skeleton for parameters X_1 and X_2 and parallel to one of the aromatic rings for parameters X_3 and X_4.

The following stereochemical parameters were chosen to describe these interrelations in terms of atomic distances: X_1 is the distance between the amino group and the center of the nearest aromatic nucleus; X_2 is the distance between the amino group and the plane of symmetry bisecting the 7-membered ring of the tricyclic skeleton; X_3 is the level of the amino nitrogen atom above the plane of the benzene ring defined by X_1; and X_4 is the level of the center of the second aromatic nucleus below the plane defined by X_1.

Method of Calculation

Conformational parameter values for each compound were measured from molecular models. The Dreiding model was chosen because of the rigidity of the bond angles and the exaggerated conformational stability, which made measurements of the parameter values less ambiguous, and because the framework model made the center of atoms and rings easily accessible.



Amitriptyline

A distance index, DI, was obtained by calculating the differences between the parameter values for each compound and an arbitrarily chosen set of reference values. The sum of the squared differences was calculated. The square root of this sum is the distance index DI_j (angstroms), in which the parameter value X_{ij} is defined as the ith distance (angstroms) of the jth compound and X_{i0} is the ith distance of the reference structure.

$$DI_j = \left[\sum_i (X_{ij} - X_{i0})^2 \right]^{1/2}$$

The distance index is a measure of the degree of fit on adaptation of the rigid and semirigid tricyclic compounds to the compound representing the chosen set of parameter values in terms of their amino group position in relation to the tricyclic skeleton. By plotting DI_j against the logarithm of the inverted value of the inhibition constant, K_i, the correlation between structure and activity was investigated. The initial set of parameter values (X_{i0}) was those of the most potent spirocyclohexylamine derivative, A 1866 (9). By systematically varying the values of X_{i0} and testing for a linear relationship, a search was made for the optimal configuration of an inhibitor of norepinephrine uptake.

Accumulation of (−)-[^{3}H]Norepinephrine in Hypothalamic Homogenate

Pooled hypothalami from male Sprague-Dawley rats (160–200 g) were homogenized in 10 volumes of ice-cold 0.25 M sucrose in an all-glass Potter-Elvehjem homogenizer. A cell-free homogenate was obtained by centrifugation at 800 × g for 10 min. The accumulation of [^{3}H]norepinephrine in the synaptosomes of the homogenate was determined as described previously (9), using a 5-min incubation. The incubation mixture consisted of 50 or 200 nM [^{3}H]norepinephrine, 100 μl of the homogenate, 12.5 μM nialamide, 1.1 mM ascorbic acid, 0.13 mM disodium EDTA, and 5.6 mM glucose in

1.85 ml of Krebs-Henseleit buffer, pH 7.4. No preliminary incubation with the inhibitor was performed. The difference between accumulation at 37° and 0° was taken as active accumulation. The inhibition constants of the compounds were obtained according to Dixon (10) and were based on five different concentrations of the compounds. The apparent K_m value for norepinephrine was determined by the double-reciprocal method with 20–200 nM [^{3}H]norepinephrine.

RESULTS

Norepinephrine Accumulation

The apparent K_m value for the uptake of (−)-norepinephrine in the cell-free homogenate employed was $1.6 \pm 0.2 \times 10^{-7}$ M (mean ± standard error of three independent determinations). The competitive nature of the inhibition was demonstrated for A 1866 by the double-reciprocal method (Fig. 1). The K_i value obtained was 1.4×10^{-8} M, which is in good agreement with the K_i value obtained from the Dixon plot (Table 1).

The results are given in Tables 1–3. The compounds are grouped according to structural flexibility and mobility of the terminal nitrogen atom. Group I consists of rigid compounds, 1–17, with very restricted positional mobility of the amino function (Table 1). Group II contains compounds (18–32) with a semirigid structure and limited positional freedom of the nitrogen atom. Group III consists of the open-chain tricyclic reference compounds, 34–40.

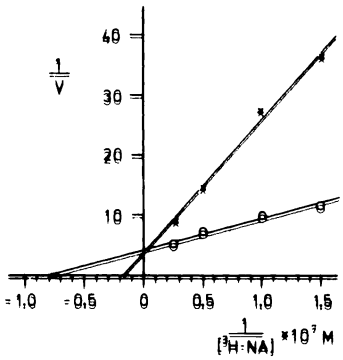


FIG. 1. *Double-reciprocal plot of inhibition by A 1866 of [^{3}H]norepinephrine (^{3}H-NA) accumulation in cell-free homogenate of rat hypothalamus*

Incubation was performed for 5 min at 37° and 0° in Krebs-Henseleit buffer containing 12.5 μM nialamide, 1.1 mM ascorbic acid, and 0.13 mM disodium EDTA. No preliminary incubation with the inhibitor was used. The difference between accumulation at 37° and 0° was taken as active accumulation. v = nanomoles per gram per minute. The regression lines were calculated, and each point is the mean of four determinations. ○, control; ×, 33 nM A 1866.

Of the rigid spiro compounds, the tertiary cyclohexene and cyclohexane derivatives 6, 9 (A 1866), and 10 were potent inhibitors of norepinephrine uptake. The corresponding secondary amines 5 and 8 were 2–3 times, and the primary amines 4 and 7 about 10 times, less active than the corresponding tertiary amines. The spirocyclopentylamines 1, 2, and 3 were 10 times less potent than the corresponding 6-membered ring analogues 7, 8, and 9 (A 1866). The same order of activity as above was found for the variously substituted amines. The saturated tertiary amine 10 also belongs to the potent class of compounds. The spirofluorene derivatives 11 and 12, with the aromatic rings in one plane, were very poor inhibitors of norepinephrine accumulation. Similar poor activity was found for the aminocyclohexylidene derivative 14, while cyproheptadine, 15, was 8 times more potent. Piroheptine, 16, and mianserine, 17, belong to the less active class of compounds.

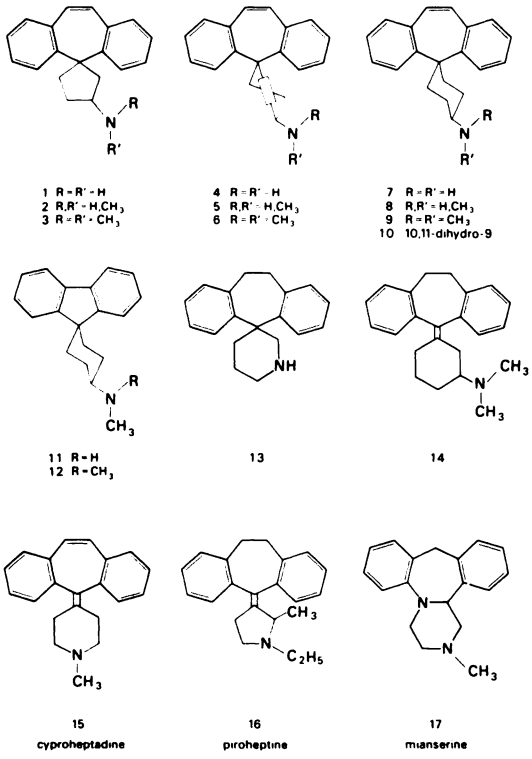
The secondary spirocyclopentane aminomethyl derivative 18 and the saturated 4-oxo analogue 20 were potent uptake inhibitors. The corresponding tertiary amine 19 had 15 times less activity. This relationship between secondary and tertiary amines was also found in the spiroazepine derivatives 21 and 22; the former was 34 times more potent. In these two series the amino function is connected via a methylene carbon atom, which is also found in the open-chain reference compounds, 34–40. In all compounds for which the reverse activity relationship is found, i.e., the tertiary amines were more potent than the secondary analogues, the amino group is attached to a methine carbon.

In the dimethylaminocyclohexyl derivative series, compounds 23, 25, and 26 were devoid of inhibitory activity while the *trans*

TABLE 1

Rigid analogues of amitriptyline with very restricted positional mobility of the nitrogen atom

Compound	Structural parameters				DI_j	K_i	log K_i
	X_1	X_2	X_3	X_4			
	A	*A*	*A*	*A*	*A*	$\times 10^8$ *M*	*(+8.000)*
Primary amines							
1	5.0	1.2	2.5	2.6	1.96	280	2.447
4	5.2	2.1	3.7	2.6	0.47	36	1.556
7	5.4	2.4	4.0	2.6	0.00	55	1.740
Secondary amines							
2	5.0	1.2	2.5	2.6	1.96	45	1.65
5	5.2	2.1	3.7	2.6	0.47	3.8	0.580
8	5.4	2.4	4.0	2.6	0.00	4.7	0.672
11	5.8	2.3	0.0	0.0	4.79	320	2.505
13	3.9	1.2	2.5	2.5	2.44		
Tertiary amines							
3	5.0	1.2	2.5	2.6	1.96	22	1.342
6	5.2	2.1	3.7	2.6	0.47	2.2	0.342
9 (A 1866)	5.4	2.4	4.0	2.6	0.00	1.7	0.230
10	5.0	2.6	3.6	2.3	0.65	6.5	0.813
12	5.8	2.3	0.0	0.0	4.79	420	2.623
14	5.5	2.5	3.7	2.3	0.45	375	2.574
15 (cyproheptadine)	6.1	0.0	3.0	2.3	2.71	44	1.643
16 (piroheptine)	5.3	0.7	3.2	2.3	1.91	230	2.362
17 (mianserine)	5.1	0.6	0.5	2.7	1.59	330	2.519



Structures of compounds listed in Table 1

isomer, **24**, displayed moderate activity. The more rigid methylidenepyrrolidine derivative, **27**, was quite potent considering the fact that it consists of a tertiary amine connected by 2 methylene carbon atoms, which might have rendered the secondary amine analogue more active (cf. compounds **21** and **22**).

The piperidine derivatives **28**, **29**, and **30**, in which the structural features of amitriptyline are preserved, were about 7 times less potent than amitriptyline. The cyclopropyl derivatives **31** and **32**, whose structures highly resemble that of amitriptyline, both were comparable in potency to amitriptyline.

In the series of reference compounds the primary analogue of imipramine, **34**, exhibited moderate potency (Table 3). Both the secondary and tertiary amines were very potent inhibitors of norepinephrine accumulation, the former having a mean activity 3.7 times higher than that of the corresponding tertiary amines.

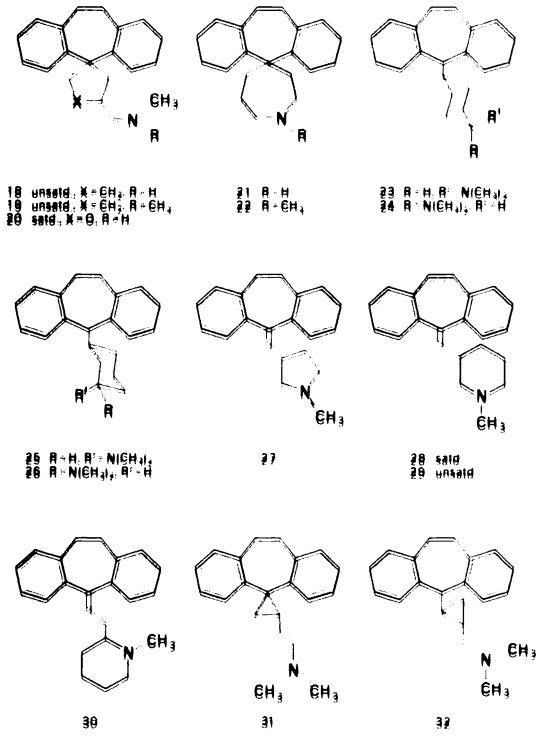
Structural Correlations

Stereochemistry. Active compounds are found among rigid, semirigid, and open-

TABLE 2

Semirigid analogues of amitriptyline with restricted positional mobility of the nitrogen atom

Compound	Structural parameters				DI$_j$	K_i	log K_i
	X_1	X_2	X_3	X_4			
	A	*A*	*A*	*A*	*A*	× 10^6 *M*	(+8.000)
Secondary amines							
18	4.8	1.4	4.4	2.6	1.23	4.3	0.663
20	4.8	1.4	4.4	2.6	1.23	6.0	0.778
21	4.7	1.5	3.3	2.6	1.34	7.5	0.875
Tertiary amines							
19	4.8	1.4	4.4	2.6	1.23	65	1.813
22	4.7	1.5	3.3	2.6	1.34	252	2.401
23	5.2	1.5	4.4	2.2	1.65	365	2.562
24	6.9	0.2	4.7	2.3	2.63	50	1.699
25	6.4	1.9	5.1	2.1	1.01	450	2.653
26	7.0	1.1	5.6	2.3	2.79	355	2.550
27	5.3	2.3	4.0	2.1	0.52	8.5	0.929
28	5.5	2.1	3.1	2.1	1.08	32	1.505
29	5.8	2.2	3.4	2.8	0.77	38	1.580
30	6.1	0.8	3.7	2.8	1.78	38	1.580
31	5.4	2.7	4.6	2.0	0.90	7.5	0.875
32	5.6	2.0	3.8	2.4	0.59	19	1.279
33	4.0	2.7	2.4	2.1	2.20		



Structures of compounds listed in Table 2

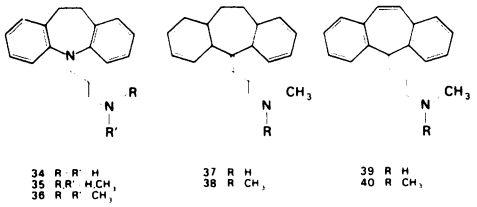
chain amines. Positional mobility of the nitrogen atom does not seem to be a structural requirement for inhibition of norepinephrine uptake. The high potencies of the very rigid spirocyclohexylamines 6 and 9 indicate that the structures of these compounds fulfill the necessary requirements for interaction with the receptor site of the norepinephrine membrane carrier. If the spatial location of the amino group of A 1866 is supposed to be optimal for this interaction, the structural similarity of other tricyclic compounds will correlate with their activities. The stereochemical parameters X_1-X_4 of each compound are listed in Tables 1 and 2. The distance indices, DI$_j$, formed by comparison with the tentatively optimal structure of A 1866, are listed in the tables. It can be easily seen that most of the inactive compounds have large distance indices while the active compounds all have low values. This relationship is illustrated in Fig. 2, where the logarithm of the inhibitory constant K_i is plotted against the square root of the sum of squared deviations (DI) from the most active spiro compound, A 1866.

In Fig. 2, primary amines are denoted by open circles, secondary amines, by open triangles, and tertiary amines, by solid circles. Identically substituted amines but with varying ring structures are connected by a line. Linear correlations between activ-

TABLE 3

Reference compounds

Compound	K_i	log K
	$\times 10^8$ M	(+8.000)
Primary amines		
34 didesmethylimipramine	19	1.279
Secondary amines		
35 desipramine	0.5	−0.301
37 nortriptyline	1.8	0.255
39 protriptyline	1.1	0.041
Tertiary amines		
36 imipramine	2.6	0.415
38 amitriptyline	5.4	0.732
40 *N*-methylprotriptyline	4.5	0.653



Structures of compounds listed in Table 3

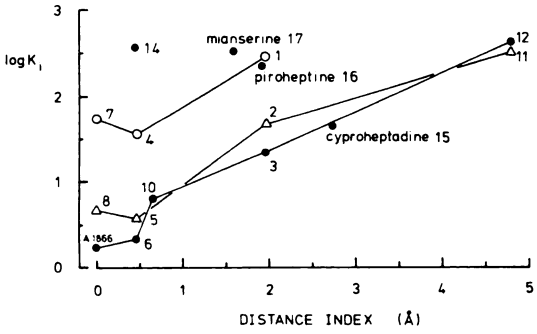


FIG. 2. *Correlation of reciprocal activity (log K_i) of the most rigid tricyclic amines with their structural similarity (distance index) to the spiro compound A 1866*

○, primary amines; △, secondary amines; ●, tertiary amines. In the series of rigid spiro compound the primary amines are on the average 20 times less active than, and the secondary amines half as active as, the corresponding tertiary amines.

ities and distance indices in the three amine series are clearly evident. Three of the very rigid compounds, i.e., the non-spiro compounds **14**, **16**, and **17**, fall outside the correlation of the tertiary spiro compounds. They belong to the inactive group of compounds that bear a false structural similarity to A 1866. This is especially pronounced

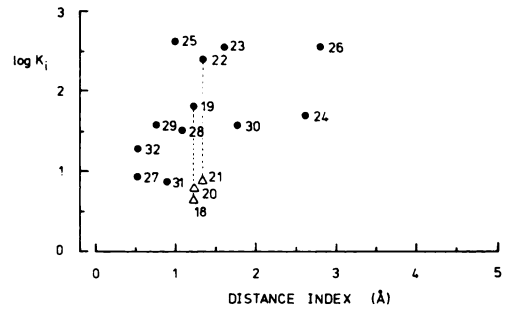


FIG. 3. *Correlation of reciprocal activity (log K_i) of semirigid tricyclic amines with their structural similarity (distance index) to A 1866*

△, secondary amines; ●, tertiary amines. Note the great differences in activity between the corresponding secondary (**18**, **21**) and tertiary (**19**, **22**) amines.

for the aminocyclohexylidene derivative **14**, which has the 6-membered ring perpendicular to that of the spirocyclohexylamine A 1866.

Correlations between activity and structural resemblance to A 1866 for 15 semirigid compounds (Fig. 3) are similar. Here also three of the compounds, **22**, **25**, and **26**, were less active than their DI values would indicate. It is interesting that two of these are the saturated derivatives of the rigid amitriptyline analogue **14** (see above).

In Fig. 4 the activities of all the tertiary amines have been correlated with their DI values, using A 1866 as the reference structure. The activities of the open-chain compounds, which are tertiary amines, are confined to the hatched area. The dashed line through compounds **6** and **12** indicates the structural border of an area where no tricyclic uptake inhibitors are found.

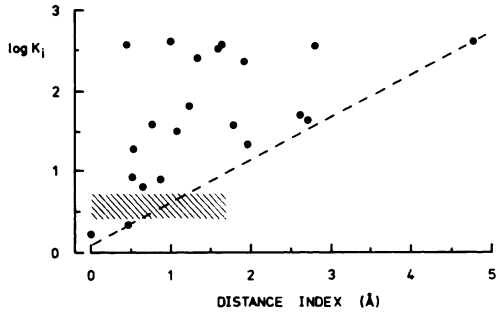
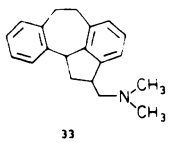


FIG. 4. *Plot of reciprocal activity (log K_i) of rigid and semirigid tertiary amines vs. distance index of the most potent spiro compound, A 1866* (**9**)

The hatched area indicates the activities of tricyclic reference compounds bearing a flexible tertiary alkylamine chain. The dashed line indicates the border of the structural area where no tricyclic uptake inhibitors were found.

The four structural parameters were regarded with equal significance. This gives a linear correlation with $r = 0.69$ ($n = 21$). Variance analysis of the individual parameter differences ($X_j - X_0$), however, reveals some interesting properties of the parameters selected in this study. The correlation found is explained about 80% by the third variable ($X_i - X_{i0}$, $i = 3$) alone; that is, the relative height of the nitrogen atom over the plane of the nearest aromatic ring compared with that found in the A 1899 molecule seems to be the most important structural feature. The residual correlation lies mainly in the distance parameter X_1. The significance of this result is, of course, dependent on the distribution of the parameter values. In the X_4 case, only the convex dibenzocycloheptene derivatives and the flat fluorene derivatives were available, thus giving a very poor basis for the correlation.



33

Spiro[10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene-5,3′-piperidine], **13**, and the semirigid 2-dimethylaminomethyl-1, 6, 7, 11*b*-tetrahydro-2*H*-dibenzo[*c,d,h*]azulene, **33**, were included in the tables without being available for test. Both compounds have been reported to be devoid of psychotropic activity (11, 12). Poor inhibitory potency toward norepinephrine accumulation is also predicted by their calculated DI values (DI_{13} = 2.4 A). The tetrahydroazulene **33** can exist in two isomeric forms, one of which (*trans*) has the nitrogen atom located in a position somewhat resembling that of A 1866. Still, its calculated distance index (DI_{33} = 2.2 A) indicates a poor adaptation to the optimal structure. The other form (*cis*), whose amino group is situated below the plane of the reference benzene ring, is completely incompatible with the structure of the active rigid compounds. In cases when several conformers seemed to be of equal probability, that conformer was chosen which had the greatest correspondence to the optimal structure.

Amine substitution. In Fig. 2 the activities (log K_i) of the most rigid compounds are correlated with the distance indices of A 1866. The regression lines for the primary, secondary, and tertiary amines in the spiro series were calculated (slope, intercept, and correlation coefficient): primary amines, 0.418, 1.575, and 0.910; secondary amines, 0.411, 0.609, and 0.977; tertiary amines, 0.490, 0.316, and 0.988. The tertiary amines are on the average 2.0 and 18.2 times more potent than the secondary and primary amines, respectively. Also, when individual structures are examined, the tertiary amine analogues of the rigid compounds are more active than the corresponding secondary amines (see DISCUSSION). In Fig. 3 the corresponding secondary and tertiary amines analogues are indicated by dashed lines. Note the greatly enhanced potencies (25-fold) of the secondary amines **18** and **21**.

Spirocyclohexylamine A 1866. Figure 4 shows the correlation between activity of the tertiary amines and their structural deviation from A 1866. The reciprocal activity (log K_i) is plotted against the distance index. A search for the optimal parameter values was performed by systematically varying the reference parameters for the 13 most active tertiary amines and minimizing the deviations from the regression line. This operation, however, did not yield results any different from the initial set of parameter values. This indicates that the apparent optimal conformation of a tricyclic in-

hibitor of norepinephrine accumulation is that of A 1866. In this molecule the amino group is situated 5.36 A from the center of the proximal benzene ring (X_1). The nitrogen atom is located 3.98 A above the plane of the previously mentioned aromatic nucleus (X_3) and 2.41 A from the plane of symmetry bisecting the tricyclic skeleton (X_2). The position of the center of one benzene ring is 2.59 A below the level of the other benzene ring (X_4), which corresponds to an angle of 120° between the two aromatic nuclei (13).

Norepinephrine. Theoretical calculations of the conformation of norepinephrine have made it possible to predict the rotamer populations, and these results are in fair agreement with the results obtained from NMR measurements in aqueous solution (12). Preferred conformations are found with the phenethylamine in the *gauche* and *anti* forms (Fig. 5). The magnitude of the energy differences between these rotamers, however, are very small (less than 1 kcal/mole) compared with the proposed forces involved in a receptor interaction (14).

In norepinephrine two of the structural parameters are identical with those of the tricyclic compounds: the distance X_1 between the nitrogen atom and the center of the proximal benzene ring, and the level X_3 over the same aromatic nucleus where the amino group is positioned. Figure 5 shows the degree of adaptation of the rigid compounds in Table 1 to the *gauche* and *anti*

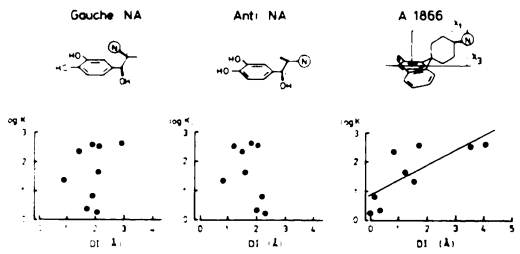


FIG. 5. *Correlation of reciprocal activity (log K_i) of nine rigid tertiary amines* (3, 6, 9, 10, 12, 14–17) *with structural features, in terms of a limited distance index (DI), of the gauche conformation of norepinephrine (NA) (X_1 = 4.1, X_3 = 2.4), the anti conformation of norepinephrine (X_1 = 5.2, X_3 = 1.7), and spiro compound A 1866 (X_1 = 5.4, X_3 = 4.0)*

Correlation coefficient r = 0.77.

conformations of norepinephrine as defined by Horn and Snyder (15) in terms of the steric parameters X_1 and X_3. Neither of them correlated with the structures of these antagonists. When correlated with the X_1 and X_3 values of the spiro compound A 1866, however, a linear relationship was found (correlation coefficient = 0.77).

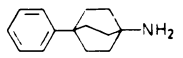
DISCUSSION

It is presumed here that tricyclic antidepressant agents, including the rigid compounds of this study, inhibit the synaptosomal accumulation of norepinephrine at the membrane level. The competitive nature of this inhibition (16) and the poor norepinephrine-releasing potency (17) support this presumption. It is also assumed that norepinephrine binds to a specific carrier at the membrane and that this complex is translocated across the membrane (1). The competitive inhibition indicates that the tricyclic antidepressants participate in a drug-receptor interaction and bind to an active site of the carrier. It has been suggested that inhibitors of norepinephrine uptake, including the tricyclic agents, like the solute (norepinephrine), form bonds to the carrier from one aromatic ring to a hydrophobic surface and from the terminal ammonium group to a negatively charged site in the same plane (3, 18). One of the objectives of the present study was to examine this hypothesis utilizing rigid and semirigid analogues of the tricyclic antidepressants and thereby to obtain some information on the topology of the active site of the norepinephrine carrier.

In the approach employed, it is assumed that structural features of importance for binding to the carrier are the lone pair electrons of the terminal nitrogen atom and the two benzene rings of these compounds (19). It is also assumed that in the case of asymmetrical molecules it is the aromatic ring adjacent to the amino group which mimics the catechol ring of norepinephrine. On these assumptions the four structural parameters (X_1, X_2, X_3, and X_4) were chosen and regarded with equal importance. The observed high potency of the very rigid spiro compound A 1866 justifies the use of the parameter values of this amine as ap-

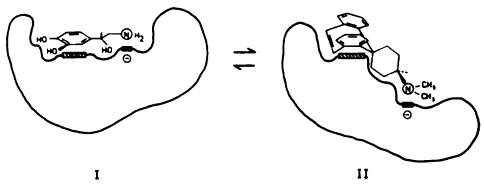
proximately optimal. The observation that there is no potent inhibitor in this series of rigid tricyclic compounds with parameters deviating appreciably from those of A 1866 shows the importance of these structural requirements for activity. However, the four structural parameters do not contain all the required structural information, since several compounds did meet the proposed optimal requirements without being very active. Other factors, including physical properties, steric hindrance, and the nature and the bulkiness of the connective aliphatic link between the amino group and the tricyclic nucleus, must be considered of importance in modulating the inhibitory activity.

By applying this approach we have defined some structural and conformational requirements for inhibition of norepinephrine accumulation in hypothalamic synaptosomes by tricyclic agents. It is obvious that a receptor topology approximately complementary to the structural features of *N,N*-dimethylspiro[5*H*-dibenzo[*a,d*]cycloheptane-5,1′-cyclohexyl]-4′-amine, A 1866, is not compatible with the receptor topology suggested by Maxwell *et al.* (18). The very potent (K_i = 1.2 × 10^{-8} M) and competitive inhibitor 1-amino-4-phenylbicyclo[2.2.2]octane (EXP 561) (20, 21),[1] in which the position of the amino group is completely limited to the same plane as the benzene ring, is, on the other hand, compatible with the concept of a flat receptor surface. The finding in the present study that the optimal conformation of the tricyclic antidepressants for inhibition of norepinephrine uptake is incompatible with the *anti* or *gauche* form of norepinephrine indicates that the receptor surface to which these inhibitors are attached is different from that to which norepinephrine is bound. There are three possible explanations of this observation. One is that the tricyclic nucleus is bound to a different site on the carrier than is the aromatic ring of the monophenylalkylamines, including norepinephrine, but that the binding site for the ammonium group is the same (19). An argument against this explanation is that the structure-activity relationship for amino substitution is different for these two types of inhibitors. Thus, in the mono- and diphenylalkylamine series, the primary amines are at least as potent as the secondary methylamino derivatives (3, 22), whereas the secondary or tertiary amine derivatives are considerably more potent than the primary amines in the tricyclic series (3, 23).



EXP 561

An interesting alternative explanation of the observed difference in the structural requirements for inhibition of norepinephrine uptake is that the receptor for the norepinephrine carrier might exist in two different conformational states, one approximately complementary to EXP 561 (and norepinephrine) and the other complementary to A 1866 but not to norepinephrine (Fig. 6). It appears likely that conformational change is a part of the functional activity of a transport carrier when binding and releasing the solute. It is accordingly possible that the inhibitor EXP 561 is bound to the conformational state that normally binds norepinephrine for transport, whereas the tricyclic agents are bound to the conformation in which the norepinephrine molecules are ejected. By occupying the carrier in this conformational state, the tricyclic agents prevent norepinephrine molecules from being transported across the membrane, giving apparent com-



I II

FIG. 6. *Tentative illustration of hypothetical conformational change of receptor for the norepinephrine carrier*

I. "Catecholamine conformation," depicted here as a planar surface to which the solutes, e.g., norepinephrine, and some inhibitors, e.g., EXP 561, are attached. II. "Tricyclic antagonistic conformation," depicted as complementary to the structure of the spiro compound A 1866.

[1] Unpublished observations.

petitive antagonism. The receptor topology outlined in Fig. 6 is also supported by the findings from statistical analysis that the height over the benzene ring (X_3) accounts for most of the linear correlation.

The third possible explanation is that the tricyclic agents are attached to the carrier at a completely different site from that binding norepinephrine, thereby inducing an allosteric transition that locks the carrier in a conformational state unable to bind norepinephrine. It is not possible from the information now available to decide which of these latter explanations is correct.

A further observation is that in the spirocycloalkylamine series tertiary amines having a methine carbon atom as the adjacent group were about twice as potent as the corresponding secondary amines. In the compounds having a methylene carbon atom adjacent to the amino group the opposite activity relationship was found. It remains to be determined whether a similar relationship also exists for the open-chain tricyclic amines.

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