Decalin Analogs of Phenethylamines as Inhibitors of Amine Uptake by Rabbit Platelets I: Uptake and Distribution of Histamine

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Abstract □ The importance of conformation with regard to the inhibition of histamine uptake by platelets was studied utilizing different conformationally rigid and semirigid inhibitors. Rabbit blood platelets were incubated in plasma for 80 min with 10^{-6} *M* ^{14}C-histamine, and the total accumulation of radioactivity with or without inhibitors was determined. Four different series were studied. The parent substances were β-phenethylamine, *N*-isopropyl-β-phenethylamine, β-hydroxy-β-phenethylamine, and *N*-isopropyl-β-hydroxy-β-phenethylamine. These structures were made conformationally rigid by incorporating the functional groups into the 2- and 3-positions of the *trans*-decalin molecule. The semirigid systems were studied using cyclohexane and *erythro*- and *threo*-butane analogs of the parent compounds. In three of the four decalin series, the most active conformation was found to have an axial phenyl and equatorial amino group. In one series, both phenyl and amino groups were equatorial in the most active isomer. Thus in all cases the active conformation possessed a *gauche*-relationship. The semirigid systems did not give consistent results, which could be due partly to their flexibility which makes it impossible to deduce the principal conformation in the binding mode. Most substances studied were racemic mixtures; the *d*- and *l*-forms of amphetamine were tested separately, and the *d*-form was slightly more active at low concentrations. The preferred conformation for uptake inhibition in most cases appears to have the phenyl and amino groups at a dihedral angle of approximately 60°, but the differences are not outstanding, which may at least in part be due to the addition of a bulky lipophilic group to the phenethylamine structures.

Keyphrases □ Phenethylamines, decalin analogs—conformationally rigid and semirigid inhibitors of amine uptake by rabbit platelets, uptake and distribution of histamine □ Histamine uptake inhibitors—conformationally rigid and semirigid inhibitors, decalin analogs of phenethylamines, uptake and distribution of histamine by rabbit platelets □ Decalin analogs of phenethylamines—uptake and distribution of histamine by rabbit platelets, conformation effects □ Structure-activity relationships—decalin analogs of phenethylamines–histamine inhibition, uptake and distribution of histamine by rabbit platelets

As a step to the characterization of the chemical structure of receptors, one recent approach has been to determine the stereochemical requirements for the effective agonist or competitive antagonist. This approach can help to ascertain the distances between the active groups of the receptor molecule, to predict some details of the receptor surface, to help explain differences in the effects of closely related substances, and to predict the efficacy of a certain drug on that particular receptor.

A great amount of work has been reported on both cholinergic and adrenergic receptors (1). The clear difference between *d*- and *l*-enantiomers of norepinephrine as adrenergic agonists is well known, and some attempts have been made to determine the possible conformational requirements. In addition to the binding to adrenergic receptors, there appear to be stereochemical requirements for norepinephrine reuptake as well. In rat heart, *l*-norepinephrine is more effectively taken up than *d*-norepinephrine (2), and the *d*-enantiomer of amphetamine is a more potent norepinephrine uptake inhibitor than the *l*-form (3). Likewise, one of four possible methylphenidate stereoisomers is by far the most active in inhibiting norepinephrine uptake by aortic strips (4). Some studies using conformationally rigid molecules were performed recently on norepinephrine uptake by various sympathetically innervated tissues[1].

In the present study an attempt was made to determine the possible stereoselectivity of another kind of uptake, that of biogenic amines by blood platelets. Histamine, which is probably taken up by the same mechanism as 5-hydroxytryptamine and catecholamines (5), was chosen as the indicator; the inhibition of its uptake by various compounds was measured. Unlike the monoamines, histamine is not metabolized or only slowly metabolized by rabbit platelets[2]. Thus, it is easy to measure changes in its intracellular distribution and to calculate whether the inhibition of uptake is taking place on the outer membrane of the cell or at the granular level (6).

Monoamines are not as suitable for the purpose because mitochondrial monoamine oxidase effectively metabolizes the portion accumulating in the cytoplasma if the granular uptake is blocked (7). However, histamine is metabolized by plasma histamine and if uptake inhibitors also have an effect on the metabolism, care must be taken in interpreting the results. In some cases, results of histamine uptake were compared with those of 5-hydroxytryptamine uptake to exclude secondary effects of metabolism.

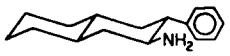
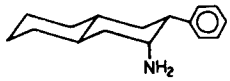
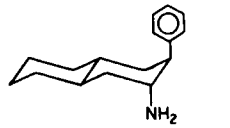
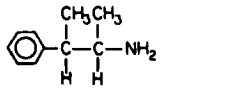
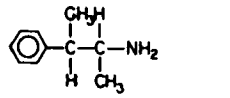
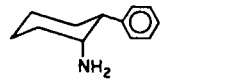
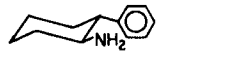
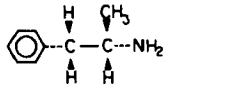
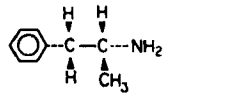
EXPERIMENTAL

Male albino rabbits, 1.8–3.0 kg, were bled, and platelet-rich plasma was prepared as previously described (8). Radioactive histamine was added to ice-cold plasma to give the final concentration of 10^{-6} *M*. Two-milliliter portions of plasma were immediately placed in polypropylene incubation tubes, which contained the substances to be tested in 0.2 ml saline or, in the case of poorly water-soluble substances, in 3–33% dimethyl sulfoxide in saline. The control samples were always incubated with the corresponding solvent. Dimethyl sulfoxide did not interfere to any detectable extent with the uptake (0–10% difference between the dimethyl sulfoxide control and the saline control).

After 80 min of incubation at 37° in oxygen with 5% CO_2, the platelets were sedimented by centrifugation for 30 min at 2000×*g* or for 5 min at 20,000×*g* and then were washed once with 2 ml of saline to remove the extracellular radioactivity. Excess saline

[1] M. Hava, E. E. Smissman, and S. El-Antably, in preparation.
[2] Unpublished results.

Table I—Inhibition of ^{14}C-Histamine Uptake in Percent of Control by β-Phenethylamine-like Compounds Made Conformationally Rigid by Incorporating Them into a *trans*-Decalin Structure[a]

Inhibitor	Structure	10^{-5} *M*	10^{-4} *M*	10^{-3} *M*
1. 2(e)-Amino-3(e)-phenyl-*trans*-decalin		24.2 ± 2.2 (6)	49.7 ± 2.5 (8)	94.8 (3)
2. 2(a)-Amino-3(e)-phenyl-*trans*-decalin		20.4 ± 1.1 (5)	38.0 ± 2.4 (8)	92.4 (3)
3. 2(e)-Amino-3(a)-phenyl-*trans*-decalin		8.6 ± 1.3 (5)	29.1 ± 2.4 (8)	77.9 (3)
4. 2(a)-Amino-3(a)-phenyl-*trans*-decalin		17.5 ± 0.7 (6)	38.7 ± 1.8 (8)	94.6 (3)
5. *erythro*-2-Amino-3-phenylbutane		19.9 ± 2.0 (5)	48.6 ± 2.1 (6)	77.3 (1)
6. *threo*-2-Amino-3-phenylbutane		13.2 ± 3.3 (5)	35.7 ± 3.1 (6)	69.3 (1)
7. *cis*-1-Amino-2-phenylcyclohexane		8.3 ± 0.7 (4)	33.0 ± 1.8 (5)	69.2 (2)
8. *trans*-1-Amino-2-phenylcyclohexane		11.8 ± 1.1 (4)	35.6 ± 1.5 (5)	71.0 (2)
9. *d*-(*S*)-Amphetamine		25.4 ± 2.6 (3)	51.8 ± 1.5 (6)	79.1 ± 1.5 (4)
10. *l*-(*R*)-Amphetamine		21.3 ± 1.3 (6)	52.8 ± 1.6 (6)	80.8 ± 1.7 (4)

[a] Platelets were incubated for 80 min at 37° under an atmosphere of oxygen with 5% CO_2. Values are means ± standard error. Number of experiments is given in parentheses.

above the platelet pellet was removed by wiping with filter paper. Washed platelets were solubilized[3], 5 ml of scintillation fluid [0.4% diphenyloxazole and 0.01% 1,4-bis(5-phenyloxazol-2-yl)benzene in toluene] was added, and the radioactivity was counted[4]. Results are given as percent inhibition compared with a control sample incubated with the solvent under the same conditions. All experiments were performed in duplicate, and the difference between duplicates was usually 0–2%.

For intracellular studies, 5-ml samples were incubated as described. Four milliliters of 0.32 *M* sucrose was pipetted onto the final washed platelet pellets and the platelets were homogenized ultrasonically[5]. Unbroken cells and other larger particles were sedimented at 2500×*g* for 20 min, and a coarse granular fraction was sedimented at 18,500×*g* for 30 min (average *g* values) (9). The two sediments and the final supernate were extracted with butanol according to the histamine assay of Shore *et al.* (10). To 0.5 ml of the final acid phase, 5 ml of scintillation fluid (11) [0.4% diphenyloxazole and 0.01% 1,4-bis(5-phenyloxazol-2-yl)benzene in equal parts of toluene and ethylene glycol monoethyl ether] was added, and the radioactivity was counted as described. In all cases at least 10,000 counts and in most at least 20,000 counts were made.

Partition Studies—The methanesulfonate salt was dissolved in 50 ml of Sørensen's phosphate buffer (pH 7.30), previously washed with the appropriate organic solvent (heptane or octanol). In the cases where the free amine was used, excess methanesulfonic acid was added to solubilize the amine and the pH was adjusted to 7.30 using 0.1 *N* NaOH.

These solutions were placed in a 60-ml separator containing a pipetted sample of an organic solvent (heptane or octanol) previously shaken with the buffer. The solutions were shaken mechanically for 22 hr. Concentrations of amines before and after shaking were determined spectrophotometrically[6]. The apparent partition coefficients were then determined by:

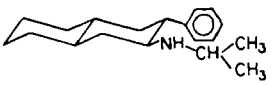
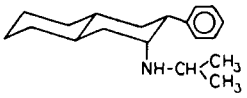
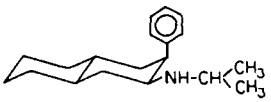
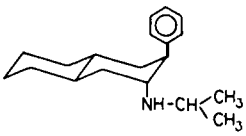
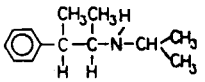
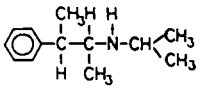
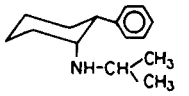
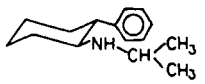
$$K_{app} = \frac{C_{init} - C_{final}}{C_{final}} \times \frac{V_w}{V_{org}} \qquad \text{(Eq. 1)}$$

where K_{app} = apparent partition coefficient, C = concentration in water layer, V_w = volume of water layer, and V_{org} = volume of organic layer.

Chemicals—The *d*- and *l*-forms of amphetamine sulfate[7], β-phenethylamine hydrochloride[8], β-hydroxy-β-phenethylamine (2-

[3] Soluene 100.
[4] Packard Tri-Carb scintillation counter.
[5] Sonifier cell disruptor, model W140D, microtip, setting 3, 1 min.
[6] Cary 14 spectrophotometer.
[7] Sigma Chemical Co.
[8] Calbiochem.

Table II—Inhibition of ^{14}C-Histamine Uptake by *N*-Isopropyl-β-phenethylamine-like Compounds[a]

Inhibitor	Structure	10^{-5} *M*	10^{-4} *M*	10^{-3} *M*
12. 2(e)-Isopropylamino-3(e)-phenyl-*trans*-decalin		18.0 ± 1.7 (5)	41.7 ± 2.0 (7)	86.2 (3)
13. 2(a)-Isopropylamino-3(e)-phenyl-*trans*-decalin		15.7 ± 2.1 (5)	32.0 ± 2.3 (7)	62.2 (3)
14. 2(e)-Isopropylamino-3(a)-phenyl-*trans*-decalin		16.5 ± 1.9 (5)	60.1 ± 1.8 (7)	95.6 (3)
15. 2(a)-Isopropylamino-3(a)-phenyl-*trans*-decalin		14.8 ± 1.7 (5)	36.9 ± 2.1 (7)	92.3 (3)
16. *erythro*-2-Isopropylamino-3-phenylbutane		12.3 ± 1.5 (6)	31.4 ± 2.3 (7)	54.4 ± 2.8 (4)
17. *threo*-2-Isopropylamino-3-phenylbutane		13.3 ± 1.5 (6)	33.2 ± 2.4 (6)	56.1 ± 1.9 (4)
18. *cis*-1-Isopropylamino-2-phenylcyclohexane		19.2 ± 1.6 (6)	40.8 ± 1.5 (6)	69.4 ± 1.4 (3)
19. *trans*-1-Isopropylamino-2-phenylcyclohexane		15.4 ± 1.5 (6)	35.7 ± 1.5 (6)	69.0 ± 2.0 (3)

[a] For details see Table I.

amino-1-phenethanol)[9], *l*-norepinephrine[9], histamine (ring-2-^{14}C) hydrochloride[10], and scintillation chemicals[11] were obtained from commercial sources. Other chemicals were synthesized as follows: Compounds 1–8 as methanesulfonates, 12–18 as hydrochlorides, 31–34 by the method of Smissman and Pazdernik (12, 13), 20–23 by the method of Smissman and Gastrock (14), and 24–30 as hydrochlorides by the method of Smissman and El-Antably (15).

RESULTS AND DISCUSSION

Amphetamine-like compounds were effective amine-uptake inhibiting agents. In this model, little evidence was found for any marked difference between the *d*- and *l*-forms of amphetamine, although in low concentrations the *d*-(*S*)-enantiomer was slightly more effective ($p < 0.05$) (Table I). In the semirigid system, β-methylamphetamine (2-amino-3-phenylbutane), the *erythro*-form, was more active (10^{-5} *M*, $p < 0.01$; 10^{-4} *M*, $p < 0.001$), but the difference was not great (Compounds 5 and 6). Because of bulky methyl groups, the transoid conformation of the phenyl ring and nitrogen would be favored for the *erythro*-form, while for the *threo*-form the preferred conformation is *gauche*. In the other semirigid system, with the amphetamine structure incorporated in cyclohexane, there was very little difference ($p > 0.05$) in activity between cisoid and transoid forms (Compounds 7 and 8). In an almost completely rigid system, where the structure is incorporated in *trans*-decalin, the most active isomer has phenyl and amino groups in the diequatorial positions (Compound 1), with the weakest compound (Compound 3) having the phenyl group axial and the amino function equatorial.

N-Isopropyl derivatives of the same substances gave slightly different results (Table II). There was little difference in semirigid systems, although the *threo*-structure seemed to be slightly more potent. In the decalin derivatives the cisoid compound with axial phenyl and equatorial amino was clearly the most effective in higher concentrations, although at 10^{-5} *M* concentration the diequatorial was the most effective.

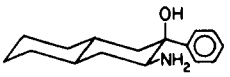
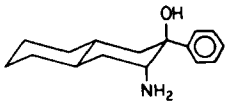
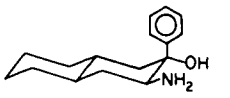
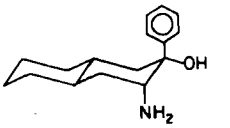
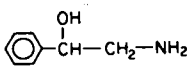
Adding a hydroxy group in the β-position appeared to decrease the effect of these β-phenethylamine derivatives, both in the parent compounds and in the decalin system. Of the β-hydroxy-β-phenethylamine derivatives (Table III), by far the most active compound was the cisoid derivative with the axial phenyl and equatorial amino group. This compound was comparable in potency to the parent compound. The *N*-isopropyl derivatives of these gave similar results (Table IV). Very little difference was found between *erythro*- and *threo*-forms of the parent compound (Compounds 29 and 30).

To determine whether various decalin structures without a complete phenethylamine structure would be active, various substances were studied (Table V). 2-Amino-*trans*-decalins as such were as potent histamine uptake inhibitors as some of the β-phenethylamine derivatives. On the other hand, 2-*trans*-decalol was without any effect and 3(a)-phenyl-2(a)-*trans*-decalol caused inhibition only at the highest concentration used.

To ascertain the localization of action of these compounds, the effects of some of them were studied on ^{14}C-histamine distribution in the platelet (Figs. 1 and 2). Reserpine was used as a substance acting mainly at the granular level. It caused a complete change in the intracellular distribution of histamine at concentrations that inhibit the total uptake only slightly or not at all. Both amphetamines and 2(e)-amino-3(a)-phenyl-3-*trans*-decalol at low concentrations caused an inhibition of uptake and only in higher concentrations did they begin to influence the distribution of histamine.

[9] Mann Research Laboratories.
[10] Amersham/Searle Corp.
[11] Packard Instrument Co.

Table III—Inhibition of ^{14}C-Histamine Uptake by β-Hydroxy-β-phenethylamine-like Compounds[a]

Inhibitor	Structure	10^{-4} *M*	10^{-3} *M*
20. 2(e)-Amino-3(e)-phenyl-3-*trans*-decalol		17.5 ± 1.3 (7)	36.4 ± 3.3 (9)
21. 2(a)-Amino-3(e)-phenyl-3-*trans*-decalol		13.4 ± 3.0 (6)	34.5 ± 3.0 (7)
22. 2(e)-Amino-3(a)-phenyl-3-*trans*-decalol		35.9 ± 3.2 (10)	80.0 ± 2.9 (11)
23. 2(a)-Amino-3(a)-phenyl-3-*trans*-decalol		7.6 ± 3.1 (7)	31.9 ± 3.0 (9)
24. β-Hydroxy-β-phenethylamine		35.4 ± 2.3 (8)	72.5 ± 0.9 (9)

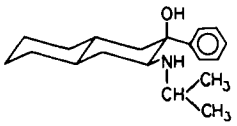
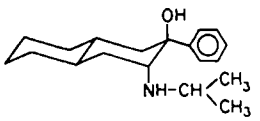
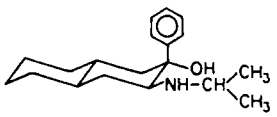
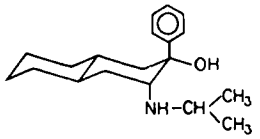
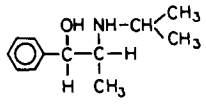
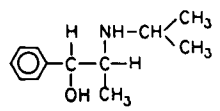
[a] For details see Table I.

It is concluded that they do not inhibit the uptake secondarily due to the inhibition of storage, because reserpine causes much less inhibition of uptake although the distribution change indicating storage failure is quite evident. The phenethylamines studied appear to act primarily on the outer cell membrane. In high concentrations they affect the granules as well, but this effect does not contribute greatly to the inhibition of the total amine uptake.

To check the possibility of platelet damage, electron microscopy was performed. After 80 min of incubation with 10^{-4} *M* 2(e)-amino-3(a)-phenyl-3-*trans*-decalol (Compound 22), no electron microscopic evidence of damage was seen.

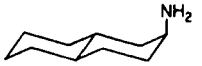
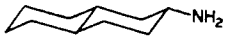
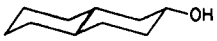
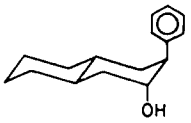
The lipid–buffer partition values of some compounds are presented in Table VI. All decalin derivatives were much more lipid soluble at physiological pH than were the parent phenethylamines. The differences among the stereoisomers are marked. Thus, the solubility and the conformation must be considered for the effect

Table IV—Inhibition of ^{14}C-Histamine Uptake by *N*-Isopropyl-β-hydroxy-β-phenethylamine-like Compounds[a]

Inhibitor	Structure	10^{-4} *M*	10^{-3} *M*
25. 2(e)-Isopropylamino-3(e)-phenyl-3-*trans*-decalol		24.1 ± 1.2 (7)	56.5 ± 0.9 (7)
26. 2(a)-Isopropylamino-3(e)-phenyl-3-*trans*-decalol		22.4 ± 1.1 (7)	56.3 ± 1.3 (7)
27. 2(e)-Isopropylamino-3(a)-phenyl-3-*trans*-decalol		41.2 ± 1.3 (7)	75.2 ± 1.7 (7)
28. 2(a)-Isopropylamino-3(a)-phenyl-3-*trans*-decalol		17.8 ± 1.4 (6)	51.8 ± 1.7 (7)
29. *erythro*-2-(*N*-Isopropylamino)-1-phenylpropanol		27.0 ± 1.1 (8)	52.9 ± 1.5 (11)
30. *threo*-2-(*N*-Isopropylamino)-1-phenylpropanol		24.8 ± 1.7 (8)	56.6 ± 0.8 (11)

[a] For details see Table I.

Table V—Inhibition of ^{14}C-Histamine Uptake by Some Substituted *trans*-Decalins[a]

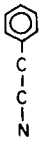
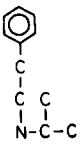
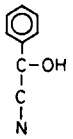
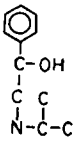
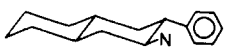
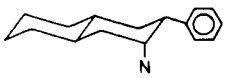
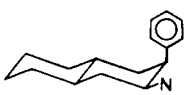
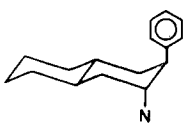
Inhibitor	Structure	10^{-5} *M*	10^{-4} *M*	10^{-3} *M*
31. 3(a)-Amino-*trans*-decalin		14.8 ± 1.4 (6)	30.1 ± 3.2 (6)	60.4 ± 1.7 (3)
32. 3(e)-Amino-*trans*-decalin		15.1 ± 2.6 (5)	42.0 ± 3.1 (6)	76.0 ± 1.7 (5)
33. 3(e)-*trans*-Decalol		—	1.8 ± 1.1 (5)	−3.0 ± 2.6 (5)
34. 3(a)-Phenyl-2(a)-*trans*-decalol		—	2.8 ± 1.1 (5)	16.3 ± 4.9 (7)

[a] For details see Table I.

Table VI—Comparison of Inhibition of ^{14}C-Histamine Uptake with Partition Coefficients

Inhibitor	Partition Coefficient		Inhibition, % at 10^{-4} *M*
	Octanol–Buffer	Heptane–Buffer	
Amphetamine			
9, 10	0.39 ± 0.08	0.051 ± 0.003	51.8 ± 1.5
Butanes			
5. *erythro*	0.59 ± 0.09	0.072 ± 0.005	48.6 ± 2.1
6. *threo*	0.47 ± 0.04	0.040 ± 0.004	35.7 ± 3.1
Hexanes			
7. *cis*	1.51 ± 0.10	0.072 ± 0.022	35.6 ± 1.5
8. *trans*	3.07 ± 0.74	0.25 ± 0.05	33.0 ± 1.8
Decalins			
1. NH_2 = e, C_6H_5 = e	>100	6.75 ± 0.77	49.7 ± 2.5
2. NH_2 = a, C_6H_5 = e	50.9 ± 5.4	10.0 ± 0.55	38.0 ± 2.4
3. NH_2 = e, C_6H_5 = a	5.1 ± 2.0	1.51 ± 0.1	29.1 ± 2.4
4. NH_2 = a, C_6H_5 = a	69.0 ± 10.5	3.49 ± 0.37	38.7 ± 1.8
20. NH_2 = e, C_6H_5 = e	58.6 ± 9.3	—	17.5 ± 1.3
21. NH_2 = a, C_6H_5 = e	>300	—	13.4 ± 3.0
22. NH_2 = e, C_6H_5 = a	∞	—	35.9 ± 3.2
23. NH_2 = a, C_6H_5 = a	47.1 ± 2.1	—	7.6 ± 3.1

Table VII—Summary of Uptake Inhibition by Compounds with Decalin Structure at a Concentration of 10^{-4} *M* (The Structure of the Phenethylamine Moiety is Shown at the Top and the Conformation at the Left Margin; the Most Active Isomer Is Underlined)

				
	<u>49.7</u>	41.8	17.5	24.1
	38.0	32.0	13.4	22.4
	29.1	<u>60.1</u>	<u>35.9</u>	<u>41.2</u>
	38.7	36.9	7.6	17.8
Parent substance (closest choice)	51.8 52.8	31.4 33.2	35.4	24.8 27.0

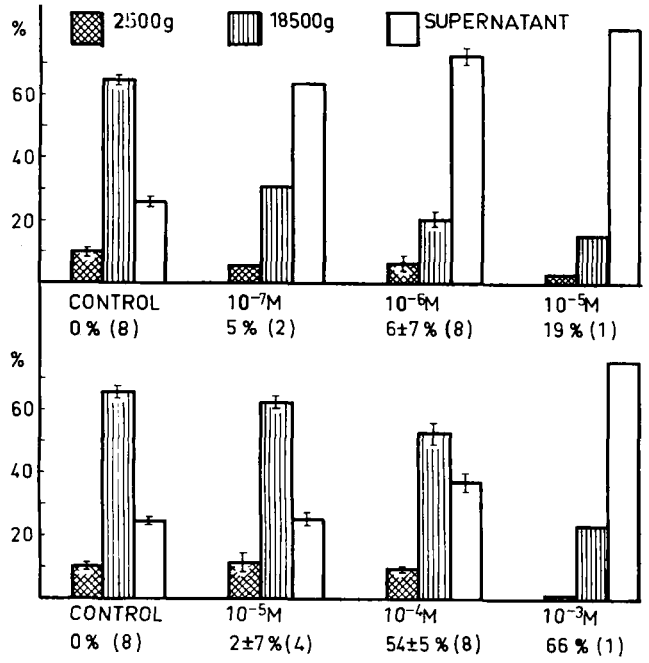


Figure 1—*Intracellular distribution of ^{14}C-histamine in platelets after 80 min of incubation with or without different concentrations of reserpine (upper line) or 2(e)-amino-3(a)-phenyl-3-*trans*-decalol (Compound 22, lower line). Inhibition of uptake in percent is shown below the respective columns. Distribution is given in percent and the sum of three columns in one group is always 100. Values are means ± standard error. Number of experiments is given in the parentheses.*

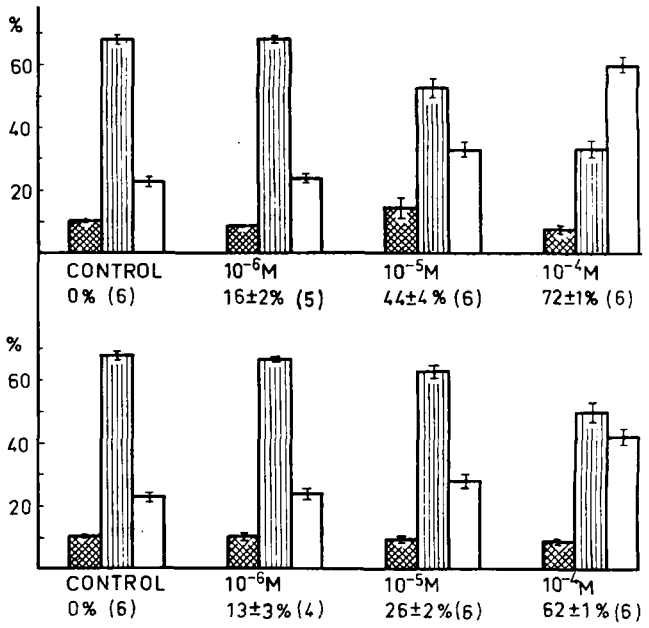


Figure 2—*Intracellular distribution of ^{14}C-histamine in platelets incubated with or without* d-*enantiomer of amphetamine (upper line) or* l-*enantiomer (lower line). Other conditions are as in Fig. 1.*

on the uptake inhibition. Compound 3, which had an exceptionally low inhibitory activity, was less lipid and more water soluble than its isomers.

There are some inherent weaknesses in the present approach. First, analogs of 5-hydroxytryptamine would be the most logical substances to study because the affinity of the parent substance for the platelet carrier is evidently much higher than that of phenethylamines. However, these compounds are difficult to obtain synthetically. Second, as shown by the experiments with 2-amino-*trans*-decalins, even substances without the phenyl ring are moderately effective. Therefore, for each compound there may be two modes of binding to the receptor: one utilizing the phenyl ring and the other the decalin moiety. In those cases where the parent substance is not very active, the "background inhibition" caused by the aminodecalin portion of the analog is so high that the conformational differences are not evident or may be distorted.

Third, the decalin derivatives are very lipid soluble compared with phenethylamines or other biogenic amines. This is probably not crucial in agonist–antagonist studies on the cholinergic or adrenergic receptors, but in uptake inhibition it definitely must be considered, since one is dealing with a transport process through a cell membrane of mainly lipid character. If the inhibitor is very lipid soluble, it may accumulate in the lipid membrane and saturate the carrier to such a degree that it is not available to transport the biogenic amines, which would render the inhibition noncompetitive. This would also cause differences among isomers because the more lipid-soluble isomers tend to be the more active.

Because the affinity of histamine to the carrier seems to be relatively low and the uptake quite slow and because histamine metabolism in plasma might influence the results in the relatively long incubations needed, a full survey of the kinetics of the uptake was impractical. However, some preliminary experiments suggest that the inhibition by decalin derivatives may be noncompetitive. A more detailed study is being performed with ^{14}C-5-hydroxytryptamine as a substrate, incubating it for 1 min with some inhibitors under the conditions described here. The results will be presented separately (16).

Decalin-type inhibitors seemed to cause mainly noncompetitive inhibition. Norepinephrine was clearly competitive and amphetamine mainly competitive, but it appeared to change the V_{max} slightly, possibly indicating some noncompetitive component. This finding could be interpreted as meaning that more lipid-soluble substances tend to cause the noncompetitive type of inhibition. This would also be in agreement with the suggestions (17) that primary binding takes place through the positively charged nitrogen and that the weaker secondary binding might be different in saturated, lipid-soluble hydrocarbons from that of catechol or heterocyclic rings. In the latter case, van der Waals-type forces might be operating; in the first case, hydrophobic binding would be the most probable.

Generally speaking, the results seem to suggest that the decalin derivatives with phenyl and amino groups in *gauche*-conformation are more active (Table VII). In three out of four cases the most active stereoisomer had an axial phenyl and equatorial amino group. In the fourth case the most active was the transoid diequatorial isomer. In all of the aforementioned compounds, the *gauche*-conformation (~60°) between the amino and phenyl groups existed.

Of the β-methylamphetamines, the *erythro*-form was more effective than the *threo*-form. The *N*-isopropyl-β-methylamphetamines gave no significant difference between *threo*- and *erythro*-forms. In the cyclohexane derivatives, no significant difference was observed between the *cis*- and *trans*-forms.

Other studies suggest that substances having phenyl and amino groups held rigidly in a *gauche*-conformation are effectively transported. Debrisoquin is effectively taken up by the platelets and, although it is probably not stored by the platelet granules, a concentration gradient of about 20 is found between the platelet and the incubation medium (18). It also inhibits 5-hydroxytryptamine uptake competitively (19). In preliminary studies[2], a number of tetrahydroisoquinoline derivatives inhibited histamine uptake; harmaline, which has an indole ring instead of a phenyl ring, was even more effective. This may be related to the higher activity of 5-hydroxytryptamine. However, these experiments did not give clear enough results to exclude the activity of *anti*-conformations. If *anti*-compounds are also transported, the carrier must have a flexible binding site to accommodate both *gauche*- and *anti*-conformations, which may have as much as 1.5 Å difference in the distance of the nitrogen from the aromatic ring.

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Decalin Analogs of Phenethylamines as Inhibitors of Amine Uptake by Rabbit Platelets II: Uptake of 5-Hydroxytryptamine

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Abstract □ The steric aspects of the uptake of 5-hydroxytryptamine by rabbit platelets were studied utilizing the conformationally rigid decalin analogs of ephedrine and amphetamine. These analogs or their parent compounds were added to platelet-rich plasma, and their influence on the uptake of ^{14}C-5-hydroxytryptamine was determined along with the kinetics of the process. Among the β-phenethanolamine-type compounds, the isomer having an axial phenyl and equatorial amino function was the most effective inhibitor and the isomer in which the phenyl and amino functions were both axial was the weakest inhibitor of uptake. In the β-phenethylamine series, the same cisoid isomer [(a) phenyl, (e) NH_2] was the weakest inhibitor and the remaining three isomers were equipotent. The most effective cisoid decalin isomer of phenethanolamine displayed a mixed type of inhibition. The inhibition by amphetamine or β-phenethanolamine was also of a mixed type but was closer to competitive than to noncompetitive inhibition. Norepinephrine was a competitive inhibitor. The differences were suggested to be due to different binding of the aromatic ring. It was also suggested that lipid solubility causes the decalin derivatives to accumulate in the cell membrane and bind part of the carrier so that it is inaccessible to the substrate. Different lipid solubility would then explain the inconsistency of the most active conformation in different series. In essence, these results are in agreement with the inhibition of histamine uptake by rabbit platelets, which was earlier studied using a number of rigid and semirigid phenethylamine derivatives.

Keyphrases □ Phenethylamines, decalin analogs—conformationally rigid inhibitors of amine uptake by rabbit platelets, uptake and kinetics of 5-hydroxytryptamine □ Histamine uptake inhibitors—conformationally rigid inhibitors, decalin analogs of phenethylamines (ephedrine and amphetamine), uptake and kinetics of 5-hydroxytryptamine □ Decalin analogs of phenethylamines (ephedrine and amphetamine)—uptake and kinetics of 5-hydroxytryptamine by rabbit platelets, conformation effects □ Structure–activity relationships—decalin analogs of phenethylamines–histamine inhibition, kinetics and uptake of 5-hydroxytryptamine by rabbit platelets

Experiments concerning stereochemical aspects of the inhibition of histamine uptake by platelets have been reported from this laboratory (1). Several amines which were conformationally rigid substituted *trans*-decalins were studied. In general, a cisoid conformation in which the phenyl group is axial and the amine group is equatorial appeared to be more favorable for uptake inhibition. In three out of four cases, the most effective isomer had an axial phenyl and an equatorial amino group. Among β-phenethylamine-like decalin isomers, the axial phenyl and equatorial amino isomer was the least active; the most active had a diaxial structure.

To determine whether these findings were due to