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Conformational Preferences of Amphetamine Analogues for Inhibition of Phenylethanolamine *N*-Methyltransferase

Conformationally Defined Adrenergic Agents. 5

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SUMMARY

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A series of conformationally defined amphetamine analogues was examined for their ability to inhibit competitively the enzyme phenylethanolamine *N*-methyltransferase (PNMT). Among these were compounds based on the benzobicyclo-[2.2.1]heptene and the benzobicyclo-[2.2.2]octene structures which, depending upon the point of the attachment of the amino group, closely approximate a *trans* antiperiplanar or a *gauche* conformation of amphetamine. Some more flexible conformationally restricted amphetamine analogues which had been previously evaluated as inhibitors of PNMT were included in the study for comparison. In all cases, those compounds which mimicked the *gauche* conformation of amphetamine failed to inhibit competitively PNMT, whereas compounds which approximated a fully extended (*trans* antiperiplanar) conformation showed significant inhibition. Therefore, the indication from the data is that phenylethylamines must be able to achieve a fully extended conformation of the side chain in order to bind to the PNMT active site.

INTRODUCTION

The enzyme PNMT[3] (EC 2.1.1.28) catalyzes the methylation of norepinephrine to produce the neurotransmitter-hormone epinephrine, utilizing SAM as the methyl donor (1). Reports that β-phenylethylamines (including amphetamines) and benzylamines competitively inhibit PNMT prompted the development of several potent PNMT inhibitors based on these structures (for reviews on PNMT inhibitors, see refs. 2 and 3). Among the most potent of these inhibitors are 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline (SKF-64139) (4), 6,7-dichloro-2-aminotetralin (5), 4,5-dichloro-1-aminoindan (6), and 8,9-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine (7), all of which are conformationally restricted analogues of amphetamine (1; Fig. 1) and/or benzylamine. Tranylcypromine (*trans*-2-phenylcyclopropylamine) is another conformationally defined amphetamine analogue, with good inhibitory activity against PNMT. 8-Amino-1,2-methanoindan (SKF-9620-A), a rigid analogue of tranylcypromine, is only slightly less potent (8). The higher activity of these compounds as compared with amphetamine and benzylamine contrasts with the relatively low potency of *cis*-2-phenylcyclopropylamine (8) and strongly suggests a conformational preference for PNMT binding of amphetamine.

Nearly all of the compounds mentioned above retain some conformational flexibility and, therefore, can only be used to define a range within which the most active conformation falls. 2-Aminotetralin, for example, is capable of assuming the conformational equivalents of both the antiperiplanar (extended) and *gauche* (folded) conformations of amphetamine and all conformations in between. 2-Aminoindan, an amphetamine analogue also reported to be a potent PNMT inhibitor (5), is only slightly more restricted in the range of conformations which it can assume. The side chain of tranylcypromine is firmly locked into a conformation ($\tau_2 \simeq 150°$) between an anti ($\tau_2 = 180°$) and *gauche* ($\tau_2 = 60°$) conformation, but the phenyl ring is unrestricted and can rotate freely (τ_1). Such flexibility makes it difficult to reach any con-

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[3] The abbreviations used are: PNMT, phenylethanolamine *N*-methyltransferase; SAM, *S*-adenosylmethionine.

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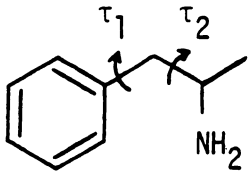

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FIG. 1. *Amphetamine (α-methylphenylethylamine)*
τ_1 and τ_2 refer to the angle of rotation about the two carbon-carbon bonds as shown.

clusions regarding the conformational aspects of PNMT binding.

Our interest in probing the conformational requirements for the various pharmacological actions of amphetamine led us to prepare several conformationally defined analogues based on the benzobicyclo-[2.2.1]heptene and the benzobicyclo-[2.2.2]octene skeletons (Fig. 2). The 2-*exo* isomers **2–4** very closely approximate the *trans* (extended) conformer of amphetamine, and the *endo* isomers **5–7** resemble a *gauche* conformation, both of which are the two most stable and abundant conformations in solution (9, 10). These structures provide excellent conformational definition, restricting both the side chain and the phenyl ring by the addition of only a few extra atoms. Important physical properties of these compounds (pK_a, partition coefficient in octanol/pH 7.4 buffer) differ only slightly from those of amphetamine (11).

Compounds **2–7** can also be regarded as conformationally defined analogues of 2-aminotetralin (**10**), mimicking the two possible boat conformations which **10** can assume. As such, these compounds can be useful in determining whether one of the two boat conformations or the more stable half-chair conformation is most important in PNMT binding.

The 9-substituted Compounds **8** and **9** are conformationally very similar to the 2-substituted Compounds **2–7.** Here, however, the side chain has been shifted slightly in line with the center of the phenyl ring, resulting in a symmetrical molecule. Because of the symmetry present in Analogues **8** and **9**, the stereoselectivity of PNMT for one enantiomer of Compounds **1–7** will not be present. In addition, Compounds **8** and **9** are conformationally defined analogues of 2-aminoindan **11.**

The side chain hydroxyl group of phenylethanolamine is reported to be an absolute requirement for substrate activity (12), implying the presence of an appropriate binding site, most probably a hydrogen-bonding functional group. Compounds **4** and **7** were evaluated as inhibitors of PNMT on the assumption that the bridging oxygen, which can act as an H-bond acceptor, could interact with this binding site. Such contribution to binding of either Compound **4** and **7** to PNMT should be reflected in a lower K_i compared with either Compound **2** or **5.** It should be pointed out that the side chain hydroxyl might act primarily as the H-bond donor at the binding site, in which case the bridging oxygen could not bind even if it were in the correct orientation. However, any positive contribution of the oxygen bridge to binding compared with the carbon-bridged analogues could be construed as evidence of the location of the hydroxyl binding site relative to the other functional group binding sites of the substrate.

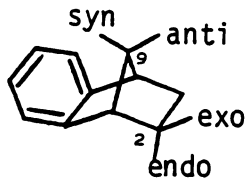
We have examined Compounds **2–9** as inhibitors of PNMT. Several compounds which had been studied previously (**10–14**; Table 1) were also included to eliminate interassay variability and to allow direct comparison of the activities of a wide range of amphetamine analogues.

MATERIALS AND METHODS

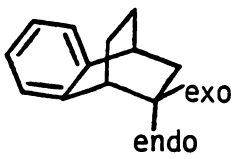
2-Aminoindan hydrochloride was purchased from Aldrich Chemical Company, Inc. (Milwaukee, Wisc.). All other compounds in the study were synthesized in our laboratory according to published procedures and purified by crystallization of their salts (13–21). Solutions of these compounds in water or in assay buffer were prepared and stored frozen prior to assay. All compounds were racemic.

PNMT was isolated as a partially purified preparation from bovine adrenal glands by the method of Connett and Kirshner (22).

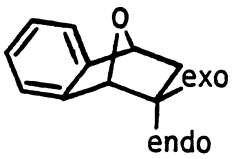
Inhibition of PNMT was determined by radiochemical assay with β-phenylethanolamine as the variable substrate. A typical incubation mixture consisted of 5 to 25 μl of 4 mM β-phenylethanolamine, 25 μl of a 10 mM solution of cold SAM (Sigma Chemical Company, St. Louis, Mo.), 5 μl of 0.8 mM [^{14}C]SAM (New England Nuclear Corporation, Boston, Mass.), variable amounts of inhibitor, 50 μl of partially purified PNMT preparation (~30 mg/ml of protein concentration), and phosphate



2: 2-exo-NH_2
5: 2-endo-NH_2
8: 9-anti-NH_2
9: 9-syn-NH_2



3: 2-exo-NH_2
6: 2-endo-NH_2



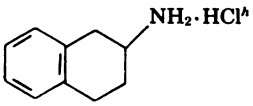
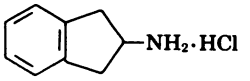
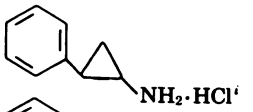
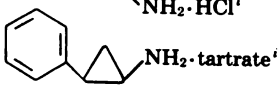
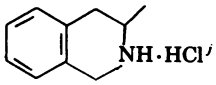
4: 2-exo-NH_2
7: 2-endo-NH_2

FIG. 2. *Conformationally defined bicyclic amphetamine analogues illustrating position of attachment of the amino groups*

TABLE I

Inhibition constants ± standard error for a series of conformationally restricted amphetamine analogues

Structure for 1 is given in Fig. 1. Structures for 2 through 9 are given in Fig. 2

Compound[a]	K_i ± SEM	Compound	K_i ± SEM
	μM		μM
1 Amphetamine·HCl	740 ± 82	10 	15 ± 2
2 ·HCl[b]	479 ± 27		
3 ·HCl[c]	1296 ± 170	11 	12 ± 1.5
4 ·HCl[d]	1297 ± 288		
5 ·HCl[b]	6721 ± 863	12 	67 ± 10
6 ·HCl[c]	10188 ± 1997		
7 ·HCl[e]	9616 ± 3420	13 	920 ± 85
8 ·HCl[f]	258 ± 27		
9 ·HCl[g]	8520 ± 1730	14 	5 ± 0.7

[a] All compounds were prepared as crystalline racemic salts.
[b] Preparation described in ref. 13.
[c] Preparation described in ref. 14.
[d] Preparation described in ref. 15.
[e] Preparation described in ref. 16.
[f] Preparation described in ref. 17.
[g] Preparation described in ref. 18.
[h] Preparation described in ref. 19.
[i] Preparation described in ref. 20.
[j] Preparation described in ref. 21.

buffer (pH 8) to a final volume of 0.25 ml. The reaction was stopped after 30 min at 37° by the addition of 0.5 M borate buffer (pH 10), and the solution was extracted with 2 ml of toluene/isoamyl alcohol (7:3). After mixing, the phases were separated by centrifugation and 1.0 ml of the organic phase was transferred to a vial containing 10 ml of scintillation cocktail for counting. At least four different concentrations of substrate were used at each of four different inhibitor concentrations for determination of kinetic constants. Data used for Lineweaver-Burke analysis were statistically weighted according to the method of Cleland (23) prior to linear regression.

RESULTS

Calculated K_i values for all of the compounds examined are listed in Table 1. Our values for **1**, **10**, and **11** are in the same range and order of potency as published values (5). All of the compounds which showed significant ability to inhibit PNMT (K_i < mM) demonstrated competitive kinetics. On the other hand, the *endo*-substituted Compounds **5**, **6**, **7**, and **9** showed uncompetitive kinetics and were extremely poor inhibitors ($K_i \simeq 10$ mM). Comparison of the *exo* Isomers **2–4** and **8** to (±)-amphetamine (**1**) revealed that Compounds **2** and **8** were significantly more potent than **1** against PNMT, whereas **3** and **4** were slightly less active. Compound **4** did not show any improvement in activity compared with **2** or **3**.

As has been previously reported, Compounds **10** and **11** were very potent inhibitors, with K_i values of 15 and 12 μM, respectively. Tranylcypromine **12** was also a very good inhibitor with a K_i value of 67 μM, in contrast to its *cis* isomer **13**, which was approximately one-fifteenth as active. The most potent compound studied was 3-methyltetrahydroisoquinoline **14**, which had a K_i value of 5 μM. Compound **14** is structurally analogous to the previously mentioned inhibitor SKF-64139, which is among the most potent PNMT inhibitors known (literature; ref. 24) ($K_i = 3 \times 10^{-9}$ M).

DISCUSSION

All of the *exo*-substituted Analogues **2–4** and **8**, which approximate a fully extended conformation of amphetamine, showed significant activity as competitive inhibitors. By contrast, the *endo* Compounds **5–7** and **9**, which mimic a *gauche* conformation of amphetamine, apparently failed to bind to the PNMT active site, as reflected in the weak uncompetitive nature of their activity. This strongly suggests that the *gauche* conformation is simply not acceptable to the active site. Compound **13**, which holds the side chain in a high-energy eclipsed conformation ($\tau_2 \approx 0°$), is considerably less active than **12**. The fact that **13** still retains some activity may be due to its smaller size as compared with **5–7** and to the ability of the phenyl ring to adopt a more favorable orientation for binding to the active site. The finding that the Symmetrical **8** was significantly better at inhibiting PNMT than any of the *exo* Compounds **2–4** implies that PNMT is somewhat stereoselective in binding **2–4**. This is not surprising, since PNMT favors the *S*(+)-isomer of amphetamine by a factor of approximately 3-fold over the

R(−)-isomer (25). The *oxo*-bridged Compound **4** was no better as an inhibitor of PNMT than either **2** or **3**, indicating that the oxygen does not participate in any favorable binding interaction. Fuller *et al.* (26) reported that *O*-methylated 3,4-dichlorophenylethanolamine retained weak substrate activity, suggesting that some binding would have been possible had the oxygen bridge been oriented properly. The implication is that the oxygen is not in the vicinity of the hydroxyl binding site, since the K_i found for **4** is actually higher than that found for **2**.

Consideration of Compounds **2–7** as conformationally defined analogues of **10** leads to the conclusion that the boat conformer mimicked by the *gauche* Conformers **5–7** is not the active site conformation. However, it is not clear whether the lower activity of the extended Conformers **2–4** compared with **10** is due to a less than optimal conformation or to steric interference of the methano or ethano bridge to approach of the molecule to the binding site. Support for a steric effect on binding can be drawn from consideration of the K_i values of **8**, **9**, and **11**. Compounds **8** and **9** are conformationally defined analogues of **11** and represent the two possible envelope conformations available to **11**. That **9** fails to bind at all to the PNMT active site must be purely a conformational effect, since neither binding group is shielded to approach of a macromolecule. Compound **8** is active; however, in this case, approach of the molecule to a relatively flat receptor is hindered by the ethano bridge. Thus, the conformation mimicked by **8** is probably close to the active conformation of **11**, and the lower activity is primarily a result of steric interference. One still cannot distinguish between conformational and steric effects on activity of the 2-aminotetralin analogues **2–7**, since similar half-chair conformational analogues are not available. However, it is apparent that steric interference of the ethano (**3**, **6**), methano (**2**, **5**), or oxo (**4**, **7**) bridge with binding is possible, and the boat conformation represented by **2–4** cannot be eliminated as a possible active site conformation of **10**.

If Compound **14** is looked upon as a conformationally restricted *gauche* analogue of amphetamine, then the result obtained for this compound is inconsistent with our earlier stated conclusion that an extended conformation is preferred for binding of amphetamine to PNMT. However, tetrahydroisoquinolines have in the past been considered to be conformationally restricted analogues of benzylamines rather than phenylethylamines (amphetamines) (27). This distinction may be important, since it is possible that the two classes of compounds bind to PNMT in different orientations.

Data reported in the literature for substituted amphetamines and benzylamines support such a proposal. For example, substituents on the aromatic ring have a greater effect on activity of benzylamines than amphetamines, and the nature of the contribution of these substituents toward binding appears different in each case, as suggested by quantitative structure-activity correlations (25, 28, 29). In addition, different substitution patterns are required for optimal activity in benzylamines and amphetamines, and PNMT exhibits a greater degree of stereoselectivity toward α-methylbenzylamines compared with amphetamines (25, 28). All of this can be construed as evidence for different binding requirements for the two structures, which suggest either different binding sites or different orientations in the same site. The activity of **14** is therefore attributable to its relationship to benzylamine, and as such does not affect conclusions based on the other compounds examined, which can only be considered as phenylethylamine analogues.

In summary, we have examined a series of conformationally defined amphetamine analogues as inhibitors of PNMT in an attempt to determine the active site conformation of amphetamine and similar compounds. Our results indicate that a *gauche* or eclipsed conformation of amphetamine is not the optimal conformation for binding. Compounds which resemble a fully extended *trans* antiperiplanar conformation of amphetamine are competitive inhibitors of PNMT and suggest that this is close to the conformation required by the inhibitor binding site of PNMT.

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