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RELATIVE SELECTIVITY OF SOME CONFORMATIONALLY CONSTRAINED TRYPTAMINE ANALOGS AT 5-HT_1, 5-HT_{1A} AND 5-HT_2 RECOGNITION SITES

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Summary

In an attempt to define pharmacophoric differences between 5-HT_1, 5-HT_{1A} and 5-HT_2 recognition sites, a number of rigid analogs were studied and compared to analogous free chain tryptamines. Racemic partial ergolines RU 27849 and RU 28306 showed reduced potency at all 5-HT_1 sites, but were at least equipotent to analogous tryptamines at the 5-HT_2 site. A non-ergoline-like constrained analog of tryptamine was similar in potency to RU 27849 at all 5-HT_1 sites, but showed a 4-fold enhancement in potency over RU 27849 and tryptamine at the 5-HT_2 site. At all 3 sites, 3-(tetrahydropyridyl) indoles (unless substituted at the indole 2-position) were the most potent rigid analogs studied and represent the most promising class for the development of selective compounds.

The recent disclosure that some atypical anti-anxiety agents (Buspirone, Ipsapirone) may exert their effects at least in part by a direct action at central serotonin (5-HT) receptors (1,2) has accentuated the importance of determining the functional correlates of the various 5-HT receptor subtypes. It is now widely accepted that central 5-HT recognition sites can be classified into 2 major types: 5-HT_1 and 5-HT_2 (3), and that the 5-HT_1 sites have been further subdivided into 5-HT_{1A}, 5-HT_{1B} (4) and 5-HT_{1C} sites (5). A class of predominantly peripheral neuronal 5-HT receptors has now been designated as 5-HT_3 (6). Attempts to determine the physiologic roles of these putative receptor types have been significantly impeded by the lack of a complete range of selective agonists and antagonists. Thus, a primary goal of research in this field is the development of selective agonists and antagonists for 5-HT_1 receptor subtypes, and potent selective agonists for 5-HT_2 receptors, which are generally lacking, with some notable exceptions (7,8,9).

Athough some simple tryptamine (TRYP) derivatives are capable of discriminating between different types and subtypes of 5-HT recognition sites, most such compounds are not potent enough to be useful as selective ligands or drugs. The problem is that most simple tryptamines that do show high affinity for 5-HT receptors (eg. 5-methoxy-N,N-dimethyltryptamine) are generally so structurally homologous to 5-HT that their activity and binding profiles are also very similar: thus their selectivity is unlikely to be much better than that of 5-HT itself. With the possible exception of the 5-HT_1 or 5-HT_{1A} selective 5-carboxamidotryptamine series (9,10), TRYP derivatives showing useful selectivity for 5-HT receptor subtypes usually feature substantial structural alterations or substitutions to the TRYP nucleus (e.g. the N'-aralkyl substituted tryptamine 5-HT antagonists we have recently reported; 11).

One of the properties of tryptamines is that they can assume different conformations, by rotations around the bonds of the ethylamino side chain. If it is assumed that 5-HT may be recognised in different conformations at different receptor sites (e.g. the antiparallel planar conformations A and B shown in Fig. 1), then clearly the problem with trying to use other tryptamines as selective ligands for 5-HT receptors is that they can potentially assume many of the same conformations as 5-HT: thus, unless

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considerably altered, they are unlikely to be much more selective than 5-HT itself. Therefore, the systematic development of compounds which are both potent and selective may be contingent upon the determination of the precise side chain conformation of 5-HT recognised at each receptor site (i.e. the pharmacophore for that receptor). In theory, an analog incorporating such a conformation into its structure, by eliminating the possibility of non-optimal conformations, would show enhanced affinity for that particular receptor type.

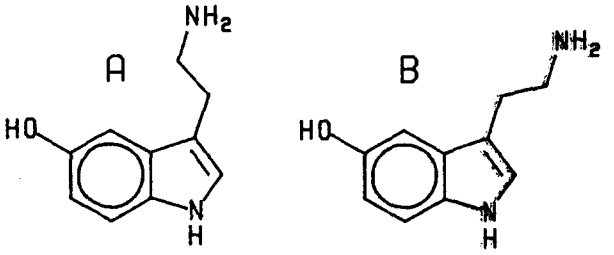

FIG. 1

Structures of 5-HT showing two antiparallel planar conformations: types A, an ergoline-like, and B, a non-ergoline-like conformation.

The comparative study of various conformationally constrained or rigid analogs is one method which can be used to attempt to determine the pharmacophore for a particular receptor. It must be kept in mind, however, that the interpretation of the results of such studies can involve the consideration of more than just conformational factors alone, since other structural features are inevitably introduced into the molecule in the process of restricting the conformation. These changes can alter the electronic and other physical properties of the molecule, obscuring the effect of the conformational change. Despite this caveat, the study of rigid analogs is probably the most effective way to map the pharmacophore of a receptor, short of x-ray studies of the ligand-receptor complex.

The choice of some prototypical rigid analogs of 5-HT is not difficult. Compared to most other indole derivatives, the high potency shown by ergolines such as d-lysergic acid diethylamide (d-LSD; Fig. 2), metergoline and methysergide at all the 5-HT recognition sites would suggest that an ergoline-like conformation of the amino nitrogen (Fig. 1A) might be optimal for recognition at 5-HT receptor sites, and thus correspond to the pharmacophore for those receptors. This view has been either tacitly or explicitly advocated by many, particularly in the hallucinogen field (12,13). It has also been used to

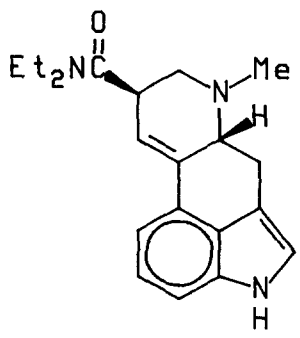

FIG. 2

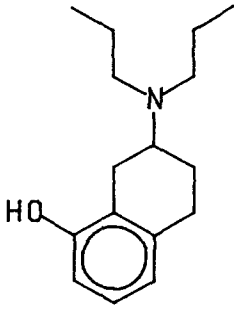

FIG. 3

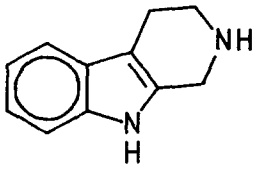

FIG. 4

Figure 2: d-LSD, an ergoline derivative and also a rigid analog of tryptamine.

Figure 3: 8-OH-DPAT, oriented to show homology to the d-LSD conformation.

Figure 4: Tetrahydro-beta-carboline (THBC), an additional conformational type.

explain the activity of at least one useful selective serotonergic agent (14): the 5-HT_{1A} agonist 8-hydroxy-(2-di-n-propylamino)tetralin (8-OH-DPAT), which is a partial ergoline without the pyrrole ring, with a hydroxy group in a position corresponding to the indole 5-position (Fig. 3).

Just as the ergolines are a promising class of compounds for this purpose, other classes of compounds can be ruled out on the basis of previous studies. In particular, derivatives of tetrahydro-beta-carboline (THBC; Fig. 4) have been found to have very low affinities for both 5-HT_1 and 5-HT_2 sites (15). At both 5-HT recognition sites, THBC is roughly an order of magnitude less potent than TRYP, its corresponding non-rigid analog. Hydroxy and methoxy THBC analogs are even less potent. In addition, indole alkaloids of the yohimbine type are conformationally similar to THBC; yohimbine also has rather low affinity (K_i > 600 nM) for both 5-HT_{1A} and 5-HT_2 sites (16,17). Thus, these types of rigid analogs have been excluded from this study.

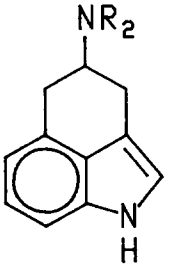

FIG. 5

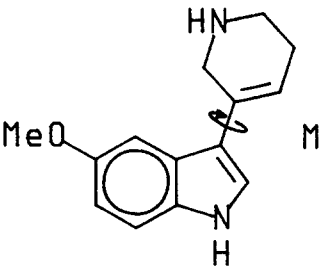

FIG. 6A

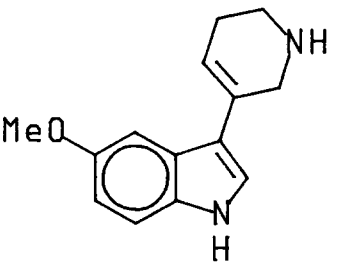

FIG. 6B

Figure 5: Structures of 2 partial ergolines studied as simplified analogs for conformation type A (Fig. 1). If R=H, RU 27849; if R= Me, RU 28306.

Figure 6: Two conformations of RU 28253, interconvertible by rotation around the bond indicated. **A:** ergoline-like; **B:** one possible non-ergoline conformation.

Fig. 5-8 show the structures of some of the analogs included in the present study. Two partial ergolines were examined: RU 27849 (Fig. 5, R=H) which is a rigid analog of TRYP, and RU 28306 (Fig. 5, R=Me), a rigid analog of N,N-dimethyltryptamine (DMT). The partially constrained tetrahydropyridylindole analog RU 28253 (Fig. 6) is very potent at both 5HT_1 and 5-HT_2 sites: it can assume an ergoline-like conformation (Fig. 6A), or, by rotation around the bond indicated, various non-ergoline-like conformations (one of which is shown as Fig. 6B). Thus, its active conformation could vary for different 5-HT receptor types. For the purpose of comparison, RU 28253 can be considered a constrained analog of 5-methoxytryptamine (5-MeO-TRYP). The compound RU 24969 (Fig. 7A, R=MeO, R'=H), the 4-pyridyl isomer of RU 28253, can also be considered a structural homolog of 5-MeO-TRYP. Two related compounds were also tested: 3(1-methyl-1,2,3,6-tetrahydro-4-pyridyl)indole (MTHPI; Fig. 7A, R=H, R'=Me) and its 2-methyl analog, 2-Me-MTHPI (Fig. 7B, R=H, R'=Me). These compounds are N'-methyl, des-methoxy, 4-pyridyl analogs of RU 28253. For comparison, MTHPI can be regarded as a constrained analog of DMT; however, since in the 4-pyridyl indoles there are 3 carbons between the indole and the amino nitrogen, the analogy to the TRYP structure is not precise. 2-Me-MTHPI was studied in order to examine the effect of forcing the pyridyl ring out of the plane of the indole ring system. This, in addition to altering the geometry of the molecule, would alter its electronic properties because of the inability of the double bond to form an extended conjugated system with the indole. The 5-methoxy derivatives of MTHPI and 2-Me-MTHPI were also studied (Fig. 7 A and B, R=MeO, R'=Me). An analog such as that shown as Fig. 8, 3-aminomethyl-1,3,4,5-tetrahydrobenz[cd]indole (3-AMTBI), which can approximate only the non-ergoline conformations of RU 28253, would be useful as an analog for conformations intermediate between the ergoline and beta-carboline types. This compound was also synthesized and tested; like the partial ergoline RU 27849, it can be regarded as a rigid analog of TRYP.

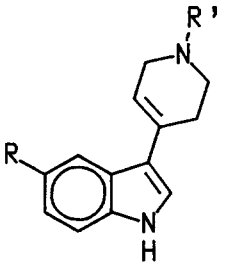

FIG. 7A

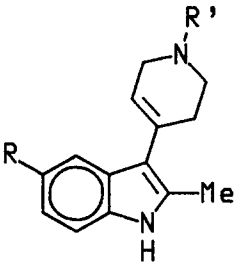

FIG. 7B

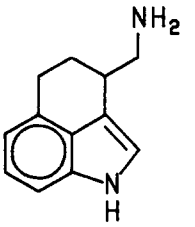

FIG. 8

Figure 7: A: Tetrahydropyridylindoles MTHPI (R=H, R'=Me), and 5-MeO-MTHPI (R=MeO, R'=Me) and RU 24969 (R=MeO, R'=H).
B: 2-Methyl derivatives 2-Me-MTHPI (R=H) and 5-MeO-2-Me-MTHPI (R=MeO).

Figure 8: 3-AMTBI, a non-ergoline-like constrained analog of tryptamine.

The approach taken in this study is the examination of these constrained analogs and the homologous tryptamines at central 5-HT recognition sites, using 3 different ligands: [^{3}H]5-HT, which labels total 5-HT$_1$ sites, [^{3}H]ketanserin, which labels 5-HT$_2$ sites, and [^{3}H]8-OH-DPAT, which labels 5-HT$_{1A}$ sites. The objective is to find which types of constrained analog, if any, show significantly enhanced or diminished potency (relative to the tryptamines) at recognition sites labeled by each of the 3 different ligands.

Methods

Chemicals and Drugs: 8-OH-DPAT was obtained fron Research Biochemicals Incorporated (Wayland, MA). RU 27849, RU 28306, RU 24969 and RU 28253 were donated by Roussel-UCLAF (Romainville, France). Ketanserin was a gift from Janssen Pharmaceutica (Beerse, Belgium). [^{3}H]8-OH-DPAT was obtained from Research Products International (Mount Prospect, IL). [^{3}H]5-HT and [^{3}H]ketanserin were obtained from New England Nuclear Corporation (Boston, MA). MTHPI, 5-MeO-MTHPI, their 2-methyl analogs and 3-AMTBI were synthesized in our laboratories; these syntheses will be described in separate publications. All other chemicals used were from standard commercial sources.

5-HT Receptor Binding Assays: These were performed as described previously (18). In brief, tissue was obtained from male Sprague-Dawley rats, which were killed by decapitation; the brains were then rapidly removed and dissected over ice. For the 5-HT$_1$ and 5-HT$_{1A}$ assays, the cortex dorsal to the rhinal sulcus was used; the 5-HT$_2$ assay was done using frontal cortex alone (17). Final tissue suspensions were in a buffer of 50 mM Tris at pH 7.6. For the 5-HT$_1$ assay, [^{3}H]5-HT to a final concentration of about 2 nM was used as ligand, and cold 5-HT at 10 µM was used to define non-specific binding. For the 5-HT$_{1A}$ assay, the ligand was [^{3}H]8-OH-DPAT at about 1 nM, and again 10 µM cold 5-HT was used to define non-specific binding. [^{3}H]Ketanserin to a final concentration of about 0.4 nM was used as the 5-HT$_2$ ligand, and non-specific binding was defined using 1 µM methysergide. The assay tubes were incubated at 37°C for 10 min (15 min for the 5-HT$_2$ assay) and filtered through Whatman GF/B filters using a Brandel cell harvester. For the 5-HT$_{1A}$ and 5-HT$_2$ assays, the GF/B filters were pre-treated with a 0.1% v/v solution of polyethyleneimine for 2 hr and allowed to dry (this was found to reduce non-specific binding to the filters). For a similar reason, the 5-HT$_2$ assays were performed in disposable polypropylene rather than glass tubes.

Analysis of Binding Data: Since none of the compounds appeared to discriminate very significantly between subtypes of [^{3}H]5-HT sites (as discussed in Results), multi-site analysis of the [^{3}H]5-HT binding data was not performed. For all three binding assays, potencies of inhibiting drugs are reported as apparent K$_i$ values, calculated from inhibitor IC$_{50}$ values using the equation

$$\text{apparent } K_i = \frac{IC_{50}}{1+(L/K_d)}$$

where L is the radioligand concentration and K_d is the dissociation constant of the ligand-receptor complex (determined by saturation studies or from the inhibition by the cold ligand for its own binding); for the 5-HT_1 assay, the apparent K_d for [^{3}H]5-HT = 2 nM; for the 5-HT_{1A} assay, the K_d for [^{3}H]8-OH-DPAT = 5.5 nM; for the 5-HT_2 assay, the K_d for [^{3}H]ketanserin = 0.4 nM. This calculation is only theoretically valid in cases of simple competitive inhibition (Hill Coefficient = 1), which is essentially the case for our $5HT_2$ and $5HT_{1A}$ data.

We have chosen to report apparent K_i rather than IC_{50} values for the 5-HT_1 site in order to make comparison of affinities between the three ligands easier, but it must be emphasized that the apparent K_i for 5-HT_1 sites is a composite value, since [^{3}H]5-HT labels a heterogeneous group of sites (Hill Coefficient $\neq$ 1). However, the composition of this group is under debate. On the one hand, Hamon et al. (19) have argued that under conditions similar to those used in the present study (2 nM [^{3}H]5-HT), both 5-HT_{1A} and 5-HT_{1B} sites are labelled, but 5-HT_{1C} sites are not significantly labeled, due to the low ligand concentration and the low density of 5-HT_{1C} sites in rat cortex (<10% of total 5-HT_1 sites in all areas; 20). However, Peroutka et al. (21) report that 5-HT_{1C} sites represented about 35% of the sites labeled by [^{3}H]5-HT in rat frontal cortex. This question remains to be resolved, so, since none of the compounds in the present study showed much selectivity between 5-HT_1 receptor subtypes, we have not attempted to analyse the components of their total 5-HT_1 binding.

Statistical analysis of data: Homologous compounds were grouped in pairs or sets of 3, as shown by the divisions of Tables 1 and 2. The relative potency of homologous compounds at each binding site was the subject of interest. Thus, for sets of 3 compounds, a 1 way ANOVA was performed for each ligand with each set of 3 drugs. If significant differences between mean drug potencies were found, Student-Newman-Keuls' (SNK) post-hoc comparison was used to determine which means were significantly different. When only 2 compounds were being compared for a given ligand, a simple t-test was applied to determine significant differences. For both these tests, differences were considered significant if $p<0.05$.

Results

For all of the compounds except 2-methyl derivatives, apparent K_i values against all 3 tritiated ligands are given in Table 1. None of these compounds show a high degree of selectivity for 5-HT_{1A} over other subtypes of 5-HT_1 binding, since for all compounds the K_i values vs. the 5-HT_{1A} ligand [^{3}H]8-OH-DPAT are close to the apparent K_i values for total 5-HT_1 sites obtained from [^{3}H]5-HT binding.

Since the object of this study was to find rigid or semi-rigid analogs which showed enhanced potency relative to the corresponding free chain compounds, the compounds grouped within each section of the table are to be compared because of their structural homology (as discussed previously). Results for each set of compounds are as follows:

Comparison of TRYP, RU 27849 and 3-AMTBI: At the 5-HT_1 site, significant differences in potency were found by one-way ANOVA ($f=19.34$, $p=0.0004$). SNK comparisons indicated that only TRYP was significantly different (being about 3 times more potent) than the other 2 compounds. Similar results were found for the 5-HT_{1A} site, where significant differences were also found ($f=8.87$, $p=0.0161$), and again TRYP was about 3 times more potent than either of the rigid analogs. At the 5-HT_2 site, significant differences were found ($f=5.58$, $p=0.0304$); in this case, the partial ergoline RU 27849 and TRYP had similar affinities, but 3-AMTBI was significantly more potent (about 4 times) than the other 2 compounds.

TABLE I

Apparent K_i Values (nM) at Central 5-HT Binding Sites: Comparison of Tryptamines and Conformationally Constrained Analogs

Compound	5-HT_1: ([^{3}H]5-HT		5-HT_{1A}: ([^{3}H]8-OH-DPAT)		5-HT_2: ([^{3}H]Ketanserin)	
RU 27849	267 ± 74	(3)	326 ± 59	(3)	1964 ± 637	(3)
TRYP	80 ± 12	(7)	125 ± 29	(3)	2481 ± 360	(5)
3-AMTBI	392 ± 55	(3)	369 ± 42	(3)	555 ± 58	(3)
RU 28306	201 ± 26	(3)	329 ± 47	(3)	314 ± 31	(6)
DMT	67 ± 12	(3)	245 ± 85	(3)	558 ± 82	(4)
MTHPI	217 ± 39	(3)	144 ± 23	(4)	71 ± 5	(3)
RU 28253	8.5 ± 2.7	(5)	5.7 ± 2.1	(3)	230 ± 58	(5)
RU 24969	9.6 ± 3.0	(4)	11.0 ± 4.7	(6)	912 ± 147	(7)
5-MeO-TRYP	5.9 ± 0.9	(5)	6.1 ± 1.4	(3)	1382 ± 86	(3)

All values are the mean ± S.E.M., followed by the number of independent experiments (n). Each set of compounds contains one tryptamine and two homologous rigid analogs (see text).

Comparison of DMT, RU 28306 and MTHPI: At the 5-HT_1 site, significant differences in potency were found by one-way ANOVA ($f=8.75$, $p=0.0166$). SNK comparisons indicated that only DMT was significantly different (being about 3 times more potent) that the other 2 compounds. However, at the 5-HT_{1A} site, the observed differences were not significant ($f=3.33$, $p=0.0963$). At the 5-HT_2 site, significant differences were found ($f=18.71$, $p=0.0004$); in this case, all 3 compounds were found to have significantly different K_i values; however, RU 28306 was only about 1.7 times as potent as DMT, whereas MTHPI was almost 8 times as potent as DMT.

Comparison of 5-MeO-TRYP, RU 24969 and RU 28253: At 5-HT_1 and 5-HT_{1A} sites, all three compounds are of essentially equal potency. At the 5-HT_2 site, all 3 compounds were found to have significantly different K_i values ($F = 15.72$, $p = 0.0004$). RU 24969 was 1.5 times as potent as 5-MeO-TRYP; the structurally more similar 3-pyridyl analog RU 28253 was 6 times as potent as 5-MeO-TRYP.

TABLE II

Apparent K_i Values (nM) at Central 5-HT Binding Sites: Effects of 2-Methylation on MTHPI and 5-MeO-MTHPI

Compound	5-HT_1: ([^{3}H]5-HT)		5-HT_{1A}: ([^{3}H]8-OH-DPAT)		5-HT_2: ([^{3}H]Ketanserin)	
MTHPI	217 ± 39	(3)	144 ± 23	(3)	71 ± 5	(3)
2-Me-MTHPI	>5,000	(3)	1890 ± 195	(3)	805 ± 96	(3)
5-MeO-MTHPI	46 ± 6	(3)	23 ± 5	(3)	814 ± 205	(3)
5-MeO-2-Me-MTHPI	1640 ± 529	(3)	365 ± 28	(4)	4003 ± 395	(3)

All values are the mean ± S.E.M.; (n) = the number of independent experiments.

Effect of 2-methylation on tetrahydropyridylindoles: In Table 2, binding data for MTHPI, 5-MeO-MTHPI and their 2-methyl analogs are compared. It is apparent that for all three ligands, the 2-Me derivatives are approximately an order of magnitude less potent than the 2-H compounds.

Discussion

The results of this study can be briefly summarized as follows:

1) At 5-HT_1 and 5-HT_{1A} sites, none of the rigid analogs show significantly enhanced potency relative to the homologous free chain compounds; however, the tetrahydropyridylindoles are approximately equipotent to the related tryptamines, at least at the 5-HT_{1A} site.

2) At 5-HT_1 sites, the partial ergolines RU 27849 and RU 28306 are both about 3 times less potent than TRYP and DMT, respectively. At the 5-HT_{1A} site, RU 27849 is still 2.5 times less potent than TRYP, but differences between RU 28306 and DMT are not significant. For both of these ligands, there is no significant difference between the affinity of RU 27849 and the analogous non-ergoline-like compound 3-AMTBI, which is also 3-4 times less potent than TRYP at both 5-HT_1 and 5-HT_{1A} sites.

3) At the 5-HT_2 site, the partial ergolines RU 27849 and RU 28306 are at least equipotent (RU 27849) or slightly greater in potency (RU 28306) than homologous tryptamines (TRYP and DMT, respectively).

4) However, at the 5-HT_2 site, the non-ergoline-like analog 3-AMTBI is significantly more potent (>4 times) than the two homologous compounds, TRYP and the partial ergoline RU 27849.

5) The tetrahydropyridylindoles MTHPI and RU 28253 both show considerably enhanced potency (6-8 fold) at 5-HT_2 sites relative to the homologous tryptamines DMT and 5-MeO-TRYP. This effect was not as pronounced for RU 24969, which was only slightly more potent than 5-MeO-TRYP.

6) When MTHPI and 5-MeO-MTHPI are altered by 2-methylation of the indole ring, the potency of the compounds is reduced by approximately an order of magnitude at all the 5-HT binding sites. In addition to steric factors (i.e. the tetrahydropyridyl ring being forced out of the plane of the indole ring system, which we have confirmed by molecular mechanics calculations), this loss of potency could also be related to altered electronic properties of the molecule when the double bond is forced out of conjugation with the indole ring. (The 2-methylation of serotonin has also been reported to cause a dramatic loss in potency at 5-HT_1 and 5-HT_2 sites (22); this effect is probably of a similar nature).

The observation that the partial ergolines do not show significantly enhanced potency at any of the 5-HT recognition sites is somewhat unexpected, considering the high affinity shown by full ergoline derivatives such as d-LSD and metergoline for those sites. Consistent results have been reported for the methoxy derivative of RU 27849, which was recently synthesised (23) and reported to have an IC_{50} of about 50 nM for the inhibition of [^{3}H]5-HT binding, which is at least 5 times less potent than typical reported values for the corresponding non-rigid analog, 5-MeO-TRYP. Our observations are particularly difficult to explain for the 5-HT_{1A} site, since the compound now used to label those sites, 8-OH-DPAT, is a partial ergoline without the pyrrole ring (Fig. 3). One factor that must be considered in interpreting the binding data for these compounds is that they are racemates. Thus, if one enantiomer is almost inactive, the potency of the other enantiomer could be as much as twice that estimated for the mixture. Also, it may be that the 5-HT_1 sites are exquisitely sensitive as far as the exact conformation required for the amino nitrogen, and that these partial ergoline derivatives are sufficiently different from that optimal conformation to explain the observed lack of potency. In this case, the very lack of rigidity posessed by the free chain compounds may confer an advantage because, although they can assume many unfavorable conformations, the exact optimal conformation can be induced during their interaction with the receptor. In an attempt to

explain the low potency of these partial ergoline derivatives, we are currently using molecular modelling techniques to determine any possible conformational differences between them and structurally related partial ergolines such as 8-OH-DPAT.

The tetrahydropyridylindoles may be useful prototypes for the design of other rigid analogs for 5-HT_1 receptors, since they are the most promising of all the analogs examined in terms of showing high potency at 5-HT_1 sites. As discussed previously, the compounds in the 3-pyridylindole series (e.g. RU 28253) are of considerable interest because of their ability to assume both ergoline-like and non-ergoline-like conformations. It is also possible that RU 28253 may bind optimally to 5-HT_1 sites in some conformation in which the two rings are roughly perpendicular (intermediate between the two rotations shown in Fig. 6). However, this seems unlikely in view of the greatly attenuated potency of 2-methyl MTHPI analogs relative to the 2-H compounds. This finding also suggests that the potency of the tetrahydropyridylindoles may originate in part from electronic effects inherent in structural features such as the conjugated double bond, and that such electronic effects may be at least as important as conformational features. In this regard, it is interesting to note that in the case of d-LSD, the conjugated double bond of the so-called D ring is known to be extremely important, at least as far as hallucinogenic potency is concerned (12,24). It is also known that the stereochemistry and alkyl substituent size on the amide of d-LSD is critically important for psychoactivity and 5-HT antagonism (24). Thus, it is possible that factors such as these, rather than an optimal position of the ergoline amino nitrogen, are most responsible for the high affinity of d-LSD and its analogs for 5-HT binding sites. The observation that the partial ergolines are very impotent compared to d-LSD are consistent with such a hypothesis. This question must remain open until more conformationally defined analogs of 5-HT become available for study.

The finding that both RU 28253 and 3-AMTBI show enhanced potency at the 5-HT_2 site is of interest, for the following reason. If the indole rings of these two structures are superimposed, there is only one possible conformation for each enantiomer of 3-AMTBI where the amino nitrogens of the two compounds can be made to superimpose. Since this conformation represents only one of many possible conformations for both RU 28253 and 3-AMTBI (neither of which is completely rigid), it would be interesting to synthesize a completely rigid analog with this conformation, a project which is currently being attempted. These observations also provide a strong incentive for the resolution of 3-AMTBI, because if its enhanced potency at 5-HT_2 sites is genuinely related to conformational factors, one might hope to see a distinct preference for one of the enantiomers. This would give insight into the stereochemistry of the 5-HT_2 receptor site.

In conclusion, although the results of this study suggest some promising leads for the development of more selective serotonergic agents, unresolved questions still remain regarding the pharmacophores of the various central 5-HT binding sites. The availability of more rigid analogs would help to resolve some of these questions; thus, the synthesis and pharmacological characterization of other conformationally defined tryptamine analogs is a continuing goal of our research.

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References

1. T. GLASER and J. TRABER, Eur. J. Pharmacol. 88: 137-138 (1983).
2. W.U. DOMPERT, T. GLASER AND J. TRABER, Naunyn-Schmiedeberg's Arch. Pharmacol. 328: 467-470 (1985).

3. S.J. PEROUTKA and S.H. SNYDER, Mol. Pharmacol. 16: 687-699 (1979).
4. N.W. PEDIGO, H.I. YAMAMURA and D.L. NELSON, J. Neurochem. 36: 220-226 (1981).
5. A. PAZOS, D. HOYER and J.M. PALACIOS, Eur. J. Pharmacol. 106: 539-546 (1984).
6. P.B. BRADLEY, G. ENGEL, W. FENIUK, J.R. FOZARD, P.P.A. HUMPHREY, D.N. MIDDLEMISS, E.J. MYLECHARANE, B.P. RICHARDSON and P.R. SAXENA, Neuropharmacol. 25:563-576 (1986).
7. D.N. MIDDLEMISS and J.R. FOZARD, Eur. J. Pharmacol. 90: 151-153 (1983).
8. M. SHANNON, G. BATTAGLIA, R.A. GLENNON and M. TITELER, Eur. J. Pharmacol. 102: 23 (1984).
9. A. HAGENBACH, D. HOYER, H.O. KALKMAN and M.P. SEILER, Br. J. Pharmacol. 87: 136P (1986).
10. G. ENGEL, M. GOTHERT, D. HOYER, E. SCHLICKER and K. HILLENBRAND, Naunyn-Schmiedeberg's Arch. Pharmacol. 332: 1-7 (1986).
11. E.W. TAYLOR and D.L. NELSON, The Pharmacologist, 27:194 (1985).
12. S. KANG and J.P. GREEN, Proc. Nat. Acad. Sci. 67: 62-67 (1970).
13. D.E. NICHOLS, W.R. PFISTER and K.W. YIM, Life Sci. 22: 2165-2170 (1978).
14. L-E. ARVIDSSON, U. HACKSELL, A.M. JOHANSSON, J.L.G. NILSSON, P. LINDBERG, D. SANCHEZ, H. WIKSTROM, K. SVENSSON, S. HJORTH and A. CARLSSON, J. Med. Chem. 27: 45-51 (1984).
15. C.S. CASIO AND K.J. KELLAR, Neuropharm. 21: 1219-1221 (1982).
16. H. GOZLAN, S. EL MESTIKAWY, L. PICHAT, J. GLOWINSKI and M. HAMON, Nature, Lond. 305: 140-144 (1983).
17. J.E. LEYSEN, J.E.NEIMEGEERS, J.M. VAN NEUTEN AND P.M. LADURON, Mol. Pharmacol. 21: 301-314 (1982).
18. E.W. TAYLOR, S.P. DUCKLES and D.L. NELSON, J. Pharmacol. Exp. Ther.236: 118-125 (1986).
19. M. HAMON, J-M. COSSERY, U. SPAMPINATO and H. GOZLAN, Trends in Pharm. Sci. 7(9): 336-338 (1986).
20. A. PAZOS and J.M. PALACIOS, Brain Res. 346: 205-230 (1985).
21. S.J. PEROUTKA, J. Neurochem. 47: 529-540 (1986).
22. B.P. RICHARDSON, G. ENGEL, P. DONATSCH and P.A. STADLER, Nature 316: 126-131 (1985).
23. L.I. KRUSE and M.D. MEYER, J. Org. Chem. 49: 4761-4768 (1984).
24. A. FANCHAMPS, in ERGOT ALKALOIDS AND RELATED COMPOUNDS, B. BERDE and H.O. SCHILD, Eds. (HANDBUCH DER EXPERIMENTELLEN PHARMAKOLOGIE, Vol. 49, pp. 567-614), Springer-Verlag, Berlin (1978).