5-HT₁ and 5-HT₂ binding properties of derivatives of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA)

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Received 7 December 1983, revised MS received 7 March 1984, accepted 14 March 1984

The affinities of a series of 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA) derivatives, most of which are hallucinogenic in man, and several related agents were determined for rat cortical serotonin (5-HT) binding sites. Competition assays were performed in which these agents were competed for the 5-HT₂ binding of [³H]ketanserin, or the 5-HT₁ binding of [³H]LSD (in the presence of ketanserin). The R-(−)-isomers of DOI, DOM and DON (i.e. the 4-iodo, -methyl and -nitro derivatives of 2,5-DMA) were found to be more potent than their racemates and demonstrated selectivity for 5-HT₂ sites. These same agents in competing for [³H]ketanserin binding resulted in Hill coefficients significantly less than unity; computer-assisted analysis indicated a two-state model better fit the data. In the presence of 10⁻⁴ M Gpp(NH)₃ the competition curve for R-(−)-DOI produced a Hill coefficient close to unity. These results are consistent with the hypothesis that certain derivatives of 2,5-DMA, in particular R-(−)-DOI, may be potent and selective agonists at 5-HT₂ sites, sites that may constitute a serotonin receptor that is regulated by a guanine nucleotide regulatory protein. Conversely, the interactions of these agents at 5-HT₁ sites was with a lower affinity and a lack of stereoselectivity. Although DOI and DOM are amongst the most potent of these agents as hallucinogens, it is still too premature to draw any conclusions regarding a possible relationship between 5-HT binding and hallucinogenic potency.

Serotonin 5-HT₁ 5-HT₂ Hallucinogens DOM

1. Introduction

A series of derivatives of 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA), most of which are hallucinogenic in man, produce behavioral (discriminative stimulus) effects in animals that are similar to those produced by 1-(2, 5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (Glennon et al., 1982, 1983a, b). Furthermore, there exists a direct linear correlation between the human hallucinogenic potencies of these agents and both their discrimination-derived ED₅₀ values, and their serotonin (5-HT) receptor affinities as determined using an isolated rat fundus preparation. Because the latter receptors are of peripheral origin, and due to the lack of knowledge concerning the nature of the relationship between these receptors and central 5-HT binding sites, the significance of the above-mentioned correlation with receptor affinity is unclear. Peroutka and Snyder (1979) have demonstrated the existence of two types of serotonin binding sites in the brain; 5-HT₁ sites may be labelled either by the high-affinity binding of [³H]5-HT, or by [³H]LSD in the presence of a 5-HT₂ blocker, while 5-HT₂ sites are defined by [³H]spiperone binding in rat frontal cortex. Tritiated ketanserin (Leysen et al., 1982) and mesulergine (Closse et al., 1983)
have now been demonstrated to be superior radio-
ligands for labelling the latter binding sites.

We recently reported that because the dis-

criminative stimulus produced by DOM could be
attenuated by pretreatment of the animals with
ketanserin, the stimulus effects of such agents
might involve a 5-HT₂-related mechanism (Glen-
non et al., 1983c). Therefore, in order to further
explore this possibility, and to investigate struc-
ture-activity relationships, the affinities of a series
of 2,5-DMA and related derivatives for rat corti-
cal 5-HT₁ and 5-HT₂ binding sites were de-
determined.  

2. Materials and methods  

2.1. Membrane preparation  

Tissue preparation was performed as described
by Leysen et al. (1982) with minor modifications.
The prefrontal and parietofrontal cortex of male
Wistar rats (200 g) were homogenized in 0.25 M
sucrose (1 : 10 w/v), and centrifuged at 1086 × g
for 10 min. The supernatant was diluted (1 : 40
w/v) in 50 mM Tris-HCl buffer (pH 7.4) and
centrifuged at 35000 × g for 10 min. The pellet
was resuspended in buffer and re-centrifuged. The
final suspension was in 50 mM Tris-HCl (pH 7.4)
at a tissue concentration of 16 mg wet weight/ml.  

2.2. Radioligand binding assay  

Assays were performed in triplicate in 2.5 ml
vol of a 50 mM Tris, 5 mM MgCl₂, 0.5 mM
EDTA Na₂ (pH 7.4 at 37°C) buffer, to which 4
mg wet weight of tissue was added last. Competition
experiments were performed using either 4 × 10⁻¹⁰ M [³H]ketanserin (defined as 5-HT₂ bind-
ing), or 10⁻⁹ M [³H]LSD in the presence of 10⁻⁷
M ketanserin (defined as 5-HT₁ binding); at this
concentration 90% of total binding (1200 cpm)
was specific for [³H]ketanserin, and 75% of
[³H]LSD binding was specific (750 cpm). Tubes
were incubated for 15 min at 37°C for
[³H]ketanserin assays, or for 30 min at 37°C for
[³H]LSD assays; filtration was accomplished using
Flow Laboratories glass fiber filters and was fol-
lowed by a wash with 10 ml buffer (as above). The
filters were counted by liquid scintillation spec-
trometry in 8 ml of aqueous counting scintillant
ACS (Amersham) at an efficiency of 32%.  

2.3. Data analysis  

Competition binding data were analyzed by a
non-linear least-squares curve-fitting procedure
based on a model of ligand binding to one or two
sites according to the law-of-mass-action. The data
were first analyzed with a two parameter logistic
equation in order to determine an IC₅₀ value and a
Hill coefficient for the competition curve. The
competition curves were then analyzed with a four
parameter equation (2 pKs, 2 populations of sites)
in order to determine the proportions of sites and
affinities of competing ligands for the two sites.
Statistical analysis of the more appropriate model
(one or two sites) was provided for ligands whose
competition curves best fit a two-site model. K_H
and K_L represent the high- and low-affinity dis-
sociation constants. R_H and R_L represent the pro-
portion of receptors corresponding to the high and
low affinity states, respectively.  

2.4. Drugs  

[³H]Ketanserin (67 Ci/mmol) was obtained
from New England Nuclear, 5'-Guanylimi-
dodiphosphate (Gpp (NH)p) was purchased from
Sigma. The 2,5-DMA derivatives are those previ-
ously described by Glennon and coworkers (1982,
1983b).  

3. Results  

Table 1 presents the structures of the seventeen
derivatives used in this study. Those agents in
table 1A are either hallucinogenic in man and/or
produce DOM-like effects in animals (i.e., result in
stimulus generalization when administered to
animals trained to discriminate DOM from saline);
these agents also possess a relatively high affinity
for the serotonin receptors of the rat fundus pre-
paration. On the other hand, those agents shown
in table 1B do not result in stimulus generalization
TABLE 1
2,5-DMA and other derivatives used in this study.

\[
\begin{array}{|c|c|c|c|c|}
\hline
R' & R'' & R_2 & R_4 & R_5 \\
\hline
\end{array}
\]

(A) 2,5-DMA derivatives

\[
\begin{align*}
(+)-2,5-DMA & : CH_3 & H & OCH_3 & H & OCH_3 \\
(+)-2,4,5-TMA & : CH_3 & H & OCH_3 & OCH_3 & OCH_3 \\
(+)-4-OEt-2,5-DMA & : CH_3 & H & OCH_3 & OCH_3 & CH_3 \\
(+)-DOF & : CH_3 & H & OCH_3 & F & OCH_3 \\
(+)-DOB & : CH_3 & H & OCH_3 & Br & OCH_3 \\
(+)-DOI & : CH_3 & H & OCH_3 & I & OCH_3 \\
R(-)-DOI & : CH_3 & H & OCH_3 & I & OCH_3 \\
(+)-DON & : CH_3 & H & OCH_3 & NO_2 & OCH_3 \\
R(-)-DON & : CH_3 & H & OCH_3 & NO_2 & OCH_3 \\
(+)-DOM & : CH_3 & H & OCH_3 & CH_3 & OCH_3 \\
R(-)-DOM & : CH_3 & H & OCH_3 & CH_3 & OCH_3 \\
\alpha\text{-Demethyl DOM} & : H & H & OCH_3 & CH_3 & OCH_3 \\
(+)-N-Methyl DOM & : CH_3 & CH_3 & OCH_3 & CH_3 & OCH_3 \\
R(-)-N-Methyl DOM & : CH_3 & CH_3 & OCH_3 & CH_3 & OCH_3 \\
\hline
\end{align*}
\]

(B) Non-2,5-DMA derivatives

\[
\begin{align*}
(+)-PMA & : CH_3 & H & H & OCH_3 & H \\
(+)-3,4-DMA & : CH_3 & H & H & OCH_3 & OCH_3 \\
(+)-4-Me PIA & : CH_3 & H & H & CH_3 & H \\
\hline
\end{align*}
\]

when administered to animals trained to discriminate DOM from saline, and possess relatively low affinities for rat fundus serotonin receptors.

In order to assess the affinities of these agents for the 5-HT_2 binding sites, competition assays were performed in homogenates of rat cortical membrane preparations using \[^3\text{H}\]ketanserin; table 2 and fig. 1A and 1B present the results of these experiments. All of the 2,5-DMA derivatives possess a greater affinity than 2,5-DMA itself; the compound with the highest affinity for 5-HT_2 sites is R(-)-DOI (K_t = 9.9 nM). Two of the non-2,5-DMA derivatives, i.e. (+)-PMA and (+)-3,4-DMA were amongst the least active at the 5-HT_2 sites.

The data in table 2 also reveal that all the 2,5-DMA derivatives display a very low affinity for 5-HT_1 binding sites (for the purposes of this report, affinity constants greater than 10^{-6} M indicate a low affinity interaction). Interestingly, with one exception, 2,5-DMA possesses the highest affinity for 5-HT_1 sites of all the 2,5-DMA derivatives evaluated; that one exception differs from the remainder of the series in being an \(\alpha\)-demethylated derivative. The ratio of affinities for 5-HT_1 and 5-HT_2 sites are listed in table 2; of those agents tested, R(-)-DOI is clearly the most selective for 5-HT_2 sites. Most of the other derivatives also are quite selective for 5-HT_2 sites, with the exception of (+)-DOF, (+)-4-Me-PIA, (+)-2,5-DMA, 3,4-DMA 25 and (+)-PMA.

Several convincing arguments have been put forth indicating that 5-HT_1 and 5-HT_2 serotonin sites are distinct molecular entities (Peroutka and Snyder, 1979). Adding to these arguments are the results presented herein revealing a lack of correlation between the affinities of the 2,5-DMA derivatives at these two sites. Clearly the structure-activity relationships at these two binding sites are quite different (see Discussion).

In a series of studies, evidence has been provided that agonist interactions with 5-HT_2 serotonin sites are regulated by guanine nucleotides, possibly through a guanine nucleotide regulatory protein (Battaglia et al., 1983, 1984). Agonist interactions with \[^3\text{H}\]ketanserin labelled 5-HT_2 sites produced competition curves with Hill coefficients less than unity; inclusion of guanine nucleotides resulted in agonist competition curves with Hill coefficients close to unity. Computer-assisted analysis indicated that 5-HT_2 sites might exist in multiple agonist-affinity states in the absence of guanine nucleotides; in the presence of guanine nucleotides high agonist affinity states (R_H) were converted to low affinity states (R_L). It was considered of interest to determine whether any of the high affinity 2,5-DMA derivatives would display binding properties characteristic of an agonist interaction at 5-HT_2 sites. The results shown in fig. 1A and 1B and table 2 indicate that several derivatives produce competition curves with Hill coefficients of approximately 0.70. Computer-assisted analysis of several of the 2,5-DMA derivative interactions at 5-HT_2 sites revealed that a two-site or two-state model produced a 'better fit' for the data than a one-site model (table 3). R(-)-DOI and (+)-DOB were found to have very high affinities for the 'high affinity site'...
### TABLE 2
Affinities of 2,5-DMA and related derivatives for 5-HT₁ and 5-HT₂ sites.

<table>
<thead>
<tr>
<th>Agent</th>
<th>5-HT₁ binding</th>
<th>5-HT₂ binding</th>
<th>Kᵢ(5-HT₁)/Kᵢ(5-HT₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kᵢ (nM)</td>
<td>Hill coefficient</td>
<td>Kᵢ (nM)</td>
</tr>
<tr>
<td><strong>Table:</strong></td>
<td><strong>Table:</strong></td>
<td><strong>Table:</strong></td>
<td><strong>Table:</strong></td>
</tr>
<tr>
<td>R(-)-DOI</td>
<td>(±) 9.9</td>
<td>(5:1±0.0) 0.72 (±0.03)</td>
<td>(5:1±130) 2290</td>
</tr>
<tr>
<td>(±)-DOI</td>
<td>(±) 18.9</td>
<td>(5:1±2.6) 0.73 (±0.04)</td>
<td>(5:1±200) 2240</td>
</tr>
<tr>
<td>R(-)-DOM</td>
<td>(±) 60</td>
<td>(5:1±5) 0.71 (±0.02)</td>
<td>(5:1±300) 3550</td>
</tr>
<tr>
<td>(±)-DOM</td>
<td>(±) 100</td>
<td>(5:1±17) 0.71 (±0.04)</td>
<td>(5:1±380) 2890</td>
</tr>
<tr>
<td>(±)-DOB</td>
<td>(±) 63</td>
<td>(5:1±2) 0.80 (±0.01)</td>
<td>(5:1±400) 3340</td>
</tr>
<tr>
<td>α-demethyl DOM</td>
<td>(±) 110</td>
<td>(5:1±8) 0.77 (±0.03)</td>
<td>(5:1±30) 350</td>
</tr>
<tr>
<td>R(-)-DON</td>
<td>(±) 210</td>
<td>(5:1±35) 0.75 (±0.05)</td>
<td>(5:1±1400) 13200</td>
</tr>
<tr>
<td>(±)-DON</td>
<td>(±) 300</td>
<td>(5:1±40) 0.79 (±0.05)</td>
<td>(5:1±950) 14100</td>
</tr>
<tr>
<td>R(-)-N-methyl DOM</td>
<td>(±) 260</td>
<td>(5:1±20) 0.91 (±0.03)</td>
<td>(5:1±320) 4300</td>
</tr>
<tr>
<td>(±)-N-methyl DOM</td>
<td>(±) 415</td>
<td>(5:1±40) 0.83 (±0.04)</td>
<td>(5:1±250) 3870</td>
</tr>
<tr>
<td>(±)-DOF</td>
<td>1110</td>
<td>(5:1±150) 0.76 (±0.04)</td>
<td>(5:1±230) 3470</td>
</tr>
<tr>
<td>(±)-2,4,5-TMA</td>
<td>1650</td>
<td>(5:1±280) 0.68 (±0.04)</td>
<td>(5:1±20500) 46600</td>
</tr>
<tr>
<td>(±)-4-OEt 2,5-DMA</td>
<td>2220</td>
<td>(5:1±300) 0.77 (±0.04)</td>
<td>(5:1±13600) 35500</td>
</tr>
<tr>
<td>(±)-4-MePIA</td>
<td>3360</td>
<td>(5:1±150) 0.89 (±0.04)</td>
<td>(5:1±6700) 14800</td>
</tr>
<tr>
<td>(±)-2,5-DMA</td>
<td>5200</td>
<td>(5:1±360) 0.85 (±0.03)</td>
<td>(5:1±150) 1020</td>
</tr>
<tr>
<td>(±)-PMA</td>
<td>33600</td>
<td>(5:1±4480) 0.87 (±0.05)</td>
<td>(5:1±13400) 79400</td>
</tr>
<tr>
<td>(±)-3,4-DMA</td>
<td>43300</td>
<td>(5:1±3300) 0.66 (±0.03)</td>
<td>(5:1±8600) 64600</td>
</tr>
<tr>
<td>R(-)-DOI + Gpp(NH)₃ (10⁻⁴ M)</td>
<td>35</td>
<td>(5:1±2) 0.90 (±0.03)</td>
<td></td>
</tr>
</tbody>
</table>

### Notes
- Kᵢ values and Hill coefficients determined by competition experiments for 0.4 nM [³H]ketanserin-labelled 5-HT₂ serotonin binding sites in rat frontal cortical homogenates. Non-radioactive drugs were tested at 23 concentrations, in triplicate, in three separate experiments. Kᵢ values were calculated from IC₅₀ values (amount of non-radioactive drug inhibiting 50% of specific binding) by the equation: Kᵢ = IC₅₀/(1 + D*/Kₛ), where D* = concentration of [³H]ketanserin, and Kₛ equals the equilibrium dissociation constant of [³H]ketanserin for the 5-HT₂ sites. Values listed are the means and ± S.E.M. for triplicate determinations.
- Kᵢ values and Hill coefficients determined by competition experiments for 1.0 nM [³H]LSD (+ 10⁻⁷ M ketanserin)-labelled 5-HT sites in rat frontal cortical homogenates. Experiments and calculations as per above footnote.

### TABLE 3
Two-site analysis of the interaction of 2,5-DMA derivatives with 5-HT₂ binding sites.

<table>
<thead>
<tr>
<th></th>
<th>K_H (nM)</th>
<th>K_L (nM)</th>
<th>% R_H</th>
<th>K_L/K_H</th>
</tr>
</thead>
<tbody>
<tr>
<td>R(-)-DOI</td>
<td>1.5 (±0.5)</td>
<td>30.0 (±5.1)</td>
<td>40 (±5)</td>
<td>20</td>
</tr>
<tr>
<td>(±)-DOI</td>
<td>2.3 (±1.2)</td>
<td>47.4 (±16.8)</td>
<td>34 (±9)</td>
<td>21</td>
</tr>
<tr>
<td>R(-)-DOM</td>
<td>2.7 (±1.6)</td>
<td>190 (±30)</td>
<td>22 (±5)</td>
<td>71</td>
</tr>
<tr>
<td>(±)-DOM</td>
<td>14.7 (±6.7)</td>
<td>245 (±90)</td>
<td>50 (±9)</td>
<td>17</td>
</tr>
<tr>
<td>(±)-DOB</td>
<td>2.4 (±0.7)</td>
<td>100 (±25)</td>
<td>19 (±1)</td>
<td>42</td>
</tr>
<tr>
<td>α-demethyl DOM</td>
<td>35 (±11)</td>
<td>400 (±110)</td>
<td>52 (±2)</td>
<td>12</td>
</tr>
<tr>
<td>R(-)-DON</td>
<td>68 (±29)</td>
<td>900 (±400)</td>
<td>55 (±18)</td>
<td>13</td>
</tr>
<tr>
<td>(±)-DON</td>
<td>137 (±49)</td>
<td>1500 (±730)</td>
<td>65 (±19)</td>
<td>11</td>
</tr>
<tr>
<td>(±)-2,4,5-TMA</td>
<td>200 (±60)</td>
<td>6250 (±1200)</td>
<td>41 (±6)</td>
<td>31</td>
</tr>
<tr>
<td>(±)-3,4-DMA</td>
<td>3100 (±950)</td>
<td>80600 (±19000)</td>
<td>25 (±5)</td>
<td>26</td>
</tr>
</tbody>
</table>

### Notes
- Data were computer-analyzed with a two-site model as described under Methods. K_H represents the dissociation constant of agonist calculated for the high affinity component of [³H]ketanserin binding. K_L is the dissociation constant calculated for the lower affinity component of [³H]ketanserin competition curves. K_L/K_H is the ratio of the two dissociation constants. % R_H represents the percentage of sites in a high-affinity form for the agonist. Values shown are the mean and S.E.M.s of the experiments, each point performed in triplicate.
Fig. 1A, B. Competition curves of six 2,5-DMA derivatives for the binding of [3H]ketanserin to rat frontal cortex membranes. Membranes were prepared as described under Methods and incubated with 0.4 nM [3H]ketanserin and increasing concentrations of the derivatives presented. The points represent experimental data determined from the means of three experiments, each point determined in triplicate. S.E.M.s were less than five percent for all points. The lines drawn represent the best fit of the data according to a model for two binding sites.

('high affinity state') of the receptors. R(-)-DOI was examined in greater detail; 10^{-4} M Gpp(NH)p was found to eliminate the high affinity phase of the R(-)-DOI competition, bringing the Hill coefficient close to unity (table 2) and shifting the competition curve to the right (fig. 2).

4. Discussion

The results reported in this study indicate that some derivatives of 2,5-DMA have very high affinity (K_D < 10^{-7} M) for [3H]ketanserin-labelled 5-HT_2 binding sites. R(-)-DOI, R(-)-DOM, (±)-DOB, and R(-)-DON were the most potent compounds. These results are consistent with previous reports indicating that these derivatives are extremely potent in interacting with brain serotonin receptors in behavioral models and serotonin receptors in the rat fundus.

Simple methylation at the 4-position of 2,5-DMA, to afford DOM, results in a dramatic increase in binding affinity to rat cortical 5-HT_2 sites; this increase is greater than that observed for
4-methoxylation. Subsequently, (+)-4-Me-PIA was evaluated and compared with (+)-PMA; again, the presence of the 4-alkyl group appears to be of greater significance than the methoxy group. Removal of the α-methyl group of (+)-DOM (i.e. α-demethyl DOM) has essentially no effect on its affinity for 5-HT2 sites, while N-methylation of either racemic DOM or its R(-)-isomer results in a several-fold decrease in affinity. In general, the R(-)-isomers of the 2,5-DMA derivatives display a two-fold higher affinity for 5-HT2 sites than do their racemates (table 2); this suggests that the α-methyl groups make a beneficial contribution to binding when in the R-configuration. These results are consistent with previous reports on human hallucinogenic (Shulgin, 1978) and/or behavioral (Glennon et al., 1983a) potencies in that the R(-)-isomers of 2,5-DMA derivatives are more potent than their racemates or enantiomers. Conversely, the R(-)-isomers of the agents evaluated are essentially equipotent with their racemates with respect to their affinities at 5-HT1 sites (table 2). In fact, the qualitative aspects of the structure activity relationships (SAR) reported herein, for this small series of 2,5-DMA derivatives, appears to echo the SAR previously reported for the serotonin receptor affinities of these agents as determined using the rat fundus preparation. However a more detailed SAR analysis must await the results of further studies. All of the compounds tested in this study displayed relatively low affinity for the [3H]LSD-labelled 5-HT1 sites.

The high-affinity 2,5-DMA derivatives displayed binding characteristics at 5-HT2 sites similar to those associated with agonists (Battaglia et al., 1983a, b). These properties include competition curves producing Hill coefficients significantly lower than unity, computer-assisted analysis supporting a two-state model for the receptor, and the observation that at least with R(-)-DOI, Gpp(NH)p converts the two-state receptor interaction into a one-state interaction. R(-)-DOI and (+)-DOB revealed equilibrium dissociation constants for the high affinity state (KH) of 1.5 and 2.4 nM, respectively. These extremely high affinities indicate that these compounds may be suitable candidates as radioactive agonist labels for 5-HT2 binding sites, especially in light of their high selectivity for 5-HT2 over 5-HT1 sites.

Finally, these results coupled with our recent findings that the selective 5-HT2 antagonists ketanserin and pirenperone effectively antagonize the discriminative stimulus effects of DOM, and DOM-stimulus generalization to several other hallucinogenic agents (Glennon et al., 1983c), support the hypothesis that certain 2,5-DMA derivatives may produce their behavioral effects via an agonistic interaction involving cortical 5-HT2 serotonin receptors.
Acknowledgements

This work was supported by MRC Term Grant No. MT-7791, and by PHS Grant DA-01642. M.S. is a recipient of a University of Toronto Open Scholarship.

References