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Molecular Orbital Studies on Tryptamines Active on the LSD Receptor of the Rat Fundus Strip

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A considerable amount of pharmacological and electrophysiological evidence suggests that *d*-lysergic acid diethylamide (LSD) may initiate its effects by reacting with a brain receptor that is normally activated by the neurotransmitter 5-hydroxytryptamine (5-HT) (see references cited in [1]). Although attempts (see [1] for review) have been made to correlate the hallucinogenic potency of certain *N*-alkylated tryptamines and methoxylated amphetamines with the molecular properties of these compounds (in particular by electronic indices derived from quantum chemical calculations), the complexities inherent in analyzing such in vivo data have encouraged pharmacologists to search for simpler biochemical or pharmacological systems as models of the LSD receptor.

The apical portion of the rat stomach (fundus), when placed in a suitable physiological medium, contracts upon addition of low concentrations (typically 10^{-9} to $10^{-6}M$) of 5-HT [2]. This contraction is blocked by LSD and it is generally assumed that LSD and 5-HT (or other tryptamine derivatives) compete for the same receptor site on the surface of the smooth muscle cells of the fundus [3]. It is reasonable that structure-activity studies upon a series of analogs of 5-HT might provide information about the forces involved in the reaction of tryptamines with the receptor in the fundus.

Structure-activity studies have been reported for ring-substituted tryptamines on the rat fundus [3]. A previous study of Vane's data [3] in this laboratory [4] showed good correlations between potency and several electronic indices calculated by molecular orbital (MO) techniques. As often happens in a limited series of derivatives, many of the MO parameters were intercorrelated and it was not possible to determine which of the parameters was the "true" correlate of biological activity. Predictions of the potencies of a large number of derivatives not yet synthesized were made, and it was noted that for several of the derivatives quite different predicted potencies were obtained, depending on which electronic parameter was used. Testing of these derivatives, a large number of which have now been synthesized, could serve as a check on the validity of the correlations.

Methods

Molecular orbital calculations were performed with the CNDO/2 method [5] with the CNINDO program (Quantum Chemistry Program Exchange, Indiana University). The atomic coordinates for each tryptamine derivative were calculated by the MBLD program (Quantum Chemistry Program Exchange) using standard bond angles and bond lengths. The conformation of the side chain was the same for all tryptamine derivatives with angles of N—C_α—C_β—C_3 = 180° and C_α—C_β—C_3—C_9 = 60° (angles defined according to the Klyne–Prelog rules [6]). This conformation is one of the three minimal energy conformations obtained in previous theoretical studies in this laboratory [7–10] and it is the conformation most likely to be present in aqueous solution (the other two minimal energy conformations involve improbable hydrogen-bonding interactions with the aromatic ring). In any case, the values for the electronic parameters of the ring atoms do not vary appreciably with changes in the conformation of the side chain unless there is intramolecular hydrogen bonding. Substituent groups were assumed to be planar with the aromatic ring and vicinal hydroxy or methoxy groups were assumed to be planar and antiparallel. All calculations were performed on a CDC 6600 computer.

The relative potencies of the tryptamine derivatives were obtained from the study of Vane [3] or were measured in this laboratory essentially as described by Vane. Details of the assay and a discussion of the pharmacological data will be published elsewhere.

Regression analysis was carried out with a stepwise multiple regression program on an interactive computer terminal connected to the Prophet* system.

Results

The original study of Green and Kang [4] demonstrating electronic correlates of tryptamine potency on the fundus was carried out with the INDO MO method. Since the complete data set now available includes two derivatives with a chlorine substituent (which cannot be handled by the standard INDO program), the calculations have been repeated with the CNDO/2 method, and it is these results that are presented here. The MO parameters that were examined in this study included total and π charges, frontier and virtual frontier densities, HOMO and LEMO energies, and total and component dipole moments. Table I shows a selected set of these parameters as well as the biological potencies.

Regression analysis on the original data of Vane [3] (Table I) yielded 12 single-parameter equations with correlation coefficients greater than 0.8 (Table II). This large number of significant correlations could be due to internal correlations of the

*The Prophet system is a specialized computer resource developed by the Chemical/Biological Information-Handling Program of the National Institutes of Health and oriented toward the needs of pharmacologists and medicinal chemists studying the relationship between chemical structure and biological activity. A detailed description of Prophet system features and applications appears in the Proceedings of the National Computer Conference and Exposition, Chicago, Ill., May 6–10, 1974.

TABLE 1. Potencies[a] and molecular orbital parameters[b] for tryptamine derivatives.

Tryptamine Derivative	LOG 1/C[a]	q_1	q_6	q_7	f_1	f_4	π_7
1. 5-OH	0.00	-.1168	-.0397	-.0004	.2384	.2228	0.00
2. 5-OCH_3	-0.06	-.1167	-.0404	-.0018	.2325	.2153	0.00
3. 5-OH, 7-CL	-0.16	-.1131	-.0331	.0717	.1951	.2107	0.80
4. 5,6-$(OH)_2$	-0.42	-.1180	.1535	-.0665	.0335	.0360	0.00
5. 5-CL	-0.88	-.1181	.0203	-.0229	.1901	.1741	0.00
6. 5-CH_3	-0.95	-.1180	-.0042	-.0169	.1855	.1743	0.00
7. 5-F	-0.98	-.1162	-.0415	-.0015	.1926	.1767	0.00
8. 4-OH	-1.15	-.1190	.0371	-.0521	.1434	.1454	0.00
9. H	-1.17	-.1188	.0138	-.0231	.1585	.1505	0.00
10. 4-NH_2	-1.54	-.1200	.0297	-.0460	.1381	.1498	0.00
11. 7-OCH_3	-1.70	-.1089	-.0588	.1638	.1761	.2070	-0.21
12. 5,7-$(OCH_3)_2$	-1.86	-.1068	-.1142	.1843	.1981	.2452	-0.21
13. 5,6-$(OCH_3)_2$	-2.17	-.1194	.1510	-.0774	.0206	.0214	0.00
14. 6-OH	-2.50	-.1216	.1992	-.0869	.0866	.0937	0.00
15. 6-OCH_3	-2.98	-.1208	.1991	-.0963	.0804	.0862	0.00
16. 5,6,7-$(OH)_3$	-3.07	-.1112	.0862	.1314	.1390	.1873	-1.07
17. 5,7-$(OH)_2$	-3.09	-.1081	-.1151	.1862	.2010	.2530	-1.07

[a]Potencies of tryptamines on the rat fundus strip. C—concentration (relative to 5-HT) giving a contraction of 50% of maximum. Compounds 1, 2, 5, 6, 8, 9, 13, 14, and 15 were studied by Vane [3]. However, we have redetermined the activities of compounds, 2, 8, 9, and 14 and it is these corrected values that appear in the table. Data for compounds 3, 4, 7, 10, 11, 12, 16, and 17 are from this study.

[b]q—net total charge on an atom; f—frontier density on an atom; π_7—hydrophobic substituent constant from the aniline system. See Figure 1 for numbering scheme of tryptamines.

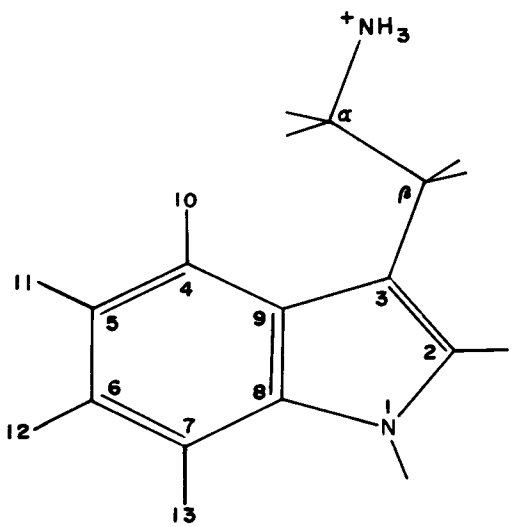

Figure 1. Numbering scheme for tryptamines.

TABLE II. Correlations of potency with the restricted data set.[a]

Correlation Equation	r	s	F
LOG (1/C)= -10.38 q_6 - 0.70	0.969	0.257	123.4
LOG (1/C)= 26.99 q_7 - 0.185	0.963	0.280	102.1
LOG (1/C)= 596.6 q_1 +69.56	0.943	0.344	65.6
LOG (1/C)= -22.86 Q_7^{π} +22.768	0.932	0.375	53.9
LOG (1/C)= -19.01 f_2 + 0.973	0.923	0.399	46.9
LOG (1/C)= 12.77 f_1 - 3.214	0.892	0.468	32.2
LOG (1/C)= 8.12 q_{12} - 0.862	0.890	0.472	31.4
LOG (1/C)= -23.72 f_9 - 0.429	0.876	0.499	27.4
LOG (1/C)= 14.06 f_4 - 3.324	0.868	0.514	25.5
LOG (1/C)= 21.77 Q_6^{π} -22.96	0.857	0.534	23.1
LOG (1/C)= -19.76 f_6 + 0.554	0.852	0.542	22.2
LOG (1/C)= -362.9 Q_1^{π} +610.98	0.829	0.579	18.6

[a]Potencies (LOG 1/C) for 9 tryptamines from original data set of Vane (see Table I). q—net total charge on an atom; Q^{π}—π-electron density on an atom; f—frontier density on an atom; r—correlation coefficient (corrected for number of degrees of freedom); s—standard error of regression; F—F test.

MO parameters. Several of the parameters in Table II can be eliminated as being unlikely correlates of activity. The correlations with f_2, f_6, and f_9 are inverse correlations (that is, increased frontier density at these positions results in decreased biological activity). Since frontier density supposedly represents electron donating ability, it appears unlikely that potency would be inversely correlated with this parameter. The π charge terms Q_1^{π}, Q_6^{π}, and Q_7^{π} are highly correlated with the corresponding total charges at these positions and hence may be eliminated. Finally q_{12}, the charge on the substituent atom attached to the 6-position of the ring, is not very suitable for regression analysis since this atom is either an oxygen or a hydrogen and hence the data consist of two clusters of points. It should also be noted that isosteres of 5-HT in which the indole nitrogen is replaced by an O, S, or CH_2 group are active on the fundus strip [11] (although considerably less active than 5-HT). Hence, it is unlikely that q_1 or f_1 are the sole determinants of potency, although these parameters may contribute to potency.

The only feasible way of approaching the problem of internal correlations of the MO parameters is to increase the size of the data set. It is particularly important to include new types of substituents and new positions of substitution in order to avoid simply extending the internal correlations. We have now tested 11 new tryptamines on the fundus. Statistically reliable estimates of potency were obtained for eight of these derivatives (Table I). Since most of the new derivatives contained substituents at the 7-position of the ring, it was hoped that the problem of multiple correlations could be avoided.

Regression analysis on the complete data set (17 compounds) did not yield any single-parameter correlations with correlation coefficients greater than 0.5. Use of multiple parameter equations (up to 4) did not improve the situation. Thus, either the correlations obtained with the limited data set were fortuitous, or in the complete data set there are some specialized effects with certain derivatives which are not accountable with the available electronic parameters. Plots of the biological data against all the likely correlates found in the original data set were examined for systematic deviations.

The correlation with q_7 [Equation (1), Table III] was markedly improved by eliminating those derivatives having a substituent at the 7-position [Equation (2)]. Such elimination could easily be justified by assuming a steric effect at this position which reduces activity from that predicted by the correlation. However, if such a steric effect exists it cannot be quantitatively accounted for by including a term in the regression to estimate substituent volume (molar volume, polarizability or Taft steric constant E_s).

TABLE III. Correlations of potency with the extended data set.[a]

Correlation Equation	n	r	s	F
(1) LOG (1/C) =-2.426 q_7 - 1.418	17	-0.225	1.052	0.6
(2) LOG (1/C) = 21.79 q_7 - 0.341	12	0.795	0.562	19.9
(3) LOG (1/C) = -111.1 q_7^2 + 8.841 q_7 - 0.592	17	0.725	0.715	9.8
(4) LOG (1/C) = -128.4 q_7^2 + 13.07 q_7 -0.462	15	0.924	0.376	41.9
(5) LOG (1/C) = 6.297 f_1 - 2.419	17	0.310	0.986	2.7
(6) LOG (1/C) = 2.187 f_4 - 1.806	17	0.0	1.060	0.3
(7) LOG (1/C) = 6.705 f_1 + 1.678 π_7 - 2.308	17	0.749	0.687	11.2
(8) LOG (1/C) = 4.996 f_4 + 1.839 π_7 - 2.070	17	0.692	0.749	8.3
(9) LOG (1/C) = 14.35 f_1 + 1.572 π_7 - 3.734	15	0.926	0.399	43.1
(10)LOG (1/C) = 10.55 f_4 + 1.948 π_7 - 3.139	15	0.811	0.618	14.5
(11)LOG (1/C) = 18.09 f_1 -74.77 q_1 + 1.182 π_7 -13.061	15	0.962	0.288	59.4
(12)LOG (1/C) = 24.36 f_4 - 188.4 q_1 + 1.410 π_7 - 27.464	15	0.935	0.375	33.4
(13)LOG (1/C) = 17.87 f_1 - 3.613 q_7 + 1.157 π_7 - 4.289	15	0.958	0.304	52.6
(14)LOG (1/C) = 24.12 f_4 - 9.451 q_7 + 1.300 π_7 - 5.405	15	0.927	0.395	29.7

[a]Potencies (LOG 1/C) from Table 1. q—net total charge on an atom; f—frontier density on an atom; π—Hansch hydrophobic substituent constant from aniline system; n—number of compounds; r—correlation coefficient; s—standard error of regression; F—F test.

Alternatively, a plot of potency against q_7 suggested that there may be a parabolic dependence of potency on this parameter such that there is an optimum charge at this position for high potency. Including a squared term for q_7 led to Equation (3) for the complete data set. Differentiating the equation with respect to q_7 indicates that potency is maximum when the charge at 7 is near zero. This result is difficult to interpret in a mechanistic way; it seems unlikely that potency would be facilitated by zero charge. One could assume that the 7-region comes into contact with a hydrophobic region of the receptor and that a charge at the 7-position (either positive or negative) would result in decreased hydrophobicity and hence decreased affinity for the receptor. This point will be discussed below.

The two major deviations from this correlation are the 5,6-$(OH)_2$ and 5,6,7-$(OH)_3$ derivatives, and elimination of these two compounds led to a marked improvement in the correlation [Equation (4)]. Despite their structural similarities there is no obvious justification for eliminating them from the regression since their deviations are in opposite directions. These results suggest that it might be profitable to examine the experimental data for these two compounds in greater detail. We have found, for example, that the dihydroxylated tryptamines are rather unstable in aqueous solution. Unless protected from oxidation with ascorbic acid, stock solutions of these derivatives rapidly turn slightly pink or yellow. Preliminary fluorescence studies on the 5,6-$(OH)_2$ derivative showed marked changes in the spectra, the implications of which are being studied. In addition to the problem of stability of the tryptamine derivatives there is the larger problem of the complexity of the biological system. For example, there is evidence for at least two types of tryptamine receptors in the fundus which may vary independently in their sensitivity to different tryptamine derivatives [12]. Thus whatever its mechanistic significance, the correlation may serve a useful purpose in revealing inconsistencies in the biological data.

The possibility that the parabolic correlation reflects a hydrophobic effect is interesting in view of the success in correlating biological data with lipid solubility parameters [13]. For this reason we included in the regression analysis terms representing the hydrophobic nature of the substituents, the Hansch π values from the aniline system [14]. Potency was not correlated with the total hydrophobicity parameter (summation of π values for all substituents). This is consistent with the observation [15] that the potencies of five derivatives were not correlated with experimentally determined partition coefficients in the octanol-water system. However, the activities of the derivatives with substituents at the 7-position correlated with the π values at the 7-position (π_7). The parabolic dependence on q_7 with an optimum near zero charge and the positive correlation with π_7 suggests that the relationship to potency may be real. Continuation of the stepwise procedure past the $q_7^2 + q_7$ stage did not result in an improvement in the correlation. These results are virtually duplicated by replacing q_7 with q_1 since these two parameters are highly correlated ($r = 0.985$), but the isostere data suggest that it is unlikely that q_1 by itself is a meaningful correlate.

The correlations involving f_1 and f_4 [Equations (5) and (6), Table III] are similar, having the same deviations (5,6-$(OCH_3)_2$, 5,6-$(OH)_2$, and the four derivatives with an oxygen at the 7-position). It is of interest that the methoxy derivatives show less

deviation than the hydroxy derivatives and that the deviations for the 6-derivatives are opposite to those of the 7-derivatives. These results suggest that there may be some specialized effects in these compounds. The limited number of compounds precludes any definitive conclusions concerning the nature of these effects. Simple steric effects at the 7-position can probably be eliminated since the 7-Cl compound is either correctly predicted (using f_4) or deviates in an opposite direction to the 7-OH or 7-OCH_3 compounds (using f_1). The correlations with f_1 and f_4 can be improved by incorporating a second parameter (π_7) in the regression to account for the hydrophobic nature of the 7-substituent [Equations (7) and (8)]. Eliminating the major deviant points, 5,6-$(OH)_2$ and 5,6-$(OCH_3)_2$, led to Equations (9) and (10). At present the only justification for eliminating these two derivatives is the possibility mentioned earlier that such multioxygenated compounds are particularly unstable in aqueous solution. While we have evidence for this in the case of the 5,6-$(OH)_2$ derivative, the 5,6-$(OCH_3)_2$ derivative is not available for examination.

Continuation of the stepwise procedure led to Equations (11)–(14) as the best three-parameter regressions. It would be difficult to eliminate any of these correlations solely on statistical grounds. We were tempted to eliminate the correlations involving f_1 and/or q_1 since, as noted before, isosteres of tryptamine are active on the fundus. However, to be certain we also carried out MO calculations on the carbon, oxygen, and sulfur isosteres of tryptamine and 5-HT. All five isosteres that were tested are less potent than 5-HT [11, 16]. The correlations noted above with the tryptamine derivatives were not observed in the isostere series, at least from a quantitative standpoint. It should be noted, however, that the isosteres will have markedly different physical properties, in particular partitioning behavior, which may influence potency. In addition, these compounds may exhibit different metabolic activity. Vane [3] has shown that the molar ratios of the tryptamines may vary dramatically depending on whether a monoamine oxidase inhibitor is used in the assay. It is not clear in the reports [11, 16] whether an inhibitor was used in the isostere studies. Thus, quantitative agreement of the isostere data with the tryptamine correlations would be unexpected and probably fortuitous. It may be of significance, however, that of the possible correlations noted above for the tryptamines, only the $f_4 + q_7$ correlation [Equation (14)] and the $f_1 + q_1$ correlation [Equation (11)] would predict all the isosteres to be less potent than 5-HT. Although this could be by chance, three of the five isosteres fall reasonably close to the regression line obtained with Equation (14). In the case of Equation (11) only one of the isosteres falls near the regression line. Equations (12) and (13) predict one or more of the isosteres to be hundreds of times more potent than 5-HT. The regressions involving q_7 [Equations (13) and (14)] may well be antifactual. The coefficient of q_7 is negative which implies that potency is increased by increasing the negative charge at this position. It is difficult to see why potency would be increased either by increasing the hydrophobic nature of the substituent at 7 or by increasing the negative charge at 7. Thus, although the predictions of the isostere data with Equation (11) are not as good quantitatively as with Equation (14), the former correlation appears to be more reasonable in a mechanistic sense.

Discussion

In any quantitative structure-activity study it is important to recognize the limitations imposed by the biological data. A number of the tryptamine derivatives used in this study showed a marked instability when dissolved in water, even when made up in the cold. Although this could be prevented to some extent by dissolving these compounds in ascorbic acid solution, there is no certainty that destruction is completely prevented. It is possible, therefore, that certain of the molar ratios reported here and previously [3] are too high. It should also be noted that, although oxidative deamination by monoamine oxidase was prevented by including an inhibitor in the assay medium, other routes of metabolism were not eliminated. In addition, there is evidence for the presence of at least two types of tryptamine receptor in the fundus [12] so that the molar ratios could reflect a composite of effects on at least two receptors. Finally, since the tryptamine receptor is apparently on the outer surface of the fundus smooth muscle cell [3], a significant uptake of the tryptamine derivatives into the cells would lead to observed molar ratios greater than the true values. Cell penetration would probably not be of significance for the very polar derivatives but could be substantial for the halogenated and methylated compounds. Because of these multiple possibilities for drug disposition and "silent receptor" binding, a strict adherence of all data points to correlation equations based upon a single, relatively simple, biologic phenomenon would be unexpected.

Of all the correlations of tryptamine potency, we feel that the "best" correlation is that of Equation (11) in Table III. However, there are significant deviations from all the correlations, and no completely satisfactory explanations for all the deviations are available. Mechanistically, the correlation with f_1 [Equation (11)] is probably most attractive since it suggests that the electron donating capacity of the indole nitrogen is a major determinant of potency. Electron donation from a heteroatom is not an unusual hypothesis to explain drug-receptor binding. It is less clear how the ring carbon 4 would be involved in electron donation. The concept of frontier density, although used extensively in structure-activity studies, is not as well founded theoretically as other MO parameters and its use has been criticized (for references see [17]). Regardless of the physical significance, the correlation equation may still have predictive use in the design of more active derivatives.

The potencies of the tryptamine derivatives appeared to be determined almost entirely by electronic effects. There was no evidence of steric effects with the derivatives studied.

The correlation results suggest that the hydrophobic nature of the substituent group except at the 7-position is not important in determining activity. It is likely, therefore, that the tryptamine receptor is near the surface of the cell membrane and may indeed be in a rather hydrophilic environment. If this is the case, one should probably consider the possible effects of a hydrophilic environment on the electronic structure of the tryptamines. For example, one might consider the effects of a solvent shell surrounding the polar oxygen functions in the 5,6-dioxygenated compounds. Similarly, one should consider the solvation of the indole nitrogen and the possible

effects of the 7-oxygen functions on the solvated structure. Such effects may well be the cause of the deviations observed in our correlations.

The large number (more than 50) of MO parameters available in this study precludes consideration of all combinations of variables. Significant correlations could have been missed by the stepwise procedure. Indeed, in the present study, by forcing certain variables into the regression that would not be entered by the stepwise procedure we have obtained correlations that are statistically significant. All these correlations involved either a large number of parameters or had no mechanistic meaning.

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