Preferred Conformation of Serotonin and a Postulate on the Nature of its Receptor from Molecular Orbital Calculations

By LEMONT B. KIER

Using extended Hückel molecular orbital theory, the preferred conformation of serotonin (5-hydroxytryptamine) I has been calculated. The internitrogen distance was calculated to be 5.84 Å. and the quaternary nitrogen to oxygen distance was calculated to be 6.96 Å. The complementary features of the serotonin receptor are predicted and the relationship of serotonin in its preferred conformation to the serotonin antagonist, LSD, is noted as an explanation of LSD's antagonism.

OVER THE past decade, an enormous amount of information has accumulated on serotonin (5-hydroxytryptamine) (I). A recent book on this subject by Garattini and Valzelli brings together much of this material (1). Of considerable interest to many scientists is the nature of the biological receptor interacting with the molecule. Gaddum and his group concluded, after a series of investigations, that the effect of serotonin on smooth muscle is specific and is not related to the effect of acetylcholine or histamine (2–5). The term "tryptamine receptors" was proposed to classify the receptors of the smooth muscle sensitive to serotonin. In reporting a study of antagonists, Barlow suggests that tryptamine and serotonin receptors may not be identical (6). This concept of the specific effect of serotonin is supported by results from numerous studies of known chemical mediators on serotonin-induced smooth-muscle stimulation (1).

A further elaboration of the serotonin-receptor concept was proposed by Gaddum, who offered evidence for two types of serotonin receptors, M and D (4). The M receptors were those blocked by morphine and the D receptors those blocked by dibenzyline. He found that the D receptors, located in the plain muscle fibers, were also blocked by LSD, gramine, or dihydroergotamine, while the M receptors, not accurately localized, were not easily blocked by these drugs (7–8).

Other authors have explained the dual-receptor-type effects by suggesting that serotonin stimulates organs containing smooth muscle by a direct action on the smooth muscle fiber, and by an indirect action on the nervous tissue (9–12).

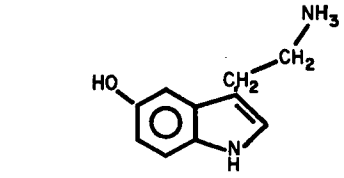
The question of whether serotonin interacts with two different types of receptors or functions both as a direct and indirect acting agent has not been resolved. The two-receptor concept is reminiscent of that proposed for the action of acetylcholine while the direct-indirect action is typified by ephedrine. In connection with the acetylcholine dual-receptor concept, Martin-Smith, Smail, and Stenlake have recently proposed that acetylcholine acts at both receptors (muscarinic and nicotinic) by being able to assume two different conformations (13).

Recently, this problem of the muscarinic and nicotinic actions has been considered by the author in studies on the preferred conformations of acetylcholine, muscarine, muscarone, and nicotine (14, 15). By performing quantum mechanical calculations using extended Hückel theory, the total energy of each molecule was minimized as a function of geometry and calculated preferred conformations were obtained. In the case of acetylcholine, the author reported that the molecule exhibited some degree of conformational flexibility and could exhibit a preferred conformation over a range of one bond-angle variation. At one point in this variable energy minimum, the acetylcholine conformation corresponded to the preferred conformations of muscarine and muscarone. The heteroatoms in each molecule bore a similar relationship to each other and were similarly related to the other muscarinics. From this it was possible to deduce the nature of the muscarinic receptor and to deduce the muscarinic conformation of acetylcholine (14).

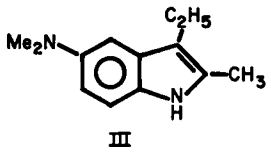
It was also found that acetylcholine in a preferred conformation different from its muscarinic conformation placed key atoms in a relationship nearly identical to that of comparable key atoms found for nicotine in its calculated preferred conformation (15).

These studies supported the idea of dual conformers functioning at two different receptors. Further support for this theory was found in the most recent study on histamine (16). In this case, it was found from these calculations that histamine exists in two different preferred conformations, and it was possible to relate one

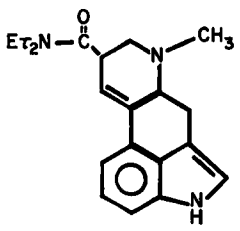
Received February 26, 1968, from Batelle Memorial Institute, Columbus, OH 43201
Accepted for publication March 21, 1968.



I



III



II

preferred conformation to a postulated receptor associated with one histamine action. The second preferred conformation was inferred to be complementary to a second histamine receptor.

From these studies it is possible to propose a theory concerning dual-receptor involvement that provides a rationale for considering the serotonin molecule by quantum mechanical methods. Essentially, it was postulated that *an observed dual action of a molecule may be explained by the presence of two preferred conformers of the molecule in equilibrium.* As a corollary, it can be stated that *if a molecule can exist in two nearly equally preferred conformations, an observed dual action of that molecule can be explained by the interaction of these different conformers with different receptors.*

If these postulates are assumed to be valid, it is readily apparent that quantum-mechanical calculations of the preferred conformation of the serotonin molecule may well lead to some understanding of its activity.

CALCULATIONS

The calculations used in this work have been described in detail elsewhere (14, 17). Briefly, the expansion of a molecular orbital as a linear combination of atomic orbitals yields

$$\Psi_i = \sum C_{ij}\phi_j$$

upon minimizing the total energy, a set of Hückel equations,

$$\sum_{i=j}^{n}[H_{ij} - ES_{ij}]C_{ij} = 0 \qquad j = 1,2 \ldots . n$$

where Ψ is the molecular orbital wave function, H the Hamiltonian operator, E the energy, S the overlap integral, and C the orbital coefficient. The calculations are performed for the valence electrons in s and p orbitals, using Slater orbitals as a basis set. The input parameters consist of the Slater exponent and the Coulomb integral, approximated by the ionization potential. All overlap integrals are considered. The resonance integrals are approximated by the equation

$$H_{ij} = 0.5\,K\,(H_{ii} + H_{jj})S_{ij}$$

with K set at 1.75. The total electronic energy is computed to be the sum of doubly occupied molecular orbitals,

$$\epsilon = 2\sum E_i$$

The total molecular energies can be written as

$$E = 2\sum E_i + \sum_{n,n'} Enn' - \sum_{e,e'} Eee'$$

where Enn' and Eee' are nuclear-nuclear and electron-electron repulsion energies. The success of the calculations in predicting preferred conformations from minimum energies lies in the fact that the method of selecting the H_{ij} values must simulate, within the calculated electronic energies, the contribution of nuclear repulsions to the total energy (17, 18). Thus, the nuclear-nuclear and electron-electron repulsion energies cancel approximately, and the simple sum of one-electron energies behaves similarly to the true molecular energy (19). A characteristic of the extended Hückel theory is the frequently correct prediction of equilibrium conformation with an exaggeration of barrier heights due to overestimation of nonbonded interactions.

For the calculations in this study, it was necessary to input precise three-dimensional coordinates for each atom. This otherwise very difficult task was expedited by a computer program that was obtained from the Indiana University Quantum Chemistry Program Exchange. The program performs a series of vector summations with previously determined planes of atoms as references. The program can be keyed to output the atom coordinates on punched cards in the fields required for the extended Hückel program. The bond angles and lengths used in the coordinates program were derived from standard values proposed by Pople and Gordon (20). Bond lengths were assumed not to vary in the different conformational models. To simulate the molecule in an *in vivo* environment, the author considered the amino-alkyl group as being protonated.

RESULTS

Calculations made every 45° for rotation of the bond joining the side chain to the ring through 180° revealed an energy minimum at $\theta_{ring-C} = 90°$ (Fig. 1). At this angle, the C—C bond of the side chain projects directly away from the plane of the ring system. Throughout this rotational pattern, the side chain is held in an all-*trans* conformation. The energy drop is not severe as the bond is rotated away from the all-planar conformation depicted in 1. An energy difference of 0.1 ev. exists between $\theta_{ring-C} = 45°$ and $\theta_{ring-C} = 90°$ (see Fig. 1). As the side chain rotates past the 90° minimum toward the benzene side of the ring system, the energy rises steeply, with a 1.5-ev. difference between $\theta_{ring-C} = 90°$ and $\theta_{ring-C} = 180°$. As has been pointed out, the energy barriers are exaggerated in these calculations; nevertheless, the values calculated must still be regarded as being considerable, based upon com-

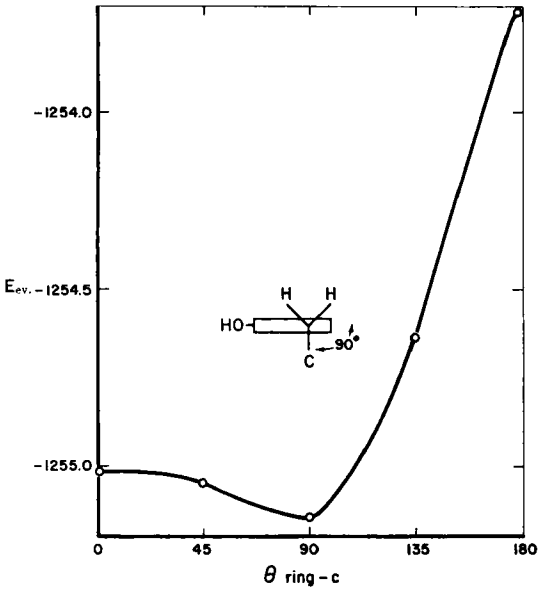


Fig. 1—Angle of rotation of ring to side chain bond vs. *energy.*

parisons of calculated with experimental values (17).

A further search for the energy minimum was conducted by varying the C—C bond of the side chain through 360°. This was done for the $\theta_{ring-C} = 90°$ and the $\theta_{ring-C} = 45°$ conformations. It was felt prudent to consider the latter conformation since the energy difference between the two was not so great as to exclude the 45° conformation as being part of a preferred conformation. The C—C rotation *versus* energy is depicted in Fig. 2. The minimum energy for this rotational model occurred for $\theta_{C-C} = 180°$, or on all-*trans* side chain. This minimum was found for both the $\theta_{ring-C} = 90°$ and the $\theta_{ring-C} = 45°$ conformations, although the former conformation had a substantially lower energy.

At the energy minimum, $\theta_{ring-C} = 90°$, $\theta_{C-C} = 180°$, the internitrogen distance was calculated to be 5.84 Å. The (*tetetete*)N-to-0 distance was calculated to be 6.96 Å. while the ($tr^2trtr\pi$)N-to-0 distance was 5.61 Å. The charge densities are displayed in Fig. 3, in which the values are for ($\sigma + \pi$) electrons. Note that the nitrogen atom of the quaternary group is negatively charged. The author has previously commented on this finding (16). The net charge of the quaternary group (including the α and β atoms) is +1.018.

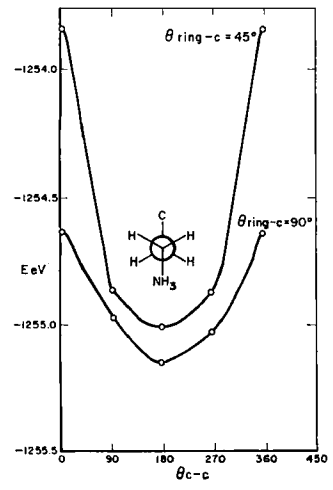


Fig. 2—Angle of rotation of side chain carbon to carbon bond vs. *energy.*

DISCUSSION

From these calculations it was found that serotonin exists in the single-energy preferred conformation shown in Fig. 4. From the energy *versus* angle profiles, it is evident that the molecule has a fair degree of rigidity in this conformation. Thus, a pattern of heteroatoms may be presented to a receptor, as shown in Fig. 5. The choice of these three atoms as being the essential ones for activity is speculative but reasonable. The inclusion of the oxygen in this category is based on the knowledge that, although tryptamine will produce some serotonin effects in the gut, its activity is feeble (2). In addition, as previously noted, antagonist actions suggest that serotonin and tryptamine receptors are different (6).

Because serotonin has only one preferred conformation, as indicated by these calculations, its

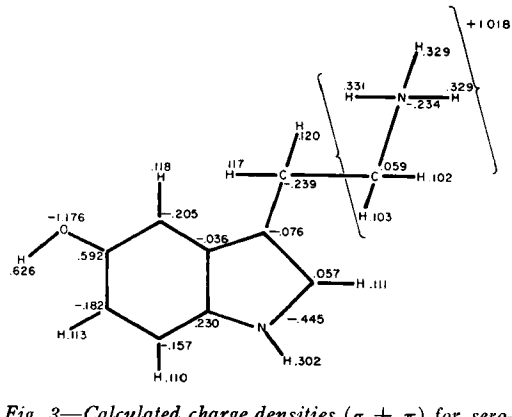


Fig. 3—Calculated charge densities ($\sigma + \pi$) for serotonin.

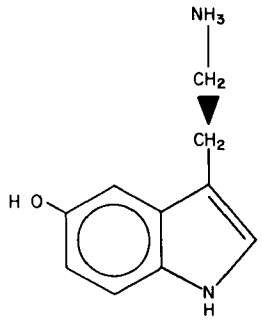


Fig. 4—Calculated preferred conformation of serotonin.

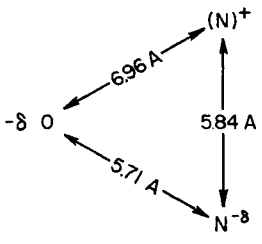


Fig. 5—Structural features proposed as being complementary to the serotonin receptor.

activity cannot be explained by the dual-conformation concept. However, the calculated inability of serotonin to exist in more than one preferred conformation does not completely exclude the possibility that the molecule could interact with two different receptors. There is the remote possibility that this could occur if the molecule presented alternate faces to each of two approximately mirror-image receptor patterns. Thus, each receptor would have its own set of stereospecific requirements for an agonist and each face of the serotonin molecule would satisfy these requirements. It remains to be conclusively demonstrated whether there are two receptors for serotonin. An explanation for the accommodation of serotonin to each is possible from this work. This possibility is considered remote since the quaternary N projects well above the plane of the rings, hence only one side of the molecule presents a planar aspect to a receptor. The alternate side of the molecule does not permit a close approach of the quaternary N if the other nitrogen and the oxygen atoms are anchored to a receptor. The alternative hypothesis of both direct and indirect effects is easier to explain since, as in the case of indirect-acting adrenergic agents, lower stereospecific requirements are present. Thus, only two of the three serotonin heteroatoms may be involved in an indirect action.

A comparison of the calculations reported here with previous calculations on the histamine molecule reveals that the two molecules in their preferred conformations do not have their heteroatoms separated by a similar distance (16). This suggests that the two molecules are not involved with the same receptor.

It is instructive to compare the results of these calculations with the dimensions of the more rigid, potent serotonin antagonists. The most prominent of these compounds is lysergic acid diethylamide (LSD) (II). It is known to be an antagonist for virtually all actions of serotonin (1). Because it is such an effective antagonist, it is reasonable to assume that the molecule successfully binds with enough of the serotonin receptor to exclude serotonin although it does not have the full complement of structural features necessary to produce an efficacious complex. Measurements on accurate scale models of LSD indicate a distance between the amino and the pyrrole nitrogen atoms to be 6.0 ± 0.2 Å. At physiological pH, the *N*-methyl group would be protonated. Therefore, it is evident that these two nitrogen atoms could interact with portions of the serotonin receptor complementary to the quaternary and pyrrole nitrogens of serotonin, based upon their positions in the calculated preferred conformation. Derivatives of lysergic acid would also present these same atoms to the serotonin receptor, which accounts for the prominence of antagonism in this class of compounds.

A second antagonist molecule from which one can readily derive values of interatomic distances is 2-methyl-3-ethyl-5-dimethylaminoindole (medmain) (III) (21). The internitrogen distance in this molecule, estimated from a scale model, is about 5.75 Å. This closely approximates the 5.61 Å. distance calculated to separate the oxygen from the pyrrole nitrogen of serotonin.

From these calculations on the serotonin molecule, it can be concluded that there is a single preferred conformation and that the relative preference is fairly high. In this preferred conformation, the molecule presents three heteroatoms to a receptor in a predictable manner, hence a complementary pattern of forces on the receptor can be deduced. The antagonism of LSD and other inhibitor molecules can now be explained in terms of this postulated receptor, since comparable interatomic distances are found in these antagonist molecules. This finding also tends to verify speculation on the serotonin receptor (Fig. 5).

REFERENCES

(1) Garattini, S., and Valzelli, L., "Serotonin," Elsevier Publishing Co., New York, N. Y., 1965.
(2) Gaddum, J. H., *J. Physiol.*, **119**, 363(1953).
(3) Fingl, E., and Gaddum, J. H., *Federation Proc.*, **12**, 320(1953).
(4) Gaddum, J. H., and Hameed, K. A., *Brit. J. Pharmacol.*, **9**, 240(1954).
(5) Gaddum, J. H., Hameed, K. A., Hathway, D. E., and Stephens, F. F., *Quart. J. Expll. Physiol.*, **40**, 49(1955).
(6) Barlow, R. B., and Kahn, I., *Brit. J. Pharmacol.*, **14**, 265(1959).
(7) Gaddum, J. H., in "5-Hydroxytryptamine," Lewis, G. P., ed., Pergamon Press, London, England, 1958, p. 195.
(8) Gaddum, J. H., and Picarelli, Z. P., *Brit. J. Pharmacol.*, **12**, 323(1957).
(9) Ambache, N., in "5-Hydroxytryptamine," Lewis, G. P., ed., Pergamon Press, London, England, 1958, p. 203.
(10) Beleshin, D., and Varagic, V., *Arch. Intern. Pharmacodyn.*, **126**, 321(1960).
(11) Innes, I. R., Kosterlitz, H. W., and Robinson, J. A., *J. Physiol. (London)*, **137**, 396(1957).
(12) Kosterlitz, H. W., and Robinson, J. A., *Brit. J. Pharmacol.*, **13**, 296(1958).
(13) Martin-Smith, M., Smail, G. A., and Stenlake, J. B., *J. Pharm. Pharmacol.*, **19**, 561(1967).
(14) Kier, L. B., *Mol. Pharmacol.*, **3**, 487(1967).
(15) *Ibid.*, **4**, 70(1968).
(16) Kier, L. B., *J. Med. Chem.*, in press.
(17) Hoffmann, R., *J. Chem. Phys.*, **39**, 1397(1963).
(18) *Ibid.*, **40**, 2480(1960).
(19) Slater, J. C., "Quantum Theory of Molecules and Solids," vol. I, McGraw-Hill Book Co., New York, N. Y., 1963, p. 108.
(20) Pople, J., and Gordon, M., *J. Am. Chem. Soc.*, **89**, 4253(1967).
(21) Shaw, E., and Woolley, D. W., *J. Biol. Chem.*, **203**, 69(1953).

Keyphrases

Serotonin—preferred conformation
Single-energy preferred conformation—serotonin
Dual-receptor theory
Receptors proposed—serotonin
LSD antagonism—serotonin