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Charge Distribution of Histamine Monocation in Its "Essential" Conformation

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With the atomic nuclei in positions earlier defined as the "essential" conformation for activity of the histamine monocation, the charge distribution is obtained by integrating the square of the molecular wave function. The results with ab initio wave functions indicate that the positive charge is evenly dispersed over the molecular skeleton and that pictures of receptors involving localized negative sites may be invalid. A detailed description of electron distribution is given.

A comparison of the conformational flexibility of the histamine monocation, which is active at both H_1 and H_2 receptors, with that of the 4-methyl compound (H_1 activity half the activity of histamine and H_2 activity $^1/_{500}$th) has led to the definition of a possible H_1 "essential" conformation.[1] The inclusion of further active species such as *N,N*-dimethylhistamine suggests restriction of attention to the single trans (antiperiplanar) conformation. These conclusions were reached using potential surfaces generated using the crude extended Huckel method which was justified by experimental criteria.[1] For the defined essential conformation we now use the more reliable ab initio molecular orbital method to compute the charge distribution.

This defined conformation may be essential in producing activity for one of two reasons. The precise spatial arrangement of atoms may be that which is required to bind to the receptor, in which case properties of the receptor binding site may be inferred. The alternative rationale for a conformation which is available to active compounds but denied to inactive species might be that the essential conformation is one through which the molecule must pass in order to gain access to the receptor site.

In either case, but particularly if the former alternative in fact represents the molecular situation, a precise definition of the relative atomic positions of the various nuclei is obviously helpful. Far more useful, however, would be a clear indication of the detailed electronic structure of the histamine monocation in its essential conformation. Any interaction between the active molecule and its receptor must involve electrons rather than nuclei which are merely positive centers to which the electronic cloud is held.

Crude theoretical methods[2] which give an indication of electronic distribution have been available for many years. Recently a method giving an accurate value for the number of electrons in any defined volume of space and suitable for pharmacological species has been devised.[3] Here we apply this technique to the histamine monocation with the nuclei in positions indicated earlier as "essential".

Charge Densities. Since the earliest days of quantum mechanics it has been realized that the square of a molecular wave function, ψ, yields a probability or electron density. The value of ψ^2, or $\psi^*\psi$ if ψ is complex, integrated over a defined region of space will give the number of electrons in that volume. The reason why this obvious source of electron density information has not been applied to molecules which are not highly symmetrical has been the practical problem of evaluating $\int\psi^*\psi d\upsilon$ over volumes of space which are removed from the origin of the molecular coordinate system.

The recent advance has been made possible by the fact that computational methods are now so powerful that it is possible to compute an accurate molecular wave function so quickly that it is not too extravagant to choose each individual atom in the molecule successively as the origin of the coordinate system and to repeat the quantum mechanical calculation for each case. Integrating $\psi^*\psi d\upsilon$ when the volume $\int d\upsilon$ is a sphere centered at the origin is relatively simple so that in this way the charge density in any sphere centered on each individual nucleus may be obtained.

There remains the problem of the arbitrary definition of the volume elements which are considered. Spheres are mathematically simplest and also sensible, although a

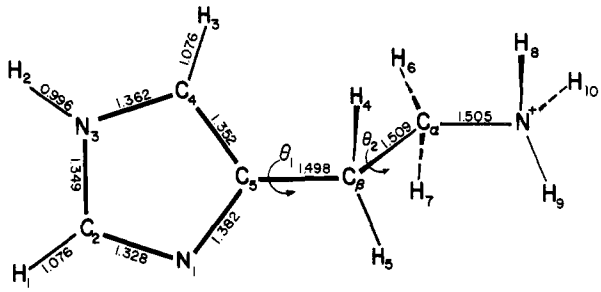

Figure 1. The molecular geometry of the histamine monocation used in the calculations. Angles refer to C_4-C_5-C_β as 0°; unless stated the C-H distance is 1.073 Å, N-H distance is 1.032 Å; $\theta_1 = 300°$ and $\theta_2 = 180°$.

Table I. Total Charge Density within Spheres of Radius r Centered on Atoms of Histamine Monocation in Its "Essential" Conformation

Atom	r, Å						
	0.77	0.70	0.68	0.65	0.63	0.33	0.30
N_1		4.631		*4.318*	4.190		
C_2	3.824	3.478	*3.384*				
N_3		4.638		*4.312*	4.180		
C_4	3.843	3.508	*3.416*				
C_5	3.507	3.256	*3.184*				
C_β	3.918						
C_α	3.902						
N^+		4.947					
H_1						*0.089*	0.073
H_2						*0.067*	0.054
H_3						*0.090*	0.074
H_4						*0.088*	0.073
H_5						*0.083*	0.069
H_6						*0.081*	0.067
H_7						*0.079*	0.065
H_8						*0.054*	0.044
H_9						*0.054*	0.044
H_{10}						*0.054*	0.044

finely structured grid of cubes could also on occasion be illuminating. If the choice of spheres is taken, then a serious question as to the appropriate radius remains. Any choice is arbitrary but the number of electrons within any sphere can be given precisely.

In the present work we have taken spheres of covalent radii, giving extra information where it is not clear what is the most appropriate value for a covalent radius, as in the case of nitrogen atoms in the imidazole ring where the covalent radius should be related to the nature of adjacent bonds.

Calculations. The conformation and labeling of the cation used in the calculations are illustrated in Figure 1. With the nuclei held in this arrangement, ab initio molecular wave functions were calculated using the Gaussian '70 molecular orbital program[4] and a basis set of STO 3G quality. Such wave functions do not involve empirical parameters and may be considered as of good rather than superlative quality. However, for charge distributions we are considering the square of the wave function at any point and there are consequently no difficulties about positive and negative portions of functions canceling, then the charge picture resulting should be very realistic.

Results

The full details of the calculations are presented in Tables I and II. From these the charge density in spheres of other diameters may be interpolated. In Table I the charge density within spheres is given while in the second table an indication is given of the gradient of charge as the distance from a particular atom increases. A plot of $(Z - q)$ against r where Z is the nuclear charge and q the calculated electronic charge in a sphere of radius r can be used to give an idea of a suitable effective atomic size.

Figure 2 illustrates the net positive charges in spheres whose radii are indicated in italics in Table I. These radii were chosen to avoid the overlapping of spheres associated with adjacent atoms.

Table II. Charges within Spheres Centered on Atoms of Histamine Monocation in Its "Essential" Conformation with Varying Sizes of the Defined Spheres

Atom	Covalent radius, Å	Charge within sphere of radius given as percentage of covalent radius			
		25%	50%	75%	100%
N_1	0.65	1.615	2.374	3.260	4.318
C_2	0.70	1.492	2.189	2.734	3.478
	0.68	1.454	2.163	2.679	3.384
N_3	0.65	1.611	2.369	3.243	4.312
C_4	0.70	1.492	2.197	2.759	3.508
	0.68	1.454	2.170	2.702	3.416
C_5	0.70	1.497	2.183	2.662	3.256
	0.68	1.459	2.159	2.615	3.185
C_β	0.77	1.607	2.293	3.003	3.918
C_α	0.77	1.606	2.288	2.993	3.902
N^+	0.70	1.700	2.505	3.614	4.947
H_1	0.33	0.0032	0.0191	0.0487	0.0890
	0.30	0.0025	0.0151	0.0394	0.0733
H_2	0.33	0.0023	0.0136	0.0351	0.0666
	0.30	0.0018	0.0107	0.0283	0.0539
H_3	0.33	0.0033	0.0195	0.0496	0.0900
	0.30	0.0025	0.0155	0.0402	0.0743
H_4	0.33	0.0033	0.0195	0.0488	0.0876
	0.30	0.0026	0.0155	0.0397	0.0727
H_5	0.33	0.0032	0.0185	0.0465	0.0834
	0.30	0.0025	0.0147	0.0378	0.0691
H_6	0.33	0.0030	0.0177	0.0447	0.0806
	0.30	0.0023	0.0141	0.0363	0.0667
H_7	0.33	0.0030	0.0174	0.0438	0.0791
	0.30	0.0023	0.0138	0.0356	0.0654
H_8	0.33	0.0019	0.0113	0.0287	0.0537
	0.30	0.0015	0.0089	0.0232	0.0437
H_9	0.33	0.0019	0.0113	0.0288	0.0538
	0.30	0.0015	0.0090	0.0233	0.0438
H_{10}	0.33	0.0019	0.0113	0.0289	0.0540
	0.30	0.0015	0.0090	0.0233	0.0440

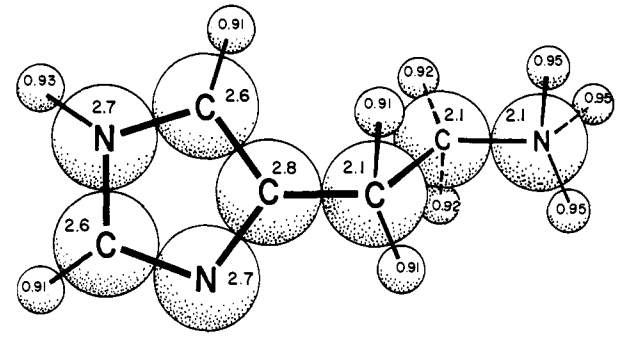

Figure 2. Net positive charges within spheres centered on atoms of histamine monocation. The conformation is as indicated in Figure 1 and the radii of the spheres correspond to the italic values in Table I.

Discussion

It must be emphasized that the calculations are performed on the isolated cation with no solvent included. However, the charge distribution shown in Figure 2 is so uniform that it seems unlikely that the inclusion of other molecules would have a dramatic effect.

The even distribution of positive charge over the molecular skeleton is the most important general result. The

positive charge is not located on the nitrogen atom conventionally written with a positive charge alongside it; the hydrogen atoms attached to it are only slightly more positive than others in the molecule.

This charge picture emphasizes the naivete of attempts to picture receptor binding sites in terms of a localized negative charge with which the onium cation must bind. A more realistic view is that the receptor site should be negatively charged so that long-range coulombic forces may be used in attracting the overall positive histamine cation but that when close-up the specificity is provided by the steeper dispersion force potential. The charge distribution may reveal something of the complimentary nature of the receptor. It may also be worthwhile to calculate the molecular electrostatic potential of the ion and to investigate the variation of charge density as a function of conformation.

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Adrenocorticotropin. 49.[1] Synthesis and Biological Activity of [2-δ-Aminovaleric acid,5-arginine]adrenocorticotropin-(2–19)

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An adrenocorticotropin analogue, [2-δ-aminovaleric acid,5-arginine]adrenocorticotropin-(2–19), has been synthesized by the solid-phase method and its biological activity has been determined. It was found that substitution of arginine for glutamic acid at position 5 of [2-δ-aminovaleric acid]adrenocorticotropin-(2–19) increased the steroidogenic potency in isolated rat adrenal cells and the lipolytic potency in isolated rat fat cells but decreased the lipolytic potency in isolated rabbit fat cells. The synthetic analogue had only 2% of the melanotropic potency of the parent molecule.

Draper et al.[2,3] reported recently that [Arg^5]-ACTH-(4–10) had a lipolytic activity in rabbit fat cells that was five times greater than that of the natural sequence, ACTH-(4–10). Since there is a good correlation between lipolytic activity and steroidogenic activity,[4] we deemed it of interest to synthesize an adrenocorticotropically active fragment of ACTH to determine if the equivalent amino acid substitution would increase steroidogenic potency. The peptide fragment that we have synthesized is [Ava^2,Arg^5]-ACTH-(2–19) (III, see Figure 1). We have previously shown[5] that [Ava^2]-ACTH-(2–19) (II) possesses a greater in vivo steroidogenic potency than does ACTH-(1–19) (I).

Peptide III was synthesized by the solid-phase procedure[6] as described for the synthesis of I.[7] Treatment of the protected peptide resin with liquid HF,[8,9] followed by purification of the crude peptide by chromatography on Sephadex G-25 and carboxymethylcellulose, gave peptide III which was shown to be highly purified by paper electrophoresis, amino acid analysis, and thin-layer chromatography.

A summary of the biological activities of peptide III is reported in Table I together with that of peptides I and II, for comparison. It may be seen that the substitution of arginine for glutamic acid (III vs. II) significantly increases the steroidogenic activity. The combination of the arginine substitution in position 5 and the substitution of δ-aminovaleric acid for the amino terminal Ser-Tyr residues of peptide I (III vs. I) doubles the steroidogenic potency.

The most remarkable aspect of the biological data is the significant decrease of melanotropic activity while increasing steroidogenic activity (III vs. II) due to the substitution of arginine for glutamic acid. Peptide III possesses approximately 2% of the melanotropic activity of peptide II in both the in vivo and in vitro assays. This contrasts with the general observation[4] that melanotropic activity is equally or less sensitive than steroidogenic activity to structural changes in ACTH peptides. It was reported earlier that amino acid substitutions in ACTH peptides which give analogues possessing only a fraction of the steroidogenic activity also result in either comparable changes[10,11] or moderate decrease[12] of melanotropic activity. The most dramatic example[13,14] is [Trp-(Nps)^9]-ACTH, which has 1% of the steroidogenic activity and 300% of the melanotropic activity of the parent molecule. A comparison of the melanotropic and steroidogenic potencies of peptides III and II shows a complete reversal of this relationship. We believe this marks the first instance thereof.

Also surprising in the biological data is the decrease in lipolytic activity in isolated rabbit fat cells (III vs. II) upon the substitution of arginine for glutamic acid in contrast to the result[2,3] obtained for the equivalent substitution in ACTH-(4–10). The most likely explanation for this difference probably relates to the different chain lengths of the peptide pairs and to the difficulties in extrapolating the results obtained for a small peptide to a larger peptide. In agreement with our observation is another recent result in this laboratory. The substitution of arginine for glutamic acid at position 8 (within the Met-Glu-His-Phe-Arg-Trp-Gly sequence) of camel β_2-melanotropin gives a peptide possessing 16% of the melanotropic activity and 41% of the lipolytic activity of the native peptide.[15,16] It is of interest that the decrease in lipolytic activity in rabbit fat cells qualitatively parallels the decrease in melanotropic activity in agreement with a previous suggestion[4,17] that the hormonal receptors in rabbit fat cells and frog melanophores are similar.

Finally, the lipolytic activity in isolated rat fat cells (III vs. II) shows an increase in potency due to the substitution of arginine for glutamic acid. This parallels the increase in steroidogenic potency and is also in agreement with a previous suggestion[4,17] that the hormonal receptors of rat