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A Molecular Orbital Study of Norepinephrine and 3,4-Dihydroxyphenethylamine: a Re-evaluation of Structure-Activity Relationships in Norepinephrine

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SUMMARY

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Complete neglect of differential overlap (CNDO) calculations show that protonated (14*R*)-norepinephrine can exhibit four minimum energy conformations, two *trans* and two *gauche*. Small relative energy differences between the *gauche* and *trans* forms were noted, in favor of the *gauche* forms. Interatomic distances in the two *gauche* and in the two *trans* forms were different. The minimum energy conformations of possible importance in the uptake process of norepinephrine at adrenergic nerve terminals of mouse cardiac tissue were found to exhibit a distance of approximately 6A between two heteroatoms. A likely distinction between (14*R*)- and (14*S*)-norepinephrine is postulated. CNDO calculations on protonated dopamine and dopamine free base indicated their existence in three minimum energy conformations, one *trans* and two *gauche*. Again, the approximately 6A distance between possibly biologically important atoms was noted in one of the *gauche* conformations. Calculations on the *meta* and *para* anions of dopamine free base indicated that the *meta* anion was more stable than the *para* anion, and that both anions displayed the same minimum energy conformations as dopamine free base. The net electronic charge distributions and interatomic distances, in minimum energy conformations, are listed for all these compounds.

INTRODUCTION

The assumption has often been made that the conformation of biological molecules *in vivo* is one determinant of their biological activity. Thus we have recently calculated the

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minimum energy conformations, interatomic bond distances, and charge distributions of 2, 4,5-trihydroxyphenethylamine (6-hydroxydopamine) and other polyhydroxyphenethylamines,[2] some of which were found to cause long-term depletion of norepinephrine at cardiac storage sites,[3] using the complete neglect of differential overlap method (1).

For comparison with the structurally similar polyhydroxyphenethylamines, we have calculated the minimum energy conformations, bond lengths, and charge distributions of the neurotransmitters norepinephrine (I) and dopamine (3,4-dihydroxyphenethylamine, II) in their salt form, as well as dopamine free base (III) and its *meta* anion (IV) and *para* anion (V), using the CNDO[4] method. The *meta* and *para* anions were included because hypotheses have been promulgated which presume interaction of the anionic species with an enzyme (2, 3). Each anion was calculated as the base structure with an appropriate proton removed. No electrons were added.

Although X-ray structure determinations have shown that dopamine and norepinephrine exist, in the solid state, in the fully extended (*trans*) form with the ethano bond perpendicular to the plane of the benzene ring (4, 5), previous molecular orbital calculations indicated that it was energetically possible for these molecules to assume other conformations. These former calculations used the extended Hückel theory for both I (6, 7) and II (8, 9), empirical potential energy calculations for I (9), perturbative configuration interaction using localized orbitals for both I and II (10), and intermediate neglect of differential overlap for I (11). We will note the major and minor differences between these results and those obtained by us using CNDO. We chose the CNDO method because it was known to give energy values (12, 13), dipole moments (14, 15), and charge distributions (7) in at least qualitative agreement with calculations *ab initio* or experimental values. The extended Hückel theory has been observed to occasionally neglect possible conformations (9). We have thus compiled and interwoven a series of programs to enable us to make efficient use of generative input to a DEC PDP-10 computer.

COMPUTER PROGRAMS AND SYSTEMS

To obtain the Cartesian coordinates necessary for the CNDO/INDO program we used the *xyz* program of Gwinn (16). X-ray crystallographic data were used as input for the catechol nucleus (17), and standard bond lengths and angles (18) were used to add the extra atoms of norepinephrine and dopamine. We decided not to use the X-ray crystallographic data available for dopamine and norepinephrine (4, 5), since the combination of methods gave us the flexibility we needed to generate the structures of various catecholamines, for which X-ray crystallographic data were not available, in a consistent manner for future work. We found little difference in the results of the CNDO calculations when these standard bond lengths and angles were compared with X-ray crystallographic data. The trends in total energy and the electronic distribution remained essentially unaffected. The highest occupied and lowest unoccupied molecular orbitals were also essentially identical.

The output of the *xyz* program was used in the DCRT X-ray modeling system (19), whose options include molecule and bond rotation and a routine to produce the input necessary for CNDO calculation.

We used the original parameterization of the CNDO/INDO program written by Dobosh (1, 20). The programming technique for array variable pickup was modified,[5] and this resulted in a 10–15% saving in CPU time. The major saving in CPU time occurred on conversion to single precision, and when convergence in the iterative calcula-

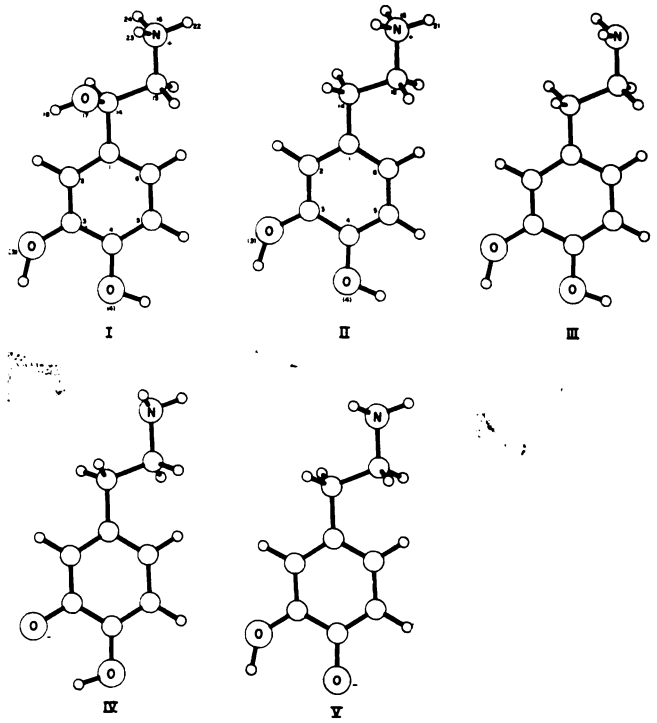
[1] Visiting Fellow, 1971–1973.

[2] R. Katz and A. E. Jacobson, unpublished results.

[3] J. Lundstrom, H. Ong, J. Daly, and C. R. Creveling, unpublished results.

[4] The abbreviations used are: CNDO and INDO, complete and intermediate neglect of differential overlap; CPU, central processing unit, EPE, empirical potential energy; PCILO, perturbative configuration interaction using localized orbitals.

[5] Modification by P. A. Dobosh (Mount Holyoke College, South Hadley, Mass.) and R. J. Feldmann, Division of Computer Research and Technology, (DCRT), National Institutes of Health.



tions of electronic energy was set at 10^{-5}. The original program used double-precision words and converged at a 10^{-6} level. Tests using double-precision words (72 bits) showed that no round-off errors occurred with the molecules used in this paper. Furthermore, calculations on these molecules from this modified program were compared with those obtained from the original program and found to be the same. However, it is possible that systems with a large number of orbitals may not converge in single precision. Thus single precision is not generally recommended for large molecules for this and other reasons. Final minimum energy conformations were displayed on an Adage AGT-30 graphics unit, and PLUTO plots[6] (an option of the DCRT X-ray modeling system) made from these images are shown in the various figures. Distances between connected atoms were obtained by additions to the program containing the output of the CNDO calculations, as were net electronic charge distributions. Also, any interatomic distance desired could be obtained through the X-ray modeling system.

For a description of our programs and the computer system, which we believe to be a simple and rapid method for data manipulation necessary for CNDO calculations, an author (S. R. H.) should be consulted.

DATA INPUT

Because of the ease of generating the coordinates for rotations around bonds by the system described above, there was no need to input structures in any specific conformation. Compounds I–V were inputted in the extended (planar) form. Atoms 2, 1, 14, 15, 16, and 21 were planar, as were atoms 15, 14, 17, and 18 in I. The dihedral angle enclosed by atoms 2, 1, 14, and 15 is referred to as the rotation angle around the 1,14 bond; that enclosed by atoms 1, 14, 15, and 16, as the rotation angle around the 14,15 bond; and so on. The minimum energy conformation of the various molecules will be referred to by the final rotation angle of the 14,15 bond (e.g., the 120-degree minimum energy conformation of protonated I repre-

[6] The PLUTO program was obtained from S. Motherwell (Chemistry Department, Cambridge University, England) and was adapted to the X-ray modeling system by R. J. Feldmann.

sents a 120-degree 14,15 rotation angle; the 1,14 rotation angles are listed in the various figures). The increments for bond rotation were initially small (30 degrees). However, for clarity, dihedral angle changes of 60 degrees are depicted in Figs. 1 and 5. We did not obtain deviations from the curves when the smaller increments were used. In no case was the increment less than 5 degrees. After initial energy minimalization of all the rotated bonds, we again minimalized the various rotation angles with the object of obtaining more accurate minimum energy conformations.

RESULTS

Minimum energy conformations. We did not find an impassable barrier to rotation from the crystalline (*trans*) conformation, as shown by X-ray crystallographic data, to the minimum energy conformation in compounds I–V. Thus the calculated minimum energy conformations are possible conformations. Although a total energy is indicated in Figs. 1 and 5 for the various molecules for comparative purposes, only the relative energies are of importance for our discussion.

Protonated (14R)-norepinephrine (I) [D(−) isomer] shows four minimum energy conformations, two *trans* and two *gauche* (Fig. 1). The two *gauche* conformations differ by a 180-degree rotation of the benzene ring (Fig. 2), as do the two *trans* conformations. In one of the *gauche* conformers the 1,14 bond was rotated by 90 degrees from its original position (Fig. 2b) and the minimum energy conformation occurred at 240 degrees (14,15 bond rotation). The other *gauche* conformer (Fig. 2d) was obtained by rotation of the 1,14 bond 270 degrees from its original position and, finally, by a 120-degree rotation of the 14,15 bond. Figure 1 shows only small differences between the relative energies of the *gauche* and *trans* forms Some of the interatomic distances between various atoms in the two *gauche* (or two *trans*) forms are, however, quite different. The PLUTO plot of these conformers is shown in Fig. 3. The L(+) (14S)-norepinephrine must, of course, display the same minimum energy conformations as its epimer (Fig. 2e–h).

Protonated dopamine displays three minimum energy conformations (Fig. 4): a *trans* or extended conformer (Fig. 4a) and two *gauche* conformers, at 120 degrees (Fig. 4b) and 240 degrees (Fig. 4c). It can be observed in Fig. 5 that the *trans* conformer is a somewhat higher energy form (by 2.5 kcal/mole)

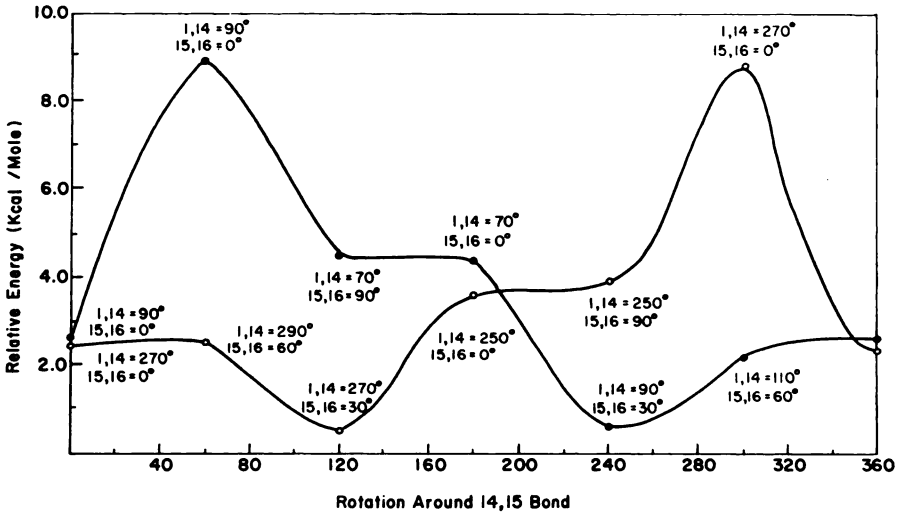


FIG. 1. *Calculated relative energies of protonated (14R)-norepinephrine (I) as a function of side chain rotation*

●, rotation around the 14,15 bond over the plane of the benzene ring; ○, this rotation under the plane of the benzene ring. Zero relative energy corresponds to −132.7770 a.u. in calculated total energy.

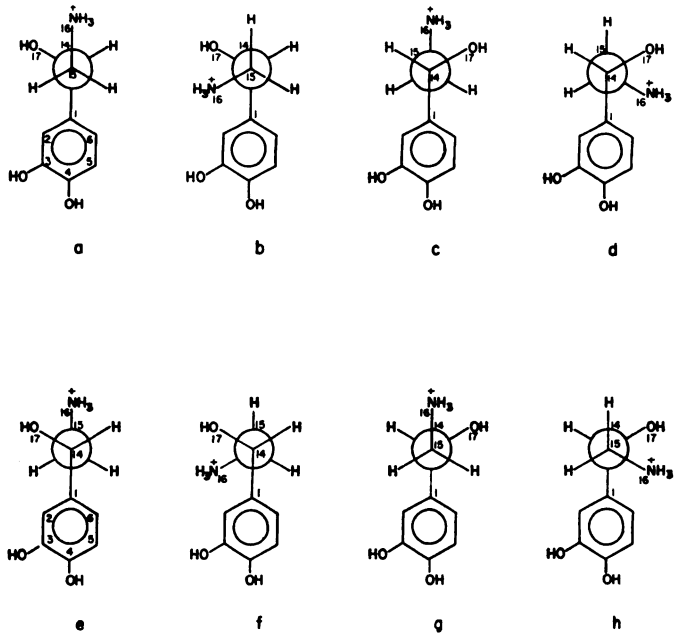


FIG. 2. *Newman projections of minimum energy conformations of protonated (14R)-norepinephrine (a–d) and protonated (14S)-norepinephrine (e–g)*

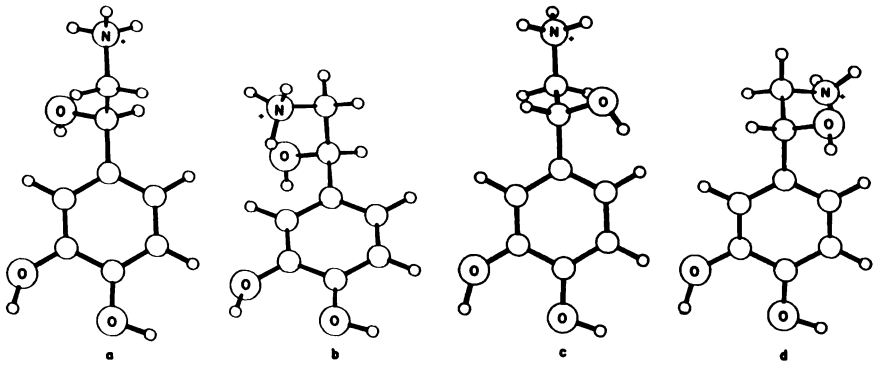


FIG. 3. *PLUTO plots of minimum energy conformations in protonated (14R)-norepinephrine*

than the folded 120- and 240-degree conformers. The energy barrier for the passage from the *gauche* to the *trans* form is a maximum of 6.4 kcal/mole. A barrier of less than 2 kcal/mole separates the *gauche* conformations. Interconversion between the 120- and 240- degree conformers can probably occur at room temperature. The 3-hydroxyl group does not appear to influence the various minimum energy conformations. That is, we observed no preference for the 240-degree *gauche* conformation.

Dopamine free base (Fig. 6) and the *meta* and *para* anions of dopamine show their minimum energy conformations in essentially the same positions as protonated dopamine. Energy barriers between these *gauche* and *trans* forms, however, are low (maximum of 3.5 kcal/mole), and no one conformer is more stable than the other. The *meta* anion of dopamine exhibits considerably greater stability (lower energy) than the *para* anion (approximately 17 kcal/mole).

It should be noted that hydrogen bonding to water *in vitro* or *in vivo* could be expected to affect the charged species more than neu-

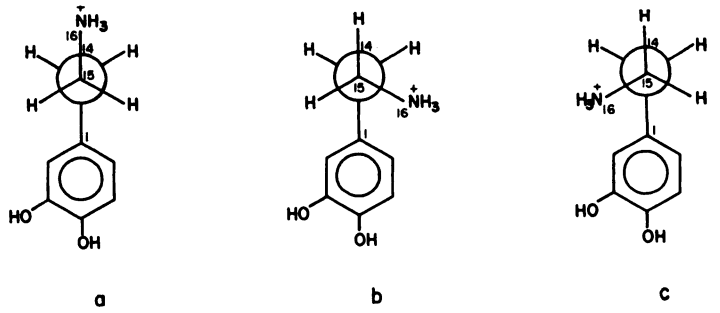


FIG. 4. *Newman projections of minimum energy confoations of protonated dopamine*

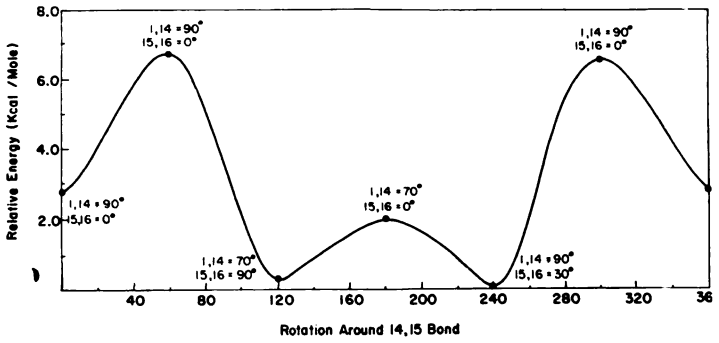


FIG. 5. *Calculated relative energies of protonated dopamine (II) as a function of side chain rotation* Zero relative energy corresponds to −114.3390 a.u. in calculated total energy.

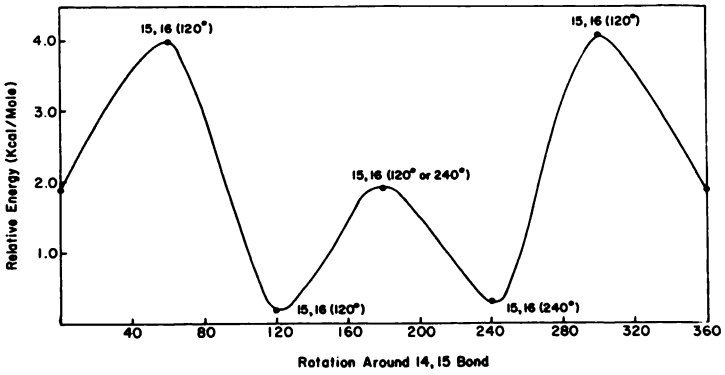


FIG. 6. *Calculated relative energies of dopamine free base (III) as a function of side chain rotation* Zero relative energy corresponds to −113.8320 a.u. in calculated total energy.

tral molecules. Practically, this could change the ratio of conformers in these charged species; we have no way of calculating this effect theoretically by CNDO.

Net electronic charge distribution. Protonated dopamine and the free base have almost identical charge distributions in minimum energy conformations, except for the (16)N atom and the associated protons. The positive charge in protonated dopamine is mostly distributed among the ammonium protons; somewhat less is present on the protons in the attached methylene group. The (16)N atom in the free base displayed a net negative charge of −0.21, which was almost the same as the net negative charge dis-

played by the oxygen atoms. The free base amine protons had a net positive charge of 0.06. The comparable net charges of −0.02 on the (16)N atom and 0.22 on the protons of the charged amine are shown in Table 1.

The net electronic charge distributions in the 0-, 120-, and 240-degree minimum energy conformations of the molecules listed in Table 1 were essentially identical, and therefore only those of the 0-degree (*trans*) conformers are shown.

The *meta* and *para* anions (IV and V), as would be expected, show greatly increased net negative charge (higher electronic density) at the anionic oxygen atom. The charge redistribution which is seen in these anions (Table 1) is that which would be postulated by resonance theory. The carbon atoms *ortho* and *para* to the carbon atom bearing the anionic group display a marked decrease in their net positive charge, or an assumption of net negative charge. Those carbon atoms *meta* to the carbon with the anionic oxygen show little or no change.

TABLE 1

Net electronic charge distribution in the "Trans" minimum energy conformation

Atom No.	Norepinephrine HCl	Dopamine HCl	Dopamine *meta* anion	Dopamine *para* anion
(1)C	0.01	0.03	0.03	−0.04
(2)C	−0.04	−0.05	−0.12	−0.05
(3)C	0.17	0.18	0.15	0.10
(4)C	0.14	0.13	0.06	0.12
(5)C	−0.03	−0.02	−0.03	−0.10
(6)C	−0.01	−0.01	−0.09	−0.01
(14)C	0.15	−0.01	0.00	0.01
(15)C	0.05	0.08	0.10	0.10
(3)O	−0.26	−0.26	−0.60	−0.29
(4)O	−0.22	−0.22	−0.28	−0.60
(17)O	−0.23			
(16)N	−0.02	−0.02	−0.21	−0.21
(22)H	0.22	0.22	0.06	0.06

The net charge distributions in protonated norepinephrine are similar to those in protonated dopamine. The side chain (17)O atom varies in electronegativity in the two extended (*trans*) conformers (Fig. 2a and c). When the (17)O atom approaches the (3)O atom in one of these *trans* conformations (Fig. 2a) its electronegativity is enhanced. The variation in the net charge is from −0.23 (Fig. 2c) to −0.28 (Fig. 2a) for the (17)O atom.

Interatomic distances. The interatomic distances noted in Table 2 are strikingly similar in the 120-degree conformation of all the listed molecules. This distance ranges between 5.97 and 6.32 A. The presumed importance of this interatomic distance is discussed in the following section.

DISCUSSION

The three conformations of protonated dopamine (Fig. 4a–c) found by these CNDO calculations were also found by Bustard and Egan (9), using both extended Hückel theory and EPE calculations, and also by Pullman *et al.* (10), using the PCILO method. Kier and Truitt (8), using extended Hückel theory, found two of the above conformations, and two other conformations not confirmed by any of these studies. They did not find a *trans* minimum energy conformer.

The major difference between our CNDO calculations on compound II and those of Bustard and Egan and of Pullman *et al.* lies in the relative stabilities of the conformers and the energy barriers between them.

TABLE 2

Interatomic Distances in Different Minimum Energy Conformations

Atoms	Norepinephrine HCl				Dopamine HCl		
	Ia	Ib	Ic	Id	IIa	IIb	IIc
	A	*A*	*A*	*A*	*A*	*A*	*A*
(3)O—(16)N	7.11	4.97	6.84	5.97	6.87	6.02	4.97
(4)O—(16)N	7.82	6.21	7.80	6.21	7.81	6.24	6.21
(3)O—(17)O	4.85	4.93	5.97	5.97			
(4)O—(17)O	6.29	6.29	6.31	6.32			

Bustard and Egan's calculations showed that their *trans* form was more stable than the *gauche* by approximately 2 kcal/mole, using extended Hückel theory, and a slight 0.39 kcal/mole by EPE calculation. They found energy barriers at 60 and 300 degrees (approximately 3 kcal/mole) and at 180 degrees (approximately 5 kcal/mole). The energy barriers and differences between conformers found by Pullman *et al.*(PCILO) were small, varying between 0 and 3 kcal/mole. We did not find a large 180-degree energy barrier between *gauche* forms (Fig. 4b and c), perhaps because CNDO may underestimate some repulsive forces (21). As we have noted, we found the *gauche* forms somewhat more stable than the *trans*, and interconversion between *gauche* conformers was likely.

The norepinephrine minimum energy conformations were noted by Kier *et al.* (6, 7) to be a *trans* and two *gauche*. Two of these conformers (a *trans* and a *gauche*) were found by our CNDO calculations (Fig. 2c and d) and by Pedersen *et al.* (11), using INDO. Pedersen *et al.* found that these two conformers were energetically equivalent and that little barrier to interconversion (1.5 kcal/mole) existed between them. A large barrier (7 kcal/mole) was found at the 300-degree position.

Our results are considerably different from those of Kier. The INDO calculations of Pedersen and colleagues gave results more comparable to our CNDO. The difference may lie in interpretation. We, like Pullman *et al.* (10) (PCILO), believe that four different conformers of norepinephrine can exist (Fig. 2a–d). Unlike Pullman, we observed small but real energy differences between the *gauche-trans* pairs (Fig. 2a and b, c and d) of approximately 2 kcal/mole and found that the *gauche* conformers were more stable. Our calculations also indicated that the energy barrier between *gauche* and *trans* forms was not great and that all four conformers can probably exist in solution.

Unlike all the previous workers, we noted that there are distinct differences in interatomic distances between various atoms (Table 2) in Fig. 2a and b vs. c and d. The previous calculations on these catecholamines appeared to use the center of the aromatic ring as their reference point, thus neglecting the possible spatial relationship between the (3)O atom and the nitrogen or (17)O atom. The polyhydroxyphenethylamines which are taken up and cause long-term depletion of norepinephrine in adrenergic nerve terminals of the mouse heart have been shown to display only one 120-degree minimum energy *gauche* conformation.[2] Both in the polyhydroxyphenethylamine series of compounds[2] and in dopamine, the 120-degree *gauche* conformation generally maintains the interatomic distance of 5.93–6.34 A between the (3)O or (4)O and nitrogen atoms. This is also true for norepinephrine but, additionally, the (3)O or (4)O and (17)O atoms display this interatomic distance (Table 2). Moreover, in norepinephrine the *trans* conformer (Fig. 2c) displays the same (3)O or (4)O and (17)O distance as in the *gauche* conformers mentioned above. It seems probable, therefore, that uptake of biogenic amines occurs only with specific conformers which have a required interatomic distance between certain atoms; i.e., the approximately 6A distance between 2 electronegative oxygen atoms or O—N atom pairs is important for the interaction of the catecholamines at uptake sites. This hypothesis is in accord with data on apparent uptake affinities of trihydroxy- and dihydroxyphenethylamines[3] and indicates that with norepinephrine both conformers c and d in Fig. 2 are biologically important.

Iversen *et al.* (22) and others (23) have shown that the uptake system in norepinephrine terminals is stereochemically specific with a higher affinity for (−)-norpinephrine than the (+) isomer in isolated, perfused mouse and rat heart. From our calculations it appears that the (17)O atom may be important for the activity of both epimers at the uptake sites; differentiation in norepinephrine activity could occur because of the spatial positioning of the ammonium and (17)hydroxyl groups above and below the plane of the aromatic ring. That is, conformations g and h (Fig. 2) of (14*S*)-norepinephrine are obviously equivalent to c and d (Fig. 2) of the (14*R*)-norepinephrine in all molecular orbital characteristics. From the viewpoint of an enzyme surface or fixed macromolecule, however, they could be very different. In (−)-norepinephrine (Fig. 2d)

the ammonium group is below the plane of the aromatic ring; the epimer (Fig. 2h) displays the ammonium group above this plane. Although interatomic distances between electronegative atoms are the same in both, each would present a different three-dimensional pattern to a fixed macromolecular surface.

ACKNOWLEDGMENT

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