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The Conformation of Catecholamines and Related Compounds in Solution

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SUMMARY

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The conformation of a series of catecholamines and related compounds in solution has been studied by nuclear magnetic resonance spectroscopy. In molecules with a β-hydroxyl group, the preferred conformation is one in which the amino group is *trans* to the aromatic ring and *gauche* to the β-hydroxyl group; the fractional population of this rotamer is approximately 0.8. The major interaction leading to the predominance of this rotamer is shown to be an electrostatic or hydrogen bonding interaction between the amino and β-hydroxyl groups. Comparison of these results with theoretical calculations shows that extended Hückel theory calculations give only qualitative agreement, while with the method of perturbative configuration interaction using localised orbitals (PCILO) the agreement is much better.

INTRODUCTION

The conformation of pharmacologically active small molecules is clearly relevant to any consideration of their interaction with a receptor. Many such molecules are potentially flexible, and thus may exist in solution in a number of conformations. Assuming that the small molecule has only a single conformation in its complex with the receptor, the formation of the complex must involve a process of conformational selection, which will influence the kinetics and energetics of complex formation. There is thus considerable interest in characterising the conformational distribution of molecules such as acetylcholine and the catecholamines [for reviews see Shefter (1) and Portoghese (2)], though it will be necessary to know the conformation of these molecules when bound to the receptor before full use can be made of this information.[1]

Both X-ray crystallography and theoretical molecular orbital calculations have been extensively used to establish the preferred conformations of pharmacologically active molecules. The method of choice for the study of conformational distributions in solution, however, is nuclear magnetic resonance spectroscopy. The use of this method is based on the fact that the spin-spin coupling constant between protons attached to two adjacent carbon atoms depends upon the dihedral angle about the

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[1] Information on the preferred conformation in the crystal or in solution has also been used to construct models of the drug-receptor complex, though there is no *a priori* relationship between these two states of the molecule.

intervening carbon-carbon bond. Thus one can determine the relative populations of different conformations produced by rotation about carbon-carbon bonds. NMR spectroscopy has been extensively used in studies of acetylcholine and related compounds (3–8), but has not hitherto been used for a detailed study of catecholamine conformation, though studies of related compounds such as amphetamines (9–11), ephedrine (12), and dopamine (13) have been reported.

We now report a study of the conformation about the α-β bond in a series of 12 catecholamines and related compounds. In view of the considerable interest in the area in recent years, we have also made a detailed comparison of our experimental results with the predictions of theoretical calculations.

METHODS

The majority of compounds studied were commercially available; we are grateful to Drs. B. Callingham and L. Iversen of the Department of Pharmacology, University of Cambridge, for giving us samples of many of them. Professor Chapman of the University of Hull kindly provided a sample of *N*,*N*-dimethyl-2(*o*-bromophenyl)ethanolamine, and 2-phenylmorpholine was a gift from Dr. J. H. Shelley, Boehringer Ingelheim, Ltd.

All compounds were examined as approximately 10% solutions of their salts (usually hydrochloride) in D_2O, acidified when necessary with DCl. Only the fully protonated form of the compounds was examined; in those instances where it was examined, the nature of the anion had no effect on the spectrum.

^1H NMR spectra were obtained at 100 MHz (Varian Associates HA-100D-15 and XL-100-15 spectrometers) and, by courtesy of the Science Research Council, at 220 MHz (Varian Associates HR-220). All spectra were run at probe temperature (24° for the HR-220, 32° for the HA-100 and XL-100); field/frequency lock was provided by a concentric capillary of hexamethyldisiloxane (HA-100) or by the deuterium in the solvent (XL-100).

Spectral analysis. In compounds such as ephedrine there is only a single α-proton, and the spectra are of the simple AX type, from which the vicinal coupling constant can be measured directly.

Compounds such as noradrenaline, having two α-protons and a single β-proton, gave a spectrum approximating to an ABX type for this part of the molecule. In adrenaline, isoprenaline, phenylephrine, and synephrine the two α-methylene protons are accidentally almost equivalent, and the spectra at 100 MHz are of the A_2X type; however, at 220 MHz the nonequivalence is sufficient to give an ABX pattern. In the majority of the ABX spectra there was considerable overlapping of lines, and in many cases one of the AB subspectra was deceptively simple; direct analysis of these spectra gave imprecise results. In spectra such as these, measured peak maxima are a poor parameter for iterative fitting. Consequently, initial estimates of the spectral parameters were obtained by standard methods (e.g., ref. 14), and these estimates were then refined by comparison of experimental and simulated spectra. The spectra were simulated (as ABC systems) using the programme SIMEQ II; we are grateful to Dr. M. J. A. de Bie of the University of Utrecht for supplying us with a copy of this programme. An illustrative comparison of experimental and "best-fit" simulated spectra is given in Fig. 1; the estimated accuracy of the coupling constants obtained in this way is better than ±0.1 Hz. The parameters obtained in this way from the 220-MHz spectra gave, in all cases, a satisfactory simultation of the 100-MHz spectra.

β-Phenylethylamine, a 1,2-disubstituted ethane, gives an $AA'BB'$ spectrum for the α- and β-protons. This was analysed iteratively using a modification of the LAOCOON programme (15). The root mean square error in line fitting was 0.11 Hz.

Calculation of rotamer populations. The conformation distribution about the α-β bond can be described in terms of the relative populations of the three classical staggered

FIG. 1. *Experimental (upper) and simulated (lower) 220-MHz spectra of the methylene protons of phenylephrine in D_2O*

The parameters used for the simulated spectrum are given in Table 1.

rotamers, shown as Newman projections in Fig. 2. The expressions relating these populations, p_I, p_{II}, and p_{III}, to the experimental coupling constants J_{12} and J_{23} are

$$p_I = \frac{J_{13} - J_g}{J_t - J_g} \quad (1)$$

$$p_{II} = \frac{J_{23} - J_g}{J_t - J_g} \quad (2)$$

$$p_{III} = 1 - (p_I + p_{III}) \quad (3)$$

where J_t and J_g are the coupling constants for dihedral angles of 180° (*trans*) and ±60° (*gauche*), respectively.

In the ephedrine series, where H_2 is replaced by an alkyl substituent, we can only distinguish rotamer I, in which H_1 and H_3 are *trans*, from rotamers II and III, in both of which they are *gauche*. Thus p_I is calculated from Eq. 1, and

$$(p_{II} + p_{III}) = 1 - p_I \quad (4)$$

Similarly, in β-phenylethylamine, we can only distinguish the "symmetrical" rotamer, in which the phenyl and amino group are *trans* (analogous to rotamer II), from the other two. Again, p_{II} is calculated from Eq. 2, and in addition we have

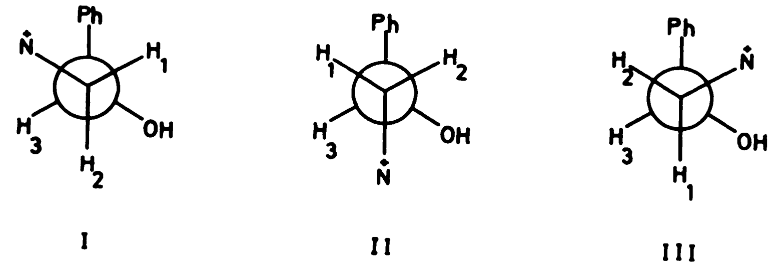
$$(p_I + p_{III}) = 1 - p_{II} \quad (5)$$

These equations are based on the assumption that single values of J_t and J_g are applicable to all rotamers; this and other assumptions involved in this approach to the study of conformational equilibria will be discussed in the following section.

RESULTS AND DISCUSSION

The coupling constants derived from the spectra of the 12 compounds studied are given in Table 1. As pointed out above, we can obtain the populations of all three rotamers shown in Fig. 2 from the two coupling constants J_{AX} and J_{BX} observed for compounds such as noradrenaline. However, examination of Fig. 2 shows that we can only associate rotamers I and II with the appropriate population values if we can assign protons H_1 and H_2 in the spectrum. Specifically, we need to know which of the protons H_1 and H_2 (defined stereochemically in Fig. 2) gives rise to which of the observed couplings J_{AX} and J_{BX}. In the absence of stereospecific isotopic substitution, this assignment cannot be made unequivocally. However, in the present instance it is possible to deduce both the qualitative distribution of rotamer populations and the assignment of protons H_1 and H_2 from the observed couplings with considerable confidence.

We assume at this stage only that J_t is large (>10 Hz) and J_g small (<4 Hz)—for which there is ample evidence (14). In noradrenaline, the observed couplings are 9.05 and 3.35 Hz; clearly rotamer III, in which *both* H_1 and H_2 are *gauche* to H_3 and

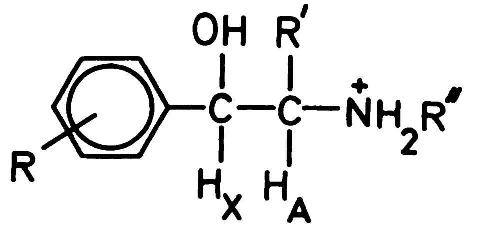


I II III

FIG. 2. *Newman projections of the three classical staggered rotamers about the* C_α—C_β *bond in catecholamines*

TABLE 1

Coupling constants in catecholamines and related compounds



Compound	R	R'	R''	J_{AB}[a]	J_{AX}[a]	J_{BX}[a]		$\lvert \nu_A - \nu_B \rvert$[b]
				Hz	*Hz*	*Hz*		*ppm*
Noradrenaline	3,4-diOH	H_B	H	13.2	9.05	3.35		0.053
Adrenaline[c]	3,4-diOH	H_B	Me	12.8	9.15	3.65		0.010
Isoprenaline[c]	3,4-diOH	H_B	iPr	12.8	9.65	3.15		0.014
β-Phenylethanolamine	H	H_B	H	13.2	9.70	3.00		0.075
Phenylephrine[c]	3-OH	H_B	Me	12.8	9.45	3.55		0.023
Synephrine[c]	4-OH	H_B	Me	12.8	9.50	3.00		0.024
N,*N*-Dimethyl-2-(*o*-bromophenyl)-ethanolamine	1-Br	H_B	diMe	13.1	10.80	2.10		0.130
Isopropanolamine	($CH_3CH(OH)CH_2NH_3^+$)			13.2	9.30	3.30		0.210
Norephedrine	H	Me	H		4.4			
Metaraminol	3-OH	Me	H		4.4			
Butanephrine	3,4-diOH	Et	H		5.0			
				$J_{AA'}$[a]	$J_{BB'}$[a]	J_{AB}[a]	$J_{AB'}$[a]	$\lvert \nu_A - \nu_B \rvert$[b]
β-Phenylethylamine	($Ph\text{-}CH_2CH_2NH_3^+$)			13.6	15.0	6.4	8.9	0.249

[a] ±0.05.

[b] ±0.005.

[c] From 220-MHz spectra.

will therefore show small coupling constants, must have a low population. We are left to choose between rotamer I, in which H_2 is *gauche* and H_1 *trans* to H_3, and rotamer II, in which H_1 is *gauche* and H_2 *trans*. Whichever of these two rotamers predominates, we should expect to observe one large and one small coupling constant (if rotamers I and II, or I, II, and III were equally populated, we should expect both coupling constants to be about 6–7 Hz). This ambiguity can be resolved by considering the results for norephedrine. In norephedrine one of the protons H_1 and H_2 in noradrenaline is replaced by a methyl group; since the stereochemistry of (−)-ephedrine and (−)-norephedrine is known to be $1R,2S$ (16), it is H_2 in Fig. 2 which is replaced. Therefore, in norephedrine, we can distinguish rotamer I, with a *trans* orientation of H_1 and H_3, from rotamers II and III, and the small coupling constant observed indicates that rotamer I has a low population. Comparison with noradrenaline indicates that in the latter case it is J_{AX} which is similar to the coupling in norephedrine, and therefore $H_A \equiv H_1$. This assumes that for noradrenaline, as for norephedrine, $p_{I} < p_{II}$; this seems reasonable, since the *trans* orientation of the α-methyl and aromatic groups of norephedrine in rotamer I would imply that, on steric grounds, the population of this rotamer would if anything be greater in norephedrine than in noradrenaline (this is in fact observed; see Table 3). We conclude, therefore, that $H_A \equiv H_1$, and as a corollary that for noradrenaline $p_{II} > p_{I}, p_{III}$. The preferred rotamer is thus that in which the amino group is *trans* to the aromatic ring and *gauche* to the β-hydroxyl group. This is supported by the results for *N*,*N*-dimethyl-2(*o*-bromophenyl)ethanolamine. In this compound the steric repulsion between the amino group and the aromatic ring will be substantially increased. The above assignment ($H_A \equiv H_1$) leads to the predicted increase in the population of rotamer II in this compound; the reverse assignment would imply a substantial increase in the population of rotamer I, in which these two bulky groups are *gauche*, which seems quite unreasonable on steric grounds.

We now turn to the problem of the quantitative estimation of the rotamer populations. As indicated by Eqs. 1–3, this requires a fairly precise knowledge of the values of J_t and J_g (the coupling constants for the *trans* and *gauche* orientation of protons, respectively), whereas up to now we have used only their approximate magnitudes. These coupling constants do not have the same value in all systems, since they depend markedly on substituent electronegativity.[2] Abraham and Gatti (17) have developed a procedure for the calculation of J_t and J_g from substituent electronegativities in 1,2-disubstituted ethanes; we have adopted their method for the case of β-phenylethylamine, and we calculate $J_t = 13.1$ Hz and $J_g = 3.6$ Hz. The majority of the compounds we are interested in here, however, are tri- or tetrasubstituted ethanes and no equations analogous to those of Abraham and Gatti (17) are available to allow us to calculate J_t and J_g in these cases. We must therefore obtain J_t and J_g from conformationally rigid model compounds. The most appropriate model compounds are the substituted morpholiniums, and the values used are shown in Table 2; the upper two compounds are models for the ephedrine series, the lower two for the noradrenaline series. The use of model compounds has two potential disadvantages: either or both the precise dihedral angle and the orientation of the electronegative substituents may be different for the model compounds and the molecules of interest. However, we have already shown qualitatively that rotamer II is the predominant one for noradrenaline and related compounds, and this rotamer is essentially identical with the conformation about the C_2—C_3 bond of the morpholines (see Table 2). Thus, for example, any deviations of the dihedral angle from 60° due to the *gauche* orientation of the amino and β-hydroxyl groups should be very similar in both cases. Similarly, the orientation of the electronegative substituents is the same as in rotamer II, so that the values of J_t and J_g from the morpholiniums are the appropriate ones for rotamer II; small differences in these values for the other rotamers will make a negligible difference to the calculated rotamer populations. However, the requirement of ring closure in the cyclic model compounds will cause a difference in dihedral angles compared to the noncyclic compounds; this is not likely to be more than about 5°.

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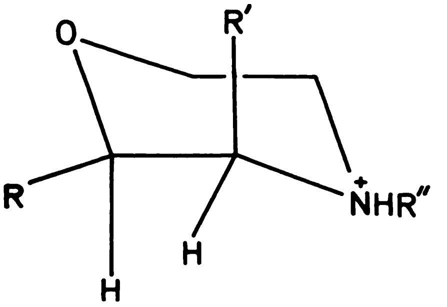
[2] Indeed, the values of J_t and J_g also depend on the *orientation* of the electronegative substituents (17), so that the present approach, assuming single values for a given system, is an approximation which we are forced to adopt in the absence of detailed studies of conformationally rigid model compounds. As argued below, adoption of the phenyl-substituted morpholines as models minimises the error from this source.

TABLE 2

Coupling constants in morpholine model compounds



R	R′	R″	J_t	J_g	Ref.
			Hz	*Hz*	
Ph	Me	H	10.5	2.8	12
Ph	Me	Me_2	10.4		18
Ph	H	H	11.0	2.6	—[a]
Me	H	Me_2	11.2	2.1	18

[a] J. Feeney and G. C. K. Roberts, unpublished work.

TABLE 3

Rotamer populations in catecholamines and related compounds

Values were calculated using J_t = 11.2 Hz and J_g = 2.1 Hz for the noradrenaline series. Using the alternative values of 11.0 and 2.6 Hz (Table 2) gave fractional populations differing from the figures below by <0.05, but led to a slightly negative value for $p_{\rm I}$ for *N*,*N*-dimethyl-2(*o*-bromophenyl)ethanolamine.

Compound	$p_{\rm I}$[a]	$p_{\rm II}$[a]	$p_{\rm III}$[a]
Noradrenaline	0.14	0.76	0.10
Adrenaline	0.17	0.77	0.06
Isoprenaline	0.11	0.83	0.06
β-Phenylethanolamine	0.10	0.84	0.06
Phenylephrine	0.16	0.81	0.03
Synephrine	0.10	0.81	0.09
N,*N*-Dimethyl-2-(*o*-bromophenyl)-ethanolamine	0.00	0.96	0.04
Isopropanolamine	0.13	0.79	0.08
Amphetamine[b]	0.45	0.50	0.05
	$p_{\rm I}$	$(p_{\rm II} + p_{\rm III})$	
Ephedrine[c]	0.10	0.90	
Norephedrine	0.21	0.79	
Metaraminol	0.21	0.79	
Butanephrine	0.28	0.72	
	$p_{\rm II}$	$(p_{\rm I} + p_{\rm III})$	
β-Phenylethylamine	0.56	0.44	
Dopamine[d]	0.43	0.57	

[a] Fractional populations of rotamers I–III (see Fig. 2). Estimated accuracy ±0.05. Temperature 32° except for adrenaline, isoprenaline, phenylephrine, and synephrine, whose spectra were obtained at 24°.

[b] Ref. 9.

[c] Ref. 12.

[d] Ref. 13.

We feel, therefore, that the morpholiniums are good model compounds, and that the calculated rotamer populations, presented in Table 3, are probably accurate to ±0.05.

Thus, in terms of the conformational distribution about the α-β bond, the catecholamines appear to exist some 80% of the time in the conformation shown as rotamer II. This is probably also true of the α-substituted compounds. It must be emphasised, however, that the conformational preferences observed are rather weak. A fractional population of 0.80 for rotamer II corresponds to an energy difference of only 0.83 kcal/mole for II compared to (I + III). The catecholamines must be regarded as having substantial conformational flexibility. From the rotamer populations in Table 3, we can now attempt to elucidate the structural features responsible for the observed conformational distribution. [For completeness, we have also included the results of other workers for ephedrine (12), dopamine (13), and amphetamine (9); we have also examined these compounds, and our results are in excellent agreement with those quoted in Table 3.]

Effect of the β-hydroxyl group. The major determinant of the conformational preference observed is clearly an interaction between the β-hydroxyl and amino groups. Thus, for β-phenylethylamine, $p_{\rm II}$ = 0.56, while for β-phenylethanolamine, $p_{\rm II}$ = 0.84; this dramatic difference is also seen on comparing dopamine with noradrenaline, and amphetamine with norephedrine. Replacement of the aromatic ring by a methyl group, to give isopropanolamine, does not appreciably reduce the population of rotamer II, so that the contribution of steric repulsion between the amino group and the aromatic ring is minimal. From the rotamer distributions in Table 3 we can estimate that the energy difference of 0.7–1.0 kcal/mole for rotamer II compared to (I + III) is made up of approximately 0.65 kcal/mole due to an interaction between the amino group and the β-hydroxyl group, and some 0.2 kcal/mole due to the steric amino group–aromatic ring interaction. These values may underestimate the contribution of steric repulsion; the low population of rotamer III, in which the N—O interaction is still possible, must presumably be ascribed to steric factors.

The nature of the N—O interaction is not clear. In the crystal structures of ephedrine (19), noradrenaline (20), and isoprenaline (21), where the molecule is in a conformation corresponding to rotamer II, the N—O distance is 2.65–3.0 Å, suggesting that a hydrogen bond might be involved. It would, however, have to be a very bent, and therefore a weak, one. The predominantly electrostatic nature of the interaction is suggested by comparison with acetylcholine; here no hydrogen bonds (except perhaps of the N—C—H···O type) are possible, yet the molecule exists in solution essentially 100% in a conformation in which the ester oxygen and the quaternary nitrogen are *gauche* to one another (1, 3–8).

Effects of α-substitution. The presence of an α-methyl or α-ethyl group seems to have the anticipated effect of increasing the population of rotamer I, in which the α-substituent is *trans* to the aromatic ring. Thus, for noradrenaline, $p_{\mathrm{I}} = 0.07$, while for norephedrine $p_{\mathrm{I}} = 0.21$ and for butanephrine $p_{\mathrm{I}} = 0.28$. Substitution of an α-methyl group into β-phenylethylamine (to give amphetamine) has a rather similar effect; the population of the rotamer in which both the amino group and the α-methyl group are *gauche* to the aromatic ring is only 0.05, whereas in β-phenyethylamine the *gauche* rotamers have an average population of 0.22 each.

Effects of N-substitution. The conformational distributions of adrenaline and noradrenaline are very similar; there is no discernible effect of the *N*-methyl group. In ephedrine, however, the *N*-methyl substituent appears to slightly reduce the population of rotamer I. The reasons for this difference are not clear.

Isoprenaline shows a slightly greater predominance of rotamer II than noradrenaline or adrenaline. This may be due to steric interference between the *N*-isopropyl group and the aromatic ring; the effect is very small, however.

Effects of substitution on the aromatic ring. The effect of introducing a bulky *ortho* substituent (together with two *N*-methyl groups) has been noted earlier; *N*,*N*-dimethyl-2(*o*-bromophenyl)ethanolamine shows an almost complete preponderance of rotamer II.

The only clear effect of the ring hydroxyl groups is seen in the comparison of dopamine ($p_{\mathrm{II}} = 0.43$) and β-phenylethylamine ($p_{\mathrm{II}} = 0.56$). Here the ring hydroxyl groups appear to favour the *gauche* conformations, so that dopamine shows a lesser conformational preference than β-phenylethylamine. A similar, though smaller, effect is seen on comparing noradrenaline ($p_{\mathrm{II}} = 0.76$) and β-phenylethanolamine ($p_{\mathrm{II}} = 0.84$). In discussing their results with dopamine, Bustard and Egan (13) concluded that the *gauche* rotamers were increased in population by an interaction between the amino group and the aromatic hydroxyl groups, probably mediated by a water molecule. This idea is certainly supported by the present results with β-phenylethylamine, though the interaction, if it exists, is clearly a very weak one —the difference in p_{II} between noradrenaline and β-phenylethanolamine is only just greater than experimental error.

Comparison with results of X-ray crystallography and theoretical calculations. In the crystal, ephedrine (19), noradrenaline (20), and isoprenaline (21) are all observed to be in a conformation corresponding to rotamer II. Thus in these instances the conformation in the crystal is the same as the preferred conformation in solution. It should be noted, however, that rotamer II is the *only* conformation observed in the crystal, while in solution the other rotamers are also significantly populated. Indeed, for dopamine (22) and β-phenylethylamine (23), too, only rotamer II is observed in the crystal, while in solution the fractional populations of this rotamer are only 0.43 and 0.56, respectively.

Molecular orbital calculations, which consider the molecule *in vacuo*, have been extensively used to elucidate conformational preferences. Calculations of the extended Hückel theory type do appear to be able to predict the referred conformation correctly. Thus Kier and his colleagues have calculated that rotamer II should be preferred for ephedrine (24), noradrenaline (25), adrenaline, and isoprenaline (26). For pseudoephedrine (24) and dopamine (27), on the other hand, the extended Hückel theory calculations reported by Kier and his colleagues did not predict rotamer II to be preferred, as is found experimentally (12, 13). [A subsequent extended Hückel theory calculation for dopamine by Bustard and Egan (13) did, however, indicate rotamer II to be preferred.] A recent calculation by Pedersen, Hoskins, and Cable (28), using the method of intermediate neglect of differential overlap (INDO), predicted that for noradrenaline rotamer II should be only slightly more populated than rotamer III. This conclusion, surprising on steric grounds, is at variance with the experimental finding (Table 3) of a very low population of rotamer III. While the extended Hückel theory calculations generally give a qualitatively correct prediction of the most preferred rotamer, they do not give a quantitatively correct picture of the rotamer distribution. For example, for noradrenaline the extended Hückel theory calculations predict that rotamer II is almost 3.5 kcal/mole more favourable than the others (26). This exaggeration of the energy differences, which makes a quantitative description of the rotamer distribution impossible, appears to be a general feature of extended Hückel theory calculations (29). It is notable that Bustard and Egan (13) achieved a much better agreement with the observed rotamer populations for dopamine using a simple semiempirical calculation, involving only pairwise van der Waals interactions and torsional potentials, than with the more complex extended Hückel theory calculations.

Recently Pullman *et al.* (30) have reported a detailed study of the conformational distributions of a number of catecholamines and related compounds using more refined molecular orbital calculations, by the method of perturbative configuration interaction using localised orbitals (PCILO). They have calculated complete conformational energy maps, considering rotation about both the α-β and β-Ar bonds, for a number of compounds; this enables us to make a detailed comparison with our experimental results. In Table 4 are shown the rotamer populations about the α-β bond calculated from the conformational energy maps of Pullman *et al.* (30) using the Boltzmann equation. The rotamer populations were taken as the total population in each energy minimum (not simply the population within some arbitrary range of the "ideal" 60° and 180° dihedral angles). Thus this calculation takes no account of the fact that for some of the compounds the predicted energy minima do not occur at precisely 180° or ±60°. A more rigorous comparison with the experimental data is possible by using the populations obtained from the theoretical energy maps as weighting factors in the Karplus

TABLE 4

Rotamer populations and coupling constants predicted by the PCILO calculations of Pullman et al.

<table>
<tr><th>Compound</th><th>p_{I}</th><th>p_{II}</th><th>p_{III}</th><th>$J_{13}{}^a$</th><th>$J_{23}{}^a$</th></tr>
<tr><td>Noradrenaline</td><td>0</td><td>0.755</td><td>0.245</td><td>7.79</td><td>4.43</td></tr>
<tr><td>Adrenaline</td><td>0</td><td>0.955</td><td>0.045</td><td>10.20</td><td>3.25</td></tr>
<tr><td>β-Phenylethylamine</td><td>0.290</td><td>0.420</td><td>0.290</td><td>8.00</td><td>6.10</td></tr>
<tr><td>Dopamine</td><td>0.345</td><td>0.352</td><td>0.303</td><td>7.70</td><td>6.35</td></tr>
<tr><td>Ephedrine</td><td>0</td><td>0.721</td><td>0.279</td><td colspan="2">3.4</td></tr>
<tr><td>Norephedrine</td><td>0</td><td>0.959</td><td>0.041</td><td colspan="2">3.0</td></tr>
</table>

^{a} Calculated using J_t = 11.2 Hz and J_g = 2.1 Hz (see legend to Table 3).

equation to calculate the predicted coupling constants J_{13} and J_{23}. The values of the numerical constants in the Karplus equation were obtained by using the coupling constants from the morpholiniums as the values for 60° and 180° dihedral angles, and the values of J_{13} and J_{23} calculated in this way are also shown in Table 4.

Comparison of the predicted rotamer populations and coupling constants in Table 4 with the observed values in Tables 1 and 3 shows that, again, the agreement is qualitatively good, rotamer II being the preferred conformation in all cases. In fact, the quantitative agreement in terms of rotamer populations is also fairly good, the main discrepancies being the underestimation of p_{II} for β-phenylethylamine and dopamine, and in particular the prediction of a zero population for rotamer I for all the compounds possessing a β-hydroxyl group. In addition, the calculations predict an appreciable difference in the rotamer populations between noradrenaline and adrenaline, notably a rather high value of p_{III} for noradrenaline, which is not borne out by experiment. The discrepancies between theory and experiment, which lead to rather large differences between predicted and observed coupling constants, cannot be explained in detail, but can probably be ascribed to the influence of solvent, which is not considered in the calculations. Indeed, it is perhaps surprising that the rotamer populations, involving as they do relatively small energy differences, can be predicted as accurately as this by calculations which do not include solvent effects.

CONCLUSIONS

It is apparent from the results reported here that the catecholamines are conformationally flexible about the α-β bond, but show a preference for rotamer II amounting to an energy difference of approximately 0.8 kcal/mole. This preference is largely due to an electrostatic and/or hydrogen-bonding interaction between the α-amino group and the β-hydroxyl, though steric interactions are also significant, as indicated by the low population of rotamer III.

The magnitude of the energy differences between the rotamers is clearly very small compared to the energy of binding of these molecules to their receptor, which must be of the order of 10–12 kcal/mole. This argues that the process of conformational selection on binding, mentioned in the INTRODUCTION, will have only a rather small influence on the over-all energetics of the binding reaction, *if* the conformation when bound is equivalent to one of the three low-energy conformations considered here. Comparison of the "potency" of, for example, dopamine and noradrenaline will, however, be influenced by the fact that they have markedly different conformational distributions, and this is clearly a factor which needs to be borne in mind in studies of the relationship between structure and activity. The possible influence of conformational selection on the kinetics of the binding reaction cannot be evaluated at the present time, since experiments of the type reported here give no information on the energy barriers between rotamers. It is interesting to note, however, that the calculations of Pullman *et al.* (30) suggest that the energy barriers may be significantly different for the various compounds studied here. In general, the theoretical calculations of catecholamine conformation, considering the molecule *in vacuo*, are in only qualitative agreement with the experimental results in solution, but those of Pullman *et al.* (30) are approaching quantitative agreement.

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