Conformational Study of Catecholamines in Solution

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Conformations of noradrenaline, dopamine and ephedrine have been studied in aqueous solutions using high resolution PMR spectroscopy. We found that for dopamine the mole fraction of trans rotamer is increased if pH is changed from acidic to basic while for noradrenaline and ephedrine the opposite trend is observed: gauche rotamer forms of the side chain become more populated. These changes are discussed in relation to the role of the benzylic hydroxyl group for conformation and/or binding to a receptor site in the biophase.

Introduction

Conformational analysis of catecholamines and related compounds in relation to their biological activity have been rather numerous in the past [1, 2]. Quantum chemical molecular orbital techniques as well as experimental X-ray and NMR methods were also used [3, 4] rather extensively. Current hypotheses of the mechanism of the catecholamine-receptor interactions [5–7] expose the amino group of the side chain as the primary point of attachment to the receptor [5–11]. It is not established whether the attachment of the aminic head at the receptor site occurs (a) in the protonated from, *i.e.* via hydrogen bonds of the N–H ... O type or (b) in the neutral form to the proton donating site, *i.e.* via hydrogen bonding of the N ... H–O type. The correct answer to this question is of importance for drug design and for the understanding of the mechanism of action. Unfortunately it can at present not be obtained from any sort of direct experiment nor from indirect evidence by studying receptor affinities of series of different side chain substituted amines [12]. It is usually assumed that the catecholamines are bound to the receptor in the protonated form because this form is predominant at the physiological pH = 7.4. Nevertheless because of the appreciable conformational freedom about the side chain C_α–C_β bond and the fact that in the immediate environment of the receptor site that exact value of pH is not accessible we have studied in this paper the conformational dependence over the whole range of pH = 2.5–11.5.

The use of proton magnetic resonance for the study of conformational differences between different catecholamines in solution is well documented [13–16]. Several authors [17–19] have treated the problem of catecholamine complex formation with ATP with this technique too, but it seems to us that no attempt has been made so far to use coupling constants for the determination of changes in conformational population at a range of pH values where the amine head nitrogen protonation/deprotonation occurs. Therefore we have elaborated three molecules of the catecholamine series: noradrenaline (NA), dopamine (DA) and ephedrine (E) with different side chain structures, to assess the role of the benzylic hydroxyl group for conformational preference of the side chain amino group relative to the

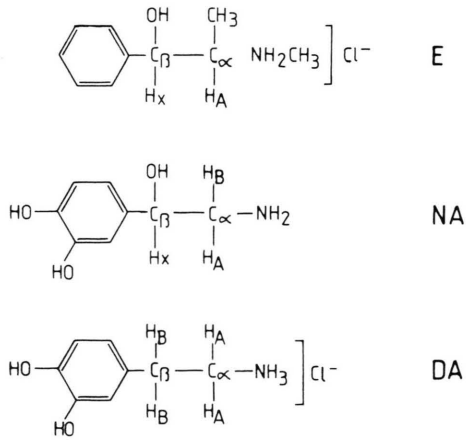

Fig. 1. Side chain structures of (1R, 2S) ephedrine (E), L (–) noradrenaline (NA), and dopamine (DA).

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aromatic ring (Fig. 1). Non-catechol analog E should indicate the importance of catechol hydroxyls for this.

Experimental Section

Materials

L-noradrenaline of highest purity (Fluka AG) as well as dopamine and ephedrine hydrochlorides (Sigma) were kindly provided by Mrs. A. Rosenberger of Krka, Pharmaceutical and Chemical Works. Experimental solutions were made up by dissolving the amines in D_2O (99.8%). pD was adjusted by adding NaOD of 98.8% isotope purity grade or DCl of 99% purity.

NMR spectra

All ^1H NMR spectra were taken at room temperature 23 °C on JEOL PS-100 and FX-90Q spectrometers using DSS and TMS in a coaxial cell as standards. Each measurement has been repeated 3–4 times at each pD to reduce random errors. A trace of Na_2SO_3 was added to prevent oxidative destruction of catechol ring hydroxyles. For chemical shift determinations arithmetic means of the multiplet lines were taken. A trace of *p*-dioxan was added as internal reference for chemical shift determination. Spectra were simulated by computer program LAOCOON 3 modified for interactive use on a minicomputer PDP 11/34 [20].

Method

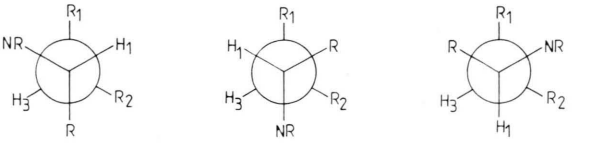
NMR spectroscopical determination of conformations is based on the connection between vicinal coupling constants J and the dihedral angle Φ [21–23]. Conformational distribution about the C_α–C_β bond of catecholamines can be described as the fractional proportion of three staggered rotamers: two *gauche* p_I and p_{III} and *trans* p_{II} (Fig. 2). For noradrenaline

$$p_I = (J_{13} - J_g)/(J_t - J_g)\,, \tag{1}$$

$$p_{II} = (J_{23} - J_g)/(J_t - J_g)\,, \tag{2}$$

$$p_{III} = 1 - (p_I + p_{II})\,, \tag{3}$$

where J_t and J_g are coupling constants for *trans* and *gauche* conformation of C_α–C_β protons, respectively.



I II III

Fig. 2. Newman projections of three staggered conformers about side chain $C_\beta - C_\alpha$ bond (R = CH_3 in E and R = H in DA, NA; R_1 = catechol in DA, NA and R_1 = benzene in E; R_2 = OH in NA, E and R_2 = H in DA).

For dopamine the two *gauche* rotamers I and III are equivalent due to rapid interconversion of the conformers by rotation about C_α–C_β bond and the geminal pairs of protons become chemically equivalent. Similarly for ephedrine only the *trans* rotamer I can be singled out from the other two *gauche* rotamers II and III [14, 15].

Results and Discussion

In Fig. 3 chemical shifts of protons of the amine side chain are plotted for NA, DA and E. It can be seen that deprotonation of the amine head takes place at pH = 8–10 for all three molecules. This is in accord with results obtained from direct pD titration [24]. Chemical shifts are presented in Table I together with coupling constants at varying pH. For all three cases considered the proportion of amine protonated species at pH = 11.5 is less than 5% [25].

The ephedrine spectrum is deceptively simple and of AX type. The vicinal coupling constant J_{AX} = 3.5 Hz, measured at acidic pH, may be compared with the previously obtained value 3.63 Hz which was reported by Porthogese [15]. If the pH is raised to pH = 10 this constant changes to 4.5 Hz and still further at pH = 11.5 to J_{AX} = 5.9 Hz. For conformational analyses one needs the values for J_t and J_g (Eqs. (1–3)). We have taken J_t = 10.5 Hz and J_g = 2.8 Hz, measured for closely related compounds of known conformational preference – rigid analogues of morpholine [15].

In Table II results of conformational analyses are presented. The fractional population of *gauche* rotamers p_{II} and p_{III} is lowered from 0.87 to 0.60 by deprotonation.

Three factors may be instrumental at changes of conformational equilibria of E in solution: steric

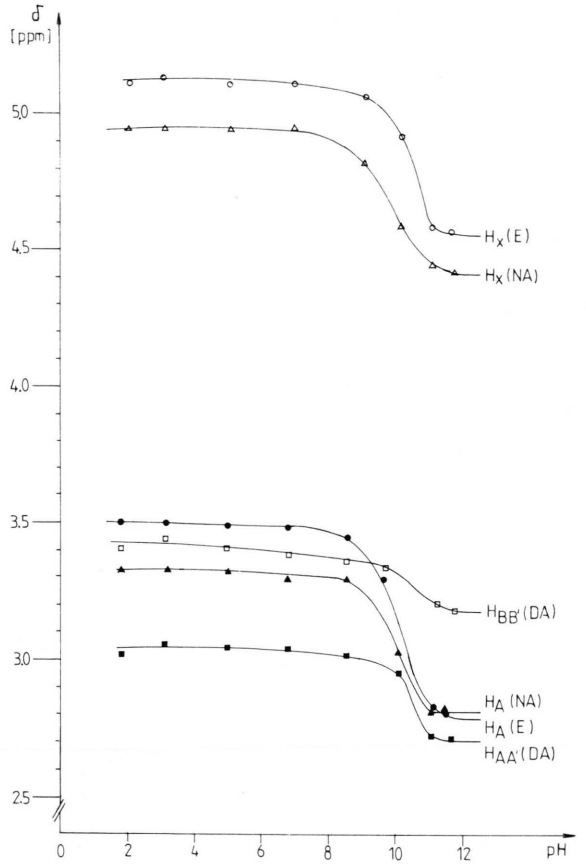


Fig. 3. Variation of chemical shifts of the side chain protons (in ppm) with pH. ○, ● ephedrine; △, ▲ noradrenaline; □, ■ dopamine.

hindrance of the C_α methyl substituent, intramolecular hydrogen bonding and solvation. From the change in p_I it may be concluded that sterical hindrance is gaining importance on deprotonation. The formation of different intramolecular hydrogen bond should explain the shift of the conformational equilibrium towards lower population of gauche conformations. If we compare coupling constants for protonated species below pH = 7.0 with those of basic ephedrine at around pH = 11, we observe that in both cases the same rotamer form is predominant. The most plausible explanation for this fact is intramolecular hydrogen bonding NH ... O also observed in the crystalline ephedrine hydrochloride [26]. While in the basic case the interaction is through the hydroxyl proton [12, 15] in case of the protonated E the acid proton of the ammonium head is involved in the H-bonding. Ison *et al.* [14] observed that methyl substituted nitrogen lowers the *trans* conformer population. This can be explained by the fact that unsubstituted nitrogen has a lower proton affinity than the NH_2–Me group. Therefore, intramolecular hydrogen bonding is less probable at higher pH in normetaephedrine than in ephedrine.

The noradrenaline spectrum is of the ABX type. For spectral analyses the assumptions $J_t > 10$ Hz and $J_g < 4$ Hz have been made, following Ison *et al.* [14]. Coupling constants $J_{AX} = 8.4$ and $J_{BX} = 3.0$ Hz lead us to the assignment that $p_{II} > p_I$, p_{III}. It favours the *trans* conformation of the amino group towards the aromatic ring and *gauche* with regard to benzylic hydroxyl. The deprotonation of the amino group at higher pH values seems to have a counterbalance in the dissociation of the acidic hydroxyl proton of the catechol ring. In consequence, low increase in *gauche* rotamer population with pH is observed from 0.30 at pH = 2.0 to 0.41 at pH = 11.5.

The assignment of AA′ BB′ multiplet arising from the two pairs of dopamine side chain methylene protons has been made on the basis of the fact that the transitions of the upfield half of the multiplet

Table I. Variation of chemical shifts (ppm) and coupling constants (Hz) for E, NA and DA, respectively, with pH.

pH	E			NA				DA			
	H_A	H_X	J_{AX}	H_A	H_X	J_{BX}	J_{AX}	$H_AH_{A'}$	$H_BH_{B'}$	J_{AB}	$J_{AB'}$
2.0	3.50	5.09	3.8	3.32	4.96	2.7	8.5	3.03	3.40	7.0	7.0
3.0	3.50	5.10	3.7	3.30	4.93	2.7	8.5	3.09	3.46	7.0	7.3
5.0	3.49	5.09	3.6	3.30	4.95	3.0	8.5	3.08	3.39	6.5	7.3
7.0	3.50	5.10	3.7	3.25	4.95	3.0	8.0	3.06	3.37	6.5	8.3
9.0	3.44	5.06	3.8	3.26	4.82	3.0	8.0	3.00	3.35	6.8	7.7
10.0	3.30	4.94	4.2	3.04	4.59	2.9	7.5	2.95	3.35	6.4	9.5
11.0	2.84	4.60	6.1	2.82	4.45	2.6	7.4	2.71	3.24	6.3	9.6
11.5	2.83	4.58	5.9	2.83	4.41	2.5	7.4	2.71	3.20	6.0	9.7

Table II. Populations of different conformations for ephedrine, noradrenaline and dopamine in aqueous solution at different pH.

pH	E		NA			DA	
	p_I	$p_{II} + p_{III}$	p_I	p_{II}	p_{III}	p_{II}	$p_I + p_{III}$
2.0	0.13	0.87	0.07	0.70	0.23	0.44	0.56
4.0	0.12	0.88	0.07	0.70	0.23	0.40	0.60
5.0	0.10	0.90	0.10	0.65	0.26	0.41	0.59
7.0	0.10	0.90	0.11	0.65	0.24	0.42	0.58
9.0	0.13	0.87	0.10	0.62	0.28	0.50	0.50
10.0	0.18	0.82	0.09	0.60	0.31	0.55	0.45
11.0	0.43	0.57	0.09	0.59	0.32	0.70	0.30
11.5	0.40	0.60	0.04	0.59	0.37	0.71	0.29

($H_BH_{B'}$) are more broadened than those of the downfield half ($H_AH_{A'}$) [13]. This was ascribed to interactions with the nitrogen nucleus [13, 18].

For dopamine coupling constants $J_g \leqq 4.6$ Hz and $J_t = 12$ Hz were assumed [13]. The evaluated population of *trans* conformer p_{II} equals 0.44 at pH = 0.3 and grows to $p_{II} = 0.70$ at pH = 11.1. These results may be compared to those of Bustard and Egan [13]. They obtained the value $p_{II} = 0.43$ at pH = 5.9. In an attempt to rationalize this result, it is concluded that here no intramolecular hydrogen bond can be formed, and the increase of the *trans* conformer population with deprotonation may be the consequence of the intermolecular solvent-solute interactions. The role of catechol ring hydroxyl groups ionization [25] may also be of importance as in the case of NA but it has not been studied in this connection as yet.

What can be the relevance of the above results in aqueous medium to the actual situation in biological receptor systems?

Our aim was primarily to find out how the conformer populations change as a function of pH, and only in the second place, how can this be connected with specificity of activity at different receptors. The results in Table II show that the nitrogen protonation/deprotonation changes the population of all three molecular species significantly. Both *trans* and *gauche* conformations are present at high pH = 11.5. However, the energy differences between these two rotamer forms are estimated to be rather small [1, 2, 14]. Therefore, ascribing differential biological activity to only one energy conformer seems to us not to be justified.

The actual pH values "felt" by catecholamine and related compounds at the receptor and/or at membranes may not equal the values in aqueous solution because the medium is not pure water but structured and partly lipoid. In fact, local conditions are responsible for the species populations at the active site. On the other hand, theoretical calculations at various levels of approximation [3] *in vacuo* and X-ray studies [4] have also given rather dispersed results. However, the results of theoretical studies corroborate with our finding, that *trans* conformer is the predominant form in NA and DA in the whole possible range of pH while in E *gauche* forms are more abundant, only if solvation has been included in the calculation. It appears that the different ability to form intra- and intermolecular hydrogen bonding with benzylic OH group and water hydration shell of the amino group, respectively, is the determining factor of the conformer population changes.

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