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ASSOCIATION OF SOME PSYCHOTOMIMETIC COMPOUNDS WITH 6-METHYLPURINE IN AQUEOUS SOLUTIONS

HELMUT SAPPER and WOLFGANG LOHMANN

Institut für Biophysik der Universität, D-63 Gießen, and Institut für Biochemie, GSF, D-8042 Neuherberg, F.R.D.

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Abstract—The interaction between 5 HT (serotonin) or stereochemically similar psychotomimetic drugs and 6-methylpurine in aqueous solutions has been investigated by means of nuclear magnetic resonance and osmometric techniques. The complex chemical shifts and the thermodynamic quantities of the association were evaluated. Relevant association models are discussed. Contrary to their mode of self-association the various drugs seem to interact with 6-methylpurine due mainly to their aromatic ring systems. In this way they might simulate the action of LSD (D-lysergic acid diethylamide).

The structural similarities as well as the serotonin antagonistic potency of various psychotomimetic compounds (e.g. D-lysergic acid diethylamide (LSD), *N*,*N*-dimethyltryptamine, and mescaline) have been considered, for some time, to be important factors for a similarity in interaction between these compounds and a hypothetical serotonin receptor [1, 2]. A common receptor for serotonin (5-hydroxytryptamine, 5 HT) and the psychotomimetic drugs as well, however, has not yet been found and it is still questionable, whether such a common receptor exists [3].

Several hypotheses have been proposed. One of them supports a direct effect of serotonin and psychotomimetic drugs upon nucleic acids [2]. On the other side, it is known that 5 HT, like other biogenic amines, are stored *in vivo* in the form of complexes with ATP [4] and it might be assumed, that the stereochemically similar psychotomimetics compete with 5 HT in regard to this interaction, too. Therefore, the investigation of the association ability of 5 HT and of the drugs mentioned above with a purine derivative such as 6-methylpurine seems to be of general interest in order to elucidate their mode of action.

Several binding sites might be involved in the molecular action of the substances investigated. These as well as the type of interaction have been studied by means of nuclear magnetic resonance (NMR) and osmometry techniques. From the experimental results obtained some of the thermodynamic parameters describing quantitatively the association formed have been calculated. They might explain the different psychic activities of the drugs investigated as has been done recently by Sung and Parker [6] in the special case of amphetamines. Since the importance as well as the arrangement of the binding sites [5] might change by the complex formation the structure of the molecules during their interaction has to be investigated, too.

MATERIALS AND METHODS

Materials

N,*N*-Dimethyltryptamine hydrogenic oxalate (DMT), 5-hydroxytryptamine hydrogenic oxalate (serotonin, 5 HT), and mescaline sulfate have been purchased from Fluka, Buchs, Switzerland, amphetamine sulfate from Merck-Schuchardt, Darmstadt, Germany. We gratefully acknowledge the generosity of Sandoz, Nürnberg, Germany, in supplying D-lysergic acid diethylamide tartrate (LSD).

6-Methylpurine (m^6Pur) has been purchased from Cyclo Chemical, Los Angeles, USA. The buffer components and the reference substance 2,2,3,3-tetradeutero-3-(trimethylsilyl)-propionic acid sodium salt (TPA) were obtained from Merck-Schuchardt, Darmstadt, Germany. The deuterated water has been purchased from Sharp and Dohme, München, Germany.

The NMR measurements were performed at pD 7.4 (phosphate buffer) with the exception of LSD, which was measured at pD 5.4 (acetate buffer) because of its relative low pK-value of protonation (the pK 7.8 of the protonation of N-6 of LSD corresponds to pK's near 10 of the protonation of the amino group of the other drugs used). The osmometric measurements were performed in distilled water. All substances used were of reagent-grade quality and were used without further purification.

Methods

The determination of association data of a two solute system (molecules A and B) has been described e.g. by Antonovsky *et al.* [7], Dimicoli and Helene [8], and Steiner [9] for either NMR experiments or colligative methods such as vapor pressure osmometry. Several models of evaluation with various restrictive assumptions were discussed. In the special case of the isodesmic model ($A_n + A \rightleftharpoons A_{n+1}$ with the equilibrium constants K_n being equal for all n and in the following defined as K_s^A) it is assumed, that the self-association of the B-molecules is negligible. In this study this assumption is justified since the self-associations of the B-molecules (in the following used as an abbreviation for the drugs investigated) were found to be very small (K^{250}: 0.5–1.0 M^{-1} [5]). Although the self-association of LSD is larger [5], the above assumption can be applied, nevertheless, since the concentration of molecules B was kept, in

general, low (ca 25 mM) compared to the concentration of molecules A (50–250 mM of m^{6}Pur).

Furthermore, the isodesmic model is very suitable for the problem to be investigated because it takes into consideration the large self-association of m^{6}Pur. This self-association has been determined analogically to that one of other purines [10]; its thermodynamic parameters were found to be $\Delta H = -22.2$ kJ/mole and $\Delta S = -57.9$ J/deg mole which agrees well with results obtained by other authors [11, 12]. Thus, the interaction between the drugs and m^{6}Pur can be described well by the isodesmic model according to which the monomeric B molecules will be attached to the stacked n-mere A_n ($n = 1,2,3,\ldots$) at either end.

In the following it is assumed that the self-association constant K_s^A of the molecules A and the total molar concentrations of A and B, a_0 and b_0, are known. With a_1, b_1 as molar concentrations of monomers of A and B, and K as the association constant of the mixed association between A and B it follows [7]:

$$a_0 = \frac{a_1(1 + K \cdot b_1)}{(1 - K_s^A \cdot a_1)^2} \tag{1}$$

$$b_0 = b_1\left(1 + \frac{K \cdot a_1}{1 - K_s^A \cdot a_1}\right). \tag{2}$$

The combination of the two equations (1) and (2) results in

$$K = \frac{(1 - K_s^A \cdot a_1)\,[a_0(1 - K_s^A)^2 - a_1]}{a_1[K_s^A \cdot a_1 \cdot b_0(1 - K_s^A \cdot a_1) - a_0(1 - K_s^A \cdot a_1)^2 + a_1]}. \tag{3}$$

In the case of the NMR experiments, the ratio between the chemical shift observed Δ_{obs}^B and the corresponding complex shift Δ_c^B of a B proton (measured relative to its monomeric shift) is given by

$$\frac{\Delta_{obs}^B}{\Delta_c^B} = 1 - \frac{b_1}{b_0}. \tag{4}$$

In the case of the osmometric experiments (freezing point depression and vapor pressure depression), the osmolarity c_{obs} is given by

$$c_{obs} = b_1 + \frac{a_1}{1 - K_s^A \cdot a_1}. \tag{5}$$

The association constants K were obtained in both of the experimental methods used in a similar way: either an initial value of the unknown concentration a_1 was chosen (osmometry) or a_1 was determined by the chemical shifts of the various A protons and their known self-association parameters (NMR). Then, a corresponding K-value was evaluated according to eqn 3. This K-value and the a_1 concentration permit the evaluation of the concentration b_1 by eqn 2. The theoretical values Δ_{obs}^B and c_{obs} were, then, calculated by using eqns 4 and 5, respectively, whereby in the first case an appropriate initial value of the complex shift Δ_c^B of each B proton observed has to be chosen. Finally, either Δ_c^B (NMR) or a_1 (osmometry) were varied until the deviation between the calculated and observed values of Δ_{obs}^B and c_{obs} exhibited its minimal value. In the first case one might assume that the Δ_c^B-value obtained in this way seems to be the correct complex shift. The association constant K was calculated by means of eqn 3.

The temperature dependence of the K-values obtained has been used for calculating the enthalpies ΔH. The absolute sizes of the Δ_c^B-values depend on the model used. For relative comparisons an error of less than ± 5 per cent has to be taken into consideration. The estimated errors of the K- and ΔH-values obtained are given in the corresponding table.

The NMR spectra were recorded with a Varian HA-100 spectrometer equipped with a variable temperature system (temperature range used: 0 to 60°). About 6 per cent of H_2O, added to the deuterated solvent, were used as a lock; its temperature dependence was corrected according to the results obtained with TPA used as a reference sample in a separate probe. Determinations of the osmolarities were made by freezing point depression (0°) and vapor pressure depression (25 to 60°), respectively, using an instrument of Knauer Co., Berlin, Germany. NaCl was used for calibration.

RESULTS AND DISCUSSION

NMR and calorimetric studies have shown that 5 HT interacts with purine and ATP, respectively, in aqueous solutions due mainly to a ring–ring interaction [4, 8]. The decrease in osmolarity as well as the shift of NMR peaks obtained by addition of m^{6}Pur to either 5 HT or structurally similar psychotomimetics, such as DMT, LSD, mescaline, and amphetamine indicate a similar interaction. As can be seen qualitatively, for instance, in the case of DMT (Fig. 1) all resonances are shifted upfield with the exception of the $N(CH_3)_2$-signal which remains nearly unchanged. The H-4 and H-7 protons of ring A are shifted stronger than the H-2 proton of ring B. Furthermore, the CH_2-β resonances are shifted stronger than the CH_2-α resonances.

For a quantitative comparison of differences in the chemical shifts due to the interaction, the complex shifts Δ_c^B of the various protons were determined by a fit of the chemical shifts observed and by varying the concentration of m^{6}Pur. These Δ_c^B-values are shown in Table 1. It can be seen that the H-4-signal of DMT, e.g., is shifted about twice as much as the CH_2-α-signal and about 1.2 times as much as the H-2-signal. Furthermore, the response of 5 HT is about the same as for DMT; the ring A of 5 HT, however, seems to be more, the side chain less influenced. It is interesting to note, that also H-14 and H-9 of LSD are influenced stronger, relative to H-2, than corresponding protons in the case of DMT. Thus, it might be assumed that the enhanced electronegativity of, or adjacent to, ring A (OH-substitution in the case of 5 HT as well as (C-9)–(C-10)-double bond in the case of LSD) changes the geometry of the complexes formed. This means, that in the case of DMT the aromatic rings of the interacting molecules overlap to a greater extent than in the cases of 5 HT or LSD, in which cases the eccentricity of the electronegativity may displace the ring systems. Unfortunately, in the case of the phenylethylamines mescaline and amphetamine such conclusions cannot be made since an averaged ring proton signal can be observed only. From Table 1 it can be seen, however, that, like in

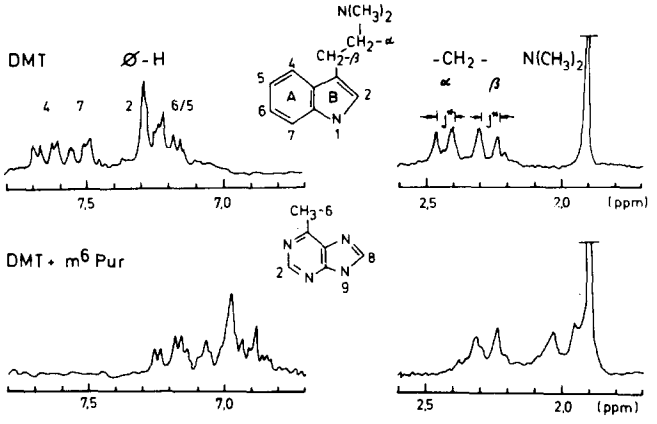

Fig. 1. NMR spectra of DMT (pD 7.4; 25 mM; 25°) without (upper spectrum) and with (lower spectrum) the addition of m^{6}Pur (200 mM).

Table 1. Complex shifts Δ_c^B (in ppm) of the various drug protons caused by the interaction with m^{6}Pur (pD 7.4, except for LSD) and calculated as described in the text

Drug	Proton H-2	H-4	H-7	CH_2-β	CH_2-α	NCH_3
5 HT	1.93	2.95	2.62	1.59	0.82	—
DMT	2.14	2.67	2.40	1.90	1.36	0
LSD (pD 5.4)	2.56	3.17*	4.05†	—	—	0
Mescaline	1.88	0.87‡	0.97§	1.09	0.60	—
Amphetamine	3.18	—	—	2.70	1.79¶	1.36‖

* H-9. † H-14. ‡ OCH_3-3/5. § OCH_3-4. ¶ CH-α. ‖ CH_3-α.

the case of the tryptamines 5 HT and DMT, the side chain protons are shifted less than the ring protons; the same applies to the protons in the α-position if compared to those ones in the β-position.

Giessner-Prettre and Pullman have used the ring current intensities of purine and various nucleobases for calculating the magnetic shielding values at a stacking interaction distance of 0.34 nm [13]. According to their results purine and adenine behave almost alike. Therefore, it might be assumed that m^{6}Pur and adenine are also very similar in regard to their ring current effects and that, thus, the shielding values calculated for adenine can be compared with the complex shifts found experimentally in this study.

The complex shifts obtained with the NMR technique are in good agreement with the calculated shielding values, if an appropriate orientation between the m^{6}Pur ring system and the interacting molecule is found. In the case of LSD such an orientation is shown in Fig. 2. The relation found experimentally (see Table 1) H-2:H-9:H-14 = 1.0:1.1:1.6 can also be obtained from the calculated isoshielding curves [13]. Furthermore, it can be seen that the NCH_3-6 group ($\Delta_c^B \approx 0$ ppm as obtained from the NMR data) is located adjacent to the paramagnetic area (shielding values <0 ppm) exerted by the ring current of m^{6}Pur. It should be pointed out, however, that the absolute size of all Δ_c^B-values seems to be too large in regard to the calculated ones. Thus, the maximum value calculated by Giessner-Prettre and Pullman is 1.28 ppm [13], whereas the complex shifts reported in this paper show values of up to about 4 ppm (H-14 of LSD).

These differences might be caused by the model used, which considers only an attachment of B to one side of A_n. Dimicoli and Helene [8] have shown that the Δ_c^B-values should be only half (the K_c-values, however, twice) the values reported here if two side attachment and an intercalation of molecules B in A_n is possible. Thus, the values of the complex shifts calculated might be in indication for intercalation or

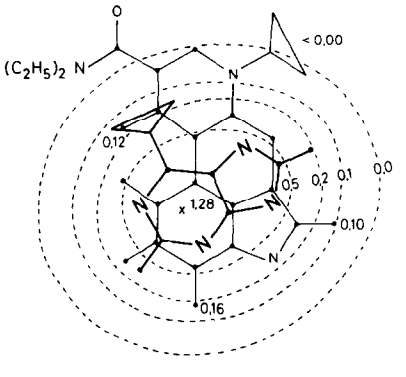

Fig. 2. Example of an association arrangement between an LSD and a m^{6}Pur molecule. The distance between the two molecules is 0.34 nm. The shielding values (in ppm) of NCH_3-6, H-2, H-9, and H-14 of LSD were estimated from the isoshielding curves calculated from the ring current intensity of m^{6}Pur [13].

Table 2. The thermodynamic quantities $K^{25°}$ (association constant at 25°) and ΔH (association enthalpy) of the interaction between m^{6}Pur and various drugs as evaluated by NMR and osmometric data

	NMR		Osmometry	
	$K^{25°}$ [M^{-1}]	$-\Delta H$ [kJ/mole]	$K^{25°}$ [M^{-1}]	$-\Delta H$ [kJ/mole]
5 HT	2.7 ± 0.3	22 ± 2	20 ± 2	21 ± 3
DMT	2.5 ± 0.3	22 ± 2	18 ± 2	20 ± 3
LSD	3.8 ± 0.4	25 ± 3	75 ± 5	26 ± 4
Mescaline	2.6 ± 0.3	21 ± 2	17 ± 2	16 ± 3
Amphetamine	~0.5	20 ± 2	< 10	*

* Too small for evaluation.

two side attachment and the complex shifts of Table 1 should be reduced, therefore, by one half for a correct comparison. Moreover, the calculations done by Giessner-Prettre and Pullman [13] are based on assumptions which perhaps cannot be applied to the problem investigated here. For instance, the ring current intensities which they have assumed to be constant could be influenced by the magnetic anisotropy of the associated molecules. Furthermore, smaller K-values can cause larger Δ_c^B-values (and vice versa) since both of these values are related to each other by the procedure described above. This is caused primarily by the limited saturation range (range of the ratio between the chemical shift observed and Δ_c^B) as has been shown by Deranleau in the case of optical spectroscopy [14]. The K-values determined by the NMR data seem to be, indeed, too small as can be seen by comparison with the results obtained from osmometric measurements. As a consequence, the Δ_c^B-values obtained might be too large.

The thermodynamic quantities of the associations investigated, the association constants K (at 25°: K^{25}) and the enthalpies ΔH, are shown in Table 2. The ΔH-values obtained from the NMR data are very similar to those ones obtained from the osmometric data. Furthermore, both of the experimental methods used seem to result in the same order of magnitude of ΔH:LSD > tryptamines (5HT, DMT) > phenylethylamines (mescaline, amphetamine). From this sequence it might be concluded that the greater the ring system of the drugs the stronger the association with m^{6}Pur. A similar sequence is obtained for the K-values; the values obtained from the osmometric technique are, however, considerably larger than those ones obtained from the NMR experiments. The greater sensitivity of the osmometric measurements in regard to the self-association of molecules B might be an explanation for these findings. This argument is supported by the fact that LSD, a substance with a great self-association ability, has a larger K^{25}-value. Moreover, the K-values obtained from the NMR technique may be too small, at least by a factor of two, as has been pointed out above.

The averaged coupling constant J^* of the A_2B_2-spectrum of the side chain (for explanation of J^* see Fig. 1 or ref. [5]) is changed by the interaction with m^{6}Pur. The value of J^* is increased from 5.5 to 6.8 Hz (5 HT), from 6.2 to 7.3 Hz (DMT), and from 6.7 to 7.2 Hz (mescaline). All other coupling constants of LSD and amphetamine seem to remain unchanged. the increase of J^* may reflect a participation of the bimethylene side chain and, especially, of the nitrogen located at its end (which corresponds to the N-6 of LSD). Such an involvement should, however, cause, more or less, a restriction of the free rotation ability and probably an extension of the side chains. The arrangement of the active sites (ring system and nitrogen atom located at the end of the side chain) should resemble, therefore, that one of the LSD.

CONCLUSIONS

It has been shown recently that LSD molecules self-associate by a ring–ring interaction and to a larger extent than some other psychotomimetics [5]. From the results obtained by this study it can be concluded that tryptamines (5 HT, DMT) as well as phenylethylamines (mescaline, amphetamine) interact with m^{6}Pur like LSD. These substances seem to reduce the rotation ability of their chains leaving them in a more extended position which resembles the LSD molecule.

The comparison between the shielding values calculated from the ring current intensity [13] and the NMR complex shifts observed exhibits some information about the type and the conformation of the complexes obtained with the various psychotomimetics and m^{6}Pur. It can be assumed, that the associations observed indicate the possibilities of one side attachments as well as two side attachments or intercalations and that an eccentric electronegativity causes a displacement of the interacting ring systems. The sequence of the thermodynamic parameters points out a correlation between the size of the ring systems of the drugs and their association ability. The differences are, unfortunately, not so significant that a correlation with the psychic activity of the compounds can be established.

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