Interactions between some Psychodrugs and Flavins

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Es wurde das Verhalten der Flavine gegenüber einiger Psychopharmaka untersucht. Die experimentellen Resultate lassen das Vorhandensein einer Wechselwirkung zwischen den zwei Molekülen annehmen. Die Ladungsübertragung spielt in diesem Prozeß eine wichtige Rolle. Eine Beziehung zwischen den berechneten ΔG^0 und der pharmakologischen Wirkung der Droge wurde festgestellt.

It has been well-known for quite a long time that flavins tend to interact with different molecules; interactions with phenols[1], indole[2], purine[3], chloropromazine[4] derivatives, etc. have been particularly studied in the last ten years.

Some of these interactions can be of considerable biological meaning.

We have therefore considered of interest to examine the reaction between flavins and some compounds similar to the above mentioned ones, which have the structural capacity of interacting somehow with the chemical reactions of the living world.

The chosen substances usually operate "in vivo" at the level of the central nervous system; some of them have also hallucinogenic effects, the specific subcellular location of which is still questionable. Nevertheless DENBER and TELLER's assumption suggesting that a molecule with hallucinogenic effects, e.g. mescaline, binds by a sort of "specific" bond to mitochondria[5], is definitely important. By this assumption we were induced to study the relation between flavins and mescaline, and then we extended our studies to the association of flavins to other molecules. Among all forms of molecular interaction theoretically to be expected, in this paper we have taken into consideration the charge transfer effect, presuming that such a mechanism, although probably not the only one, is of considerable importance in the reaction between the two molecules.

Material and Methods

The substances studied, all chromatographically pure, were: DL-adrenaline (K e K), DL-amphetamine (K e K), DL-noradrenaline (Fluka), mescaline hydrochloride (Sigma), LSD 25 (Delysid-Sandoz S.A.). These substances were made react with Riboflavin (Fluka), FMN (Sodium salt, Fluka), FAD (Fluka) in buffered aqueous solution. The interaction was studied spectrophotometrically with a Beckman DU Spectrophotometer in a 1 cm quartz cell.

The applied concentrations were limited by the low solubility of the substances used (for Riboflavin, a $0.7 \cdot 10^{-3}$ M solution is very near to the saturation point); in all cases, the substance interacting with flavin was added in large excess. All the solutions were deaerated by a stream of nitrogen.

The measurements were performed (at least when possible) in buffered solutions at pH 0 — 7 — 13 respectively, since Riboflavin shows several absorption spectra as an index of different molecular arrangement[6] in different pH-values.

Furthermore, the measurements were done at 3 °C, 20 °C, and 50 °C in order to observe the influence of temperature on the interaction. Simply in order to compare, some well-known interactions of flavin with indole ring molecules were measured again in several conditions of pH and temperature[2, 7]. For this purpose, measurements analogous to those described above were made with: Indole (Fluka), DL-tryptophane (Fluka), DL-hydroxytryptophane (Fluka), Serotonin hydrogenoxalat (Fluka). In fig. 1, the spectrum of the combination: Riboflavin-Mescaline (pH 7; 3 °C) is reported. Such a spectrum can be considered as an example of the general behaviour, the spectral variations being the same in all cases studied.

In the spectrum a broadening of the last band can be observed (≈ 450 nm) which seems to be due to the interaction between the two molecules. All extinction values normalized to the maximum of the difference spectrum (ΔE) and the wave-lengths corresponding to the most significant conditions of pH and temperature, are reported in table 1. Finally, the fluorescence of the flavin solutions and the recorded quenching

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Donor-acceptor	Donor concentration Mole/$l \cdot 10^2$	pH	t [°C]	λ max·nm	ΔE max·nm
DL-Amphetamine-Riboflavin	10	7	3	475—480	0.110
		7	25	480	0.110
		7	50	480	0.055
		0	3	475	0.035
		0	25	475	0.050
		13	3	480—485	0.070
		13	25	480—495	0.050
DL-Amphetamine-FMN	10	7	3	475—480	0.100
		7	25	480	0.080
DL-Adrenaline-Riboflavin	2	7	3	480—485	0.055
		7	25	480—485	0.035
		0	3	455—460	0.040
DL-Adrenaline-FMN	2	7	3	470	0.100
		7	25	470	0.080
DL-Noradrenaline-Riboflavin	2	7	3	480—490	0.035
		7	25	475—480	0.035
DL-Noradrenaline-FMN	2	7	3	475—480	0.095
		7	25	470—475	0.050
Mescaline Hydrochloride-Riboflavin	7	7	3	480	0.250
		7	25	485—490	0.190
		7	50	490	0.120
		0	3	460	0.360
		0	25	460	0.300
		0	50	460	0.235
Mescaline-Hydrochloride-FMN	7	7	3	475	0.300
		7	25	475—485	0.245
		7	50	480	0.180
Mescaline-Hydrochloride-FAD	7	7	3	486	0.120
		7	25	490—500	0.090
Indole-Riboflavin	2	7	3	495	0.150
		7	25	500	0.120
DL-Tryptophane-Riboflavin	0.7	7	3	500—505	0.100
		7	25	500—505	0.070
DL-5-Hydroxytryptophane-Riboflavin	0.7	7	3	500	0.210
		7	25	505	0.125
		7	50	505—510	0.075
		0	3	470	0.190
		0	25	465—470	0.170
		0	50	465	0.170
DL-Hydroxythryptophane-FMN	0.7	7	3	495	0.200
		7	25	500—505	0.185
		7	50	505	0.090
DL-Hydroxytryptophane-FAD	0.7	7	3	505	0.080
Serotonin Hydrogenoxalat-Riboflavin	0.7	7	3	505	0.250
		7	25	505—510	0.160
		7	50	510	0.100
		0	3	475	0.080
Serotonin Hydrogenoxalat-FMN	0.7	7	3	495	0.300
		7	25	500	0.200
Serotonin Hydrogenoxalat-FAD	0.7	7	3	505—510	0.150
		7	25	500—510	0.090
LSD-Riboflavin	0.14	7	3	495	0.130
		7	25	490—500	0.090
		7	50	495—500	0.050
LSD-FMN	0.14	7	3	490	0.150
		7	25	495	0.120
		7	50	495	0.090
		0	3	490	0.090

Tab. I. Maxima of the difference spectra for several donor-acceptor couples. Concentration of Riboflavin, FMN, FAD: 0.7×10^{-3} M.

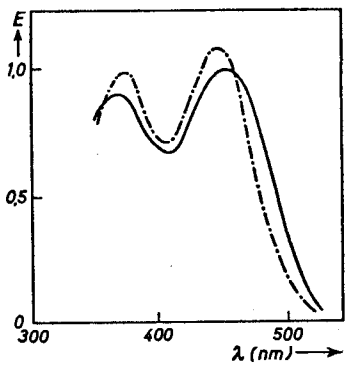


Fig. 1. Optical absorption spectra of neutral flavin and a complex in aqueous solution ($t=3$ °C). ——— Riboflavin (0.35×10^{-3} M); – – – Riboflavin (0.35×10^{-3} M)-Mescaline Hydrochloride (0.35×10^{-1} M).

caused by addition of the interacting molecule were measured.

These measurements were done by means of a Lange fluorimeter. Table 2 reports the fluorescence quenching values together with the data of qualitative observations made at −190 °C, and the colour changes of the flavin solutions as a consequence of the interaction.

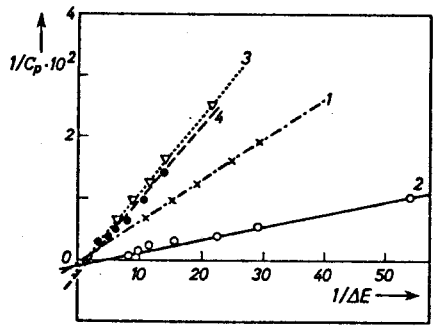
Then, in order to characterize the extent of the interaction, the constant of the possible association was calculated by the Benesi-Hildebrand's formula (fig. 2), which is pertinent to the case in which one of the components is in great excess and does not show any absorption at the examined wave length. ΔE measurements were made for each couple of reagents for various partner concentrations at a constant wave length at the maximum of absorption of the difference spectrum. K values were obtained from the $1/C_p$ against $1/\Delta E$ plot. These values are reported in table 3.

Partner	K [mole^{-1}]	ΔG^0 [cal mole^{-1}]
DL-Amphetamine	8	−1214
DL-Noradrenaline	13,3	−1516
DL-Adrenaline	20	−1755
Mescaline hydrochloride	25	−1886
Indole	35	−2083
DL-Tryptophane	60	−2398
DL-Hydroxytryptophane	110	−2753
Serotonin Hydrogenoxalat	190	−3074
LSD	140	−2085

Tab. III. Stability constants and ΔG^0 of flavins in different molecular complexes in aqueous solution.

Partner	conc. [Mole/lx10^2]	pH	Quenching [%]	Colour
DL-Amphetamine-Riboflavin	10	7	0	Yellow-green (−)
	10	0		Yellow (−)
	10	13		Yellow-light brown (−)
	20	7	0	Yellow-green (−)
	30	7	0	Yellow-green (+)
DL-Amphetamine-FMN	10	7	0	Yellow-green (−)
	10	0		Yellow-green (−)
	10	13		Yellow-light brown (−)
DL-Adrenaline-Riboflavin	2	7	32.8	Yellow-orange (+)
	2	0		Yellow-orange (+)
DL-Adrenaline-FMN	2	7	36.2	Yellow-orange (+)
DL-Noradrenaline-Riboflavin	2	7	26.5	Deep orange (+)
	2	0		Deep orange (+)
DL-Noradrenaline-FMN	2	7	29.8	Deep orange (+)
Mescaline Hydrochloride-Riboflavin	7	7	65	Light brown (+)
Mescaline Hydrochloride-FMN	0.7	7	67.2	Light brown (+)
Indole-Riboflavin	0.7	7	22.2	Yellow-orange (+)
	2	7	47.7	Yellow-red (+)
Indole-FMN	0.7	7	37.1	Yellow-orange (+)
DL-Tryptophane-Riboflavin	0.7	7	20	Red-orange (+)
DL-Tryptophane-FMN	0.7	7	28.1	Deep orange (+)
DL-5-Hydroxytryptophane-Riboflavin	0.7	7	44	Red-brown (+)
DL-Hydroxytryptophane-FMN	0.7	7	38.7	Brick red (+)
Serotonin Hydrogenoxalat-Riboflavin	0.7	7	49.3	Dark orange (−)
Serotonin Hydrogenoxalat-FMN	0.7	7	50	Deep orange (+)
LSD-Riboflavin	0.14	7	22.6	Dark yellow (+)
LSD-FMN	0.14	7	26	Dark yellow

Tab. II. Flavin fluorescence quenching in riboflavin-partner mixtures at 18 °C. Assumed colour at different pH. The mark (+) indicates that the colour is varied in comparison to the one assumed by the acceptor alone in the same conditions. The mark (−) indicates that the coulour is not notably varied. Riboflavin and FMN concentration : 0,0007 M.



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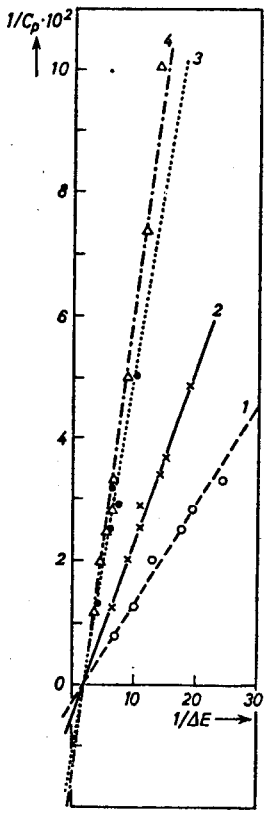

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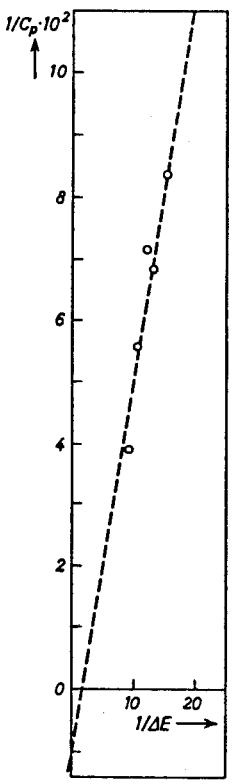

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Fig. 2. Plot of inverse concentration of Riboflavin partner, 1/Cp, versus inverse extinction $1/\Delta E$. The Riboflavin was 0.25×10^{-3} M in both the sample and the reference cell. a) 1: Riboflavin-Amphetamine; 2: Riboflavin-Noradrenaline; 3: Riboflavin-Adrenaline; 4: Riboflavin-Mescaline. b) 1: Riboflavin-Indole; 2: Riboflavin-Tryptophane; 3: Riboflavin-Hydroxytryptophane; 4: Riboflavin-Serotonin, c) Riboflavin-LSD.

Results and Discussion

The fluorescence quenching as well as the change in the absorption spectra of riboflavin, FMN and of FAD strongly suggest the existence of an interaction between flavins and the compounds studied.

Such a behaviour which was regularly observed in our experiments (see fig. 1 as an example of obtained results) is characteristic of the formation of complexes (WEBER [8]).

To get some information about the interaction, we calculated the free energy variation for the associative process from the association constant values. Such values were subsequently compared to those of the energy ascribable to the highest occupied level of the interacting molecules whose parameters are well-known (for β values see fig. 3 [9]).

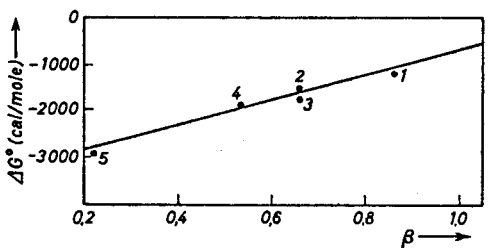

Fig. 3. Reaction ΔG^0 between some psychodrugs and flavins as a function of the HOMO. 1: Amphetamine; 2: Noradrenaline; 3: Adrenaline; 4: Mescaline; 5: LSD.

Although the data are not yet complete and the calculation is still approximate, the values of ΔG^0 seem to be linear function of the electron-donor power of the drug.

Consequently we may also state that the interactions between flavins and the substances examined are of the charge transfer type; more precisely, we should say that the charge transfer plays quite an important role in this kind of association.

As for the compounds which have a benzene ring as well as for indole derivatives (using the same flavin acceptor) it is likely that the electrons involved in the charge transfer are the π electrons of the ring. As a matter of fact, the spectrum variations observed for the examined molecules seem to be similar to those observed under the same conditions for solutions containing pyrocatechine as a donor.

On the contrary, no variation occurs when ethylamine is used as a donor. As for indole ring compounds it seems evident that the more the electron

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[9] S. H. SNYDER and C. R. MERRIL, Proc. nat. Acad. Sci. USA 54, 258 [1965].

transfer process is effective, the more the ring substitutes increase the conjugation of the indole.

The presence of associative processes other than charge transfer cannot be excluded. General analysis of the obtained data permit the following conclusion: the reaction of Adrenaline, Noradrenaline, Amphetamine, Mescaline with flavins involves a charge transfer process, which is, however, less effective than between flavins and indole derivatives.

Biological Implications

If the substances examined, although in different ways, can react with flavins, such reactions will be of biological meaning. Of course it is necessary that the molecules face one another. At the neuronal level, Adrenaline, Noradrenaline, Amphetamine, Mescaline, LSD and Serotonin inhibit the synaptic transmission [10, 11]. In this effect, Serotonin is 6 – 9 times more effective than LSD 25, and about 25 – 30 times more effective than Adrenaline [11].

Some of these compounds – however in this connection not Serotonin because it shows a particular behaviour not yet completely defined – can show hallucinogenic action with a progressive increase of effectiveness starting from Amphetamine, to Mescaline and LSD [11]. In other words, both inhibitor and hallucinogenic powers seem to go at the same rate. Furthermore, the pharmacological power of the drug and the ΔG^0 calculated for the interactions between these drugs and flavins, follow the same order. We may therefore reasonably state that the studied charge transfer process is, in some way, involved in the biochemical mechanisms of the drug. Nevertheless, this process cannot be considered as the fundamental mechanism through which the molecule acts. As a matter of fact, even if ΔG^0 values are changed in the same order as the pharmacological activity, the extent of this variation seems to be insufficient to allow the hypothesis that the differences of the pharmacological behaviour might be caused by some mechanism of this type (or even exclusively of this type).

If the compounds studied act as electron-donors in relation to flavins or other acceptors, it might be possible to assume that such a mechanism of electronic transfer exists at a certain stage of the biochemical process as a common base of the pharmacological action.

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[11] A. G. Marazzi and E. R. Hart, Science [Washington] **121**, 365 [1955].