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Psychotropic drug competition for [^{3}H]imipramine binding further indicates the presence of only one high-affinity drug binding site on human α_1-acid glycoprotein

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The role of α_1-acid glycoprotein (α_1-AGP) as a high affinity, but low capacity, binding component in human plasma was recognized only a few years ago and α_1-AGP has since received increasing attention from clinical pharmacologists as a parameter of the pharmacokinetics of many basic drugs (De Leve & Piafsky 1981: Piafsky 1980). In attempts to characterize molecular aspects of the interaction of drugs with this protein only one high-affinity binding site has been reported for nearly all drugs so far investigated, e.g. for dipyridamole (El-Gamal et al 1982), perazine (Brinkschulte & Breyer-Pfaff 1980; Schley et al 1980), chlorpromazine (El-Gamal et al 1983), imipramine (Kornguth et al 1981), thioridazine (Nyberg & Martensson 1982), quinidine (Fremstad et al 1976), disopyramide (Lima et al 1981); propranolol (Glasson et al 1980; Sager et al 1979), pindolol and other β-blockers (Lemaire & Tillement 1982) nicergolin (Robert et al 1983), methadone (Abramson 1982), and even some acidic drugs like warfarin and phenylbutazone (Urien et al 1982). Moreover, during some of these studies mutual displacement reactions have been observed for some of these drugs. All these findings are in agreement with our recent conclusion about the presence of only one single drug binding site at the α_1-AGP molecule which is common for nearly all drugs investigated so far (Müller & Stillbauer 1983). This conclusion is further substantiated by the data reported herein indicating that this site is also important for the binding of several tricyclic antidepressants as well as for a variety of other psychotropic drugs.

Materials. α_1-acid glycoprotein (α_1-AGP) (orosomucoid, human) was obtained from Behringwerke (Marburg, Federal Republic of Germany) (electrophoretic purity 99%). [^{14}C]Chlorpromazine (specific activity 79 mCi mmole^{-1} and [^{3}H]imipramine (specific activity 21 Ci mmole^{-1}) were obtained from Amersham Buchler (Braunschweig, Federal Republik of Germany). The radiochemical purities were >98% as determined by thin layer chromatography. All other drugs were generous gifts of the manufacturers.

Equilibrium dialysis measurements. The binding of radio-labelled drugs to α_1-AGP was determined by equilibrium dialysis using a protein concentration of 12·5 μM. All solutions were prepared in 0·07 M phosphate buffer pH 7·4. Aliquots (0·9 ml) of the protein solution were dialysed for 16 h at 22 °C in the dark against 0·9 ml buffer using cellophane dialysis membranes (Union Carbide) (Müller & Stillbauer 1983). The radioactivity at both sites was determined by liquid scintillation spectrometry.

Circular dichroism measurements. Circular dichroism (CD) measurements were carried out with a Cary 61 CD spectropolarimeter (El-Gamal et al 1982). All measurements were made in 0·07 M phosphate buffer pH 7·4. Results are expressed as molar ellipticity ([θ]) calculated with reference to the α_1-AGP concentration (25 μM).

RESULTS AND DISCUSSION

As indicated by Scatchard analysis, [^{3}H]imipramine binds to α_1-AGP via one single site with an intermediate association constant of about 24 × 10^4 M^{-1} (data not shown, see also Müller & Stillbauer 1983). Binding of imipramine to this site generates a biphasic extrinsic Cotton effect (Fig. 1). Several other tricyclics also interact with this site as indicated by the pronounced

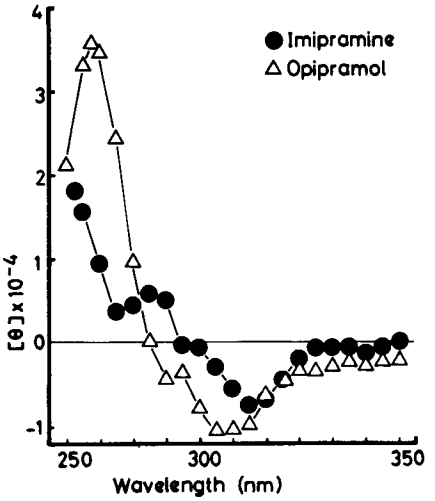

FIG. 1. Induced circular dichroism spectra of imipramine and opipramol (25 μM) in the presence of α_1-acid glycoprotein (25 μM). The data are difference values, using the Cotton effects of the protein at each wavelength as blank.

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Table 1. Structural requirements for the interaction of several tricyclic antidepressants with α_1-acid glycoprotein

Drug	Formula	nm[a]	$[\Theta]^{bx}$ 10^{-4}	$\Delta\alpha^c$ [%]
Imipramine	I	305 275	−3·5 −0·5	24
Desipramine	R_1 = H R_2 = H R_3 = H	305	−0·3	12
Trimipramine	R_1 = CH_3 R_2 = CH_3 R_3 = H	305 280	−1·7 +1·9	35
Clomipramine	R_1 = CH_3 R_2 = H R_3 = Cl	305 280	−0·4 +1·7	40
Amitriptyline	II R = CH_3	— — 250	— — +3·6	33
Nortriptyline	R = H	— —	— —	11
Protriptyline	III R = N $\begin{matrix}CH_3\\H\end{matrix}$	— 280	— +1·8	6
Opipramol	R = N C_4H_8N–CH_2–CH_2–OH	305 — 258	−0·8 — +7·2	25
Doxepine	IV	295 265	+0·9 −1·1	24

[a] Wavelength of the induced circular dichroism bands.
[b] Intensity of the induced circular dichroism bands at a molar drug/protein ratio of five.
[c] Increase of the free fractions × 100 (α) of [^{3}H]imipramine (6·3 μM) in the presence of several other tricyclics (25 μM). Without displacer, the free fraction × 100 was 29·3 ± 0·8 (n = 8).

displacement of [^{3}H]imipramine (Table 1). This interaction also generates extrinsic Cotton effects, which all reach maximal intensity at a molar ratio of five, but which are quantitatively and sometimes also qualitatively different from those of imipramine (Table 1). In spite of these two variable parameters for the binding to α_1-AGP (Table 1) the structural requirements for high affinity binding (high displacing potency) of the tricyclics are not completely obvious. It is remarkable that

Table 2. Increase of the free fractions × 100 of [^{3}H]imipramine and [^{14}C]chlorpromazine (6·3 μM) in the presence of several other psychotropic drugs (25 μM). Without displacer the free fractions × 100 were for [^{3}H]imipramine 29·3 ± 0·8 (n = 8) and for [^{14}C]chlorpromazine 6·8 ± 0·5 (n = 8). The data are means of four determinations.

	Increase of the fraction free × 100	
Displacing drug	[^{3}H]Imipramine	[^{14}C]Chlorpromazine
Chlorpromazine	44	33
Perazine	25	13
Haloperidol	20	8
Imipramine	26	10
Desipramine	13	4
Trihexyphenidyl	56	43
Biperiden	46	24
Diphenhydramine	20	6
Methaqualone	17	9
Glutethimide	16	8
L-Tryptophan	5	1
Flurazepam	28	8
Chlordiazepoxid	16	5
Diazepam	16	5
Carbamazepine	16	6
Benzoctamine	0	0
Cimetidine	0	0

all derivatives with a secondary amine group exhibit a low displacing activity and give only small extrinsic Cotton effects (desipramine, nortriptyline, protriptyline).

On the other hand, not only the aliphatic side chain but also substitution at the aromatic nucleus can affect the binding pattern (desipramine, trimipramine, clomipramine). Interestingly, the most important difference of the physicochemical parameters between the tertiary and secondary amine derivatives is the much smaller lipophilicity of the latter derivatives (Sharples 1976). Moreover, there is a fairly good correlation between the displacing potency (Table 1) and lipophilic parameters (Sharples 1976) for imipramine and the three closely related derivatives desipramine, trimipramine and clomipramine. On the other hand, the middle part of the tricyclic system is obviously not a crucial point as indicated by the comparable displacing potencies of imipramine, amitriptyline, opipramol, and doxepine (Table 1) and the qualitatively comparable Cotton effects of imipramine and opipramol (Fig. 1). Thus, although additional factors cannot be ruled out, the lipophilicity seems to be an important determinant for the binding of the tricyclics to α_1-AGP. This contrasts with the interaction of these drug with human serum albumin, where electronic parameters rather than the lipophilicity are the major binding factors (Sharples 1976).

As reported previously, the imipramine binding site of α_1-AGP is identical with the single basic drug binding site common for many different drugs (Müller & Stillbauer 1983). Accordingly, several neuroleptics as well as the tricyclics imipramine and desipramine inhibit the binding of [^{3}H]imipramine as well as of [^{14}C]chlorpromazine to this site (Müller & Stillbauer 1983) in a similar rank order of potencies (Table 2). Moreover, several other psychotropic drugs also displace both

marker ligands in a similar pattern, while cimetidine, benzoctamine, and L-tryptophan show no or only negligible effects (Table 2). These observations strongly support the concept of only one drug binding site at this protein. Furthermore, similar competition phenomena taking place in-vivo would be of considerable pharmacokinetic interest. However, for most psychotropic drugs therapeutic plasma concentrations might be too low to suggest any relevant displacing activity in-vivo, possibly except methaqualone (Delong et al 1976) and tricyclics like thioridazine (Nyberg & Martensson 1982) which reach therapeutic plasma values in the micromolar range. On the other hand, because of the predominant role of α_1-AGP for the plasma binding of many psychotropic drugs, pharmacokinetically relevant displacement reactions could take place by a concomitant therapy with other drugs also binding to this protein and reaching plasma concentrations in the micromolar range e.g. dipyridamole (Niewiarowski et al 1975), disopyramide (Lima et al 1981), or quinidine (Fremstad et al 1976). Moreover, since binding to α_1-AGP is sometimes not only a determinant for the free but also for the total plasma concentration of basic drugs (De Leve & Piafsky 1981), drug competition for α_1-AGP might contribute to the sometimes extremely large variations of the therapeutic plasma values of many psychotropic drugs in man.

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