J. Pharm. Pharmacol. 1992, 44: 28–33
Received April 18, 1991

Investigation of the Interaction Mode of Phenothiazine Neuroleptics with α_1-Acid Glycoprotein

TOSHIMI MIYOSHI, KATSUAKI SUKIMOTO AND MASAKI OTAGIRI

Faculty of Pharmaceutical Science, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862, Japan

Abstract—The interaction of phenothiazine neuroleptics with α_1-acid glycoprotein (AGP) and desialylated AGP (asialoAGP) has been investigated by fluorescence, circular dichroism spectroscopy and by equilibrium dialysis. The binding parameters of phenothiazines obtained from fluorescence agreed closely with those obtained from circular dichroism and equilibrium dialysis. The binding affinities (nK) to AGP were slightly higher than binding affinities to asialoAGP. Attempts to correlate binding affinities with partition coefficients suggested that hydrophobic forces were mainly involved in the binding of phenothiazine neuroleptics to AGP and asialoAGP. However, electrostatic interaction was also found to be involved as suggested by experimental data obtained from the influence of oleic acid and caesium chloride on the drug binding to the two proteins.

The binding of basic drugs to α_1-acid glycoprotein (AGP) can strongly affect their pharmacokinetic behaviour (Piafsky 1980; Routledge 1986; Kremer et al 1988). One common binding site on AGP has been found for basic drugs including the phenothiazine neuroleptics (El-Gamel et al 1983; Brunner & Müller 1985, 1987; Maruyama et al 1990). Hydrophobic forces have been mainly considered for the interaction of basic drugs with AGP (El-Gamel et al 1982; Urien et al 1982; Otagiri et al 1987, 1990; Maruyama et al 1990). However, in contrast to the binding of drugs to serum albumin, the mechanism of drug binding to AGP has not been clarified, and the role of the sialic acid component of AGP in the binding has been little investigated. In this paper, we have studied the binding of basic drugs to AGP and to desialylated AGP (asialoAGP) using the phenothiazine neuroleptics as model basic drugs because they can strongly bind to AGP (El-Gamel et al 1983; Schley & Müller-Oerlinghausen 1986).

Materials and Methods

Materials

AGP was donated by the Chemo-Sera-Therapeutic Research Institute (Kumamoto, Japan). AGP (mol. wt 44 100) gave only one band in SDS–PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis). Phenothiazines (Table 1) were gifts from Yoshitomi Co. Ltd (Fukuoka, Japan) and Shionogi Co. Ltd (Osaka, Japan), and were used without further purification. All other materials were of reagent grade and all solutions were prepared in deionized and distilled water. All protein and drug solutions were prepared in 67 mM phosphate buffer, pH 7·4.

Preparation of asialoAGP

AGP was enzymatically desialylated as outlined by Primazic & McNamara (1985), using an acylneuramininyl hydrolase enzyme obtained from *Clostridium perfrigens*. AGP in phosphate buffer (1·5 mg mL^{-1}) was added to the enzyme (0·357 units suspended in 5 mL of buffer). This solution was incubated at 37°C in a water bath, while being gently rotated at 30 rev min^{-1} for 2 h. The protein solution was filtered over an 8 μm filter to remove the enzyme. The concentration of sialic acid in the filtrate was determined using thiobarbituric acid (Warren 1959). The product was dialysed against deionized water and the dialysate lyophilized. Approximately 90% of sialic acid was removed, leaving an average of one sialic acid residue per protein molecule. The mol. wt of asialoAGP was therefore 40 000.

Physicochemical properties of phenothiazine derivatives

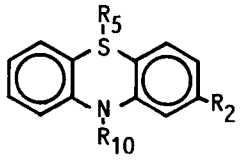
The pK_a values used were as reported by Hulshoff & Perrin (1977) and Whelpton (1989). The van der Waals volumes (V_w) were calculated according to the method of Moriguchi et al (1976). The partition coefficients (PC) were determined by the method of Fujita et al (1964). Ten mL of buffer solution containing phenothiazine was added to 1 mL of n-octanol saturated with buffer and the mixture was agitated for 30 min at 25°C. After centrifugation for 30 min the clear phases were assayed. Experiments were carried out in triplicate.

Equilibrium dialysis

Dialysis experiments were performed using a Sanko plastic dialysis cell (Fukuoka, Japan). The two cell compartments were separated by Visking cellulose membranes. AGP solution (10 μM, 1·5 mL) was poured into one compartment and 2 mL of drug solution (0·4–80 μM) was poured into the opposite compartment. After 6 h dialysis at 25°C, the drug concentrations in each compartment were assayed by HPLC. The HPLC system consisted of a Hitachi 655A-11 pump and Hitachi 655A variable wavelength UV monitor. A column of LiChrosorb CN (E. Merck, Darmstadt, Germany) was used as the stationary phase. The detection wavelength was 250 nm and the mobile phase consisted of 1·5% acetic acid-methanol (3:7, v/v). To determine absorption to the mem-

Correspondence: M. Otagiri, Faculty of Pharmaceutical Science, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862, Japan.

Table 1. Structures of phenothiazine neuroleptics.



Compound	R_2	R_{10}	R_5
1 Promazine	—H	$-(CH_2)_3N(CH_3)_2$	
2 Chlorpromazine	—Cl	$-(CH_2)_3N(CH_3)_2$	
3 Triflupromazine	$-CF_3$	$-(CH_2)_3N(CH_3)_2$	
4 Methoxypromazine	$-OCH_3$	$-(CH_2)_3N(CH_3)_2$	
5 Perazine	—H	$-(CH_2)_3-N$	$N-CH_3$
6 Prochlorperazine	—Cl	$-(CH_2)_3-N$	$N-CH_3$
7 Trifluoperazine	$-CF_3$	$-(CH_2)_3-N$	$N-CH_3$
8 Perphenazine	—Cl	$-(CH_2)_3-N$	$N-CH_2CH_2OH$
9 Fluphenazine	$-CF_3$	$-(CH_2)_3-N$	$N-CH_2CH_2OH$
10 Promethazine	—H	$-CH_2CH(CH_3)N(CH_3)_2$	
11 Propericiazine	—CN	$-(CH_2)_3-N$	—OH
12 Opromazine	—Cl	$-(CH_2)_3N(CH_3)_2$	—O

brane, concentrations in both compartments were measured.

Bound drug concentrations were calculated as:

bound concentration (D_b) =
drug concentration in protein compartment −
drug concentration in buffer compartment (D_f)

Circular dichroism

Circular dichroism measurements were made on a Jasco J-50A recording spectropolarimeter (Tokyo, Japan) using a 10 mm cell at 25°C. The induced ellipticity is defined as the ellipticity of the drug protein mixture minus the ellipticity of protein alone at the same wavelength and is expressed in degrees.

Bound drug concentration (D_b) and free drug concentration (D_f) were calculated according to the method of Rosen (1970).

Fluorescence

Fluorescence measurements were made using a Jasco FP-770 fluorescence spectrophotometer (Tokyo, Japan) and carried out at 10, 25 and 40°C. Thermodynamic examinations were carried out at the same temperatures. The fluorimetric titrations were as follows: protein solution (5 μM, 3 mL) was titrated by the successive addition of drugs (to give a final concentration of 0·1 to 20 μM), and the fluorescence intensity of protein was measured (excitation 290 nm and emission 340 nm). The total volume was less than 3 mL + 20 μL; corrections of protein concentrations to the total volume were not made as they were insignificant. At the selected wavelength, the drugs did not contribute to the fluorescence.

The fraction of drug bound, X, was determined according to Weber & Young (1964):

$$X = \frac{F_0 - F_b}{f_a \cdot F_0} \qquad (1)$$

where F_b and F_0 are the fluorescence intensities of protein in a solution with given concentration of drug and without drug, respectively, and f_a is the maximum fraction of quenchable fluorescence. The f_a value for chlorpromazine and dipyridamole was reported as 2/3 by Friedman et al (1985); all phenothiazines used in this study gave approximately this same value of f_a. F_b was determined for a range of drug concentrations.

Data analysis

The results obtained by the above methods were plotted according to the Scatchard equation:

$$r/D_f = nK - rK \qquad (2)$$

where r is the mol of bound drug per mol of protein, n is the number of binding sites per protein molecule, K is the binding constant, and D_f is the free drug concentration.

Thermodynamic analysis

Thermodynamic analysis was carried out according to the method of Cho et al (1971). Assuming that there is no significant temperature dependence of enthalpy change within the temperature range in which the interaction was carried out, it is possible to estimate the standard enthalpy change (ΔH°) for the association of 1 mol of the drug with 1 mol of the binding site from the following equation:

$$\log \frac{nK \text{ at } T_1}{nK \text{ at } T_2} = \frac{-\Delta H^\circ}{2{\cdot}303R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \qquad (3)$$

The standard free energy, ΔG°, for complex formation is estimated from:

$$\Delta G^\circ = -RT \ln nK \qquad (4)$$

and the entropy change, ΔS° is obtained by substituting ΔH° and ΔG° into the Gibbs-Helmholtz equation:

$$\left(\frac{\Delta G^\circ}{T}\right)_p = -\Delta S^\circ \frac{\Delta G^\circ - \Delta H^\circ}{T} \quad (5)$$

Multiple regression analysis

The characteristics of the binding constant were quantitatively examined by the use of multiple regression analysis. The variations in values of the log PC and the V_w parameters were sufficient to attempt correlation analysis. The reliability of regression analysis was judged by the F-test.

Results

The physicochemical properties of phenothiazine derivatives in this study are listed in Table 2. The Scatchard plots for the binding to AGP and asialoAGP obtained by equilibrium dialysis, circular dichroism and fluorescence are shown in Fig. 1. The binding parameters are shown in Table 3.

The AGP and asialoAGP binding parameters for the 12 phenothiazines studied are shown in Table 4.

The thermodynamic parameters of three phenothiazines are shown in Table 5.

Table 2. Physicochemical properties of phenothiazine neuroleptics.

Compound	log PC	pK_a	V_w(Å^3)
Promazine	5·22	9·42*	256
Chlorpromazine	5·32	9·36*	274
Triflupromazine	5·93	9·21*	285
Methoxypromazine	4·85	9·41*	281
Perazine	4·52	8·1*	290
Prochlorperazine	4·59	8·1*	306
Trifluoperazine	4·96	8·1*	319
Perphenazine	4·57	7·9*	317
Fluphenazine	4·53	7·9*	330
Promethazine	5·09	9·1*	256
Propericiazine	4·44	8·5*	353
Opromazine	2·32	9·0#	294

* Values from Hulshoff & Perrin (1977). # Value from Whelpton (1989).

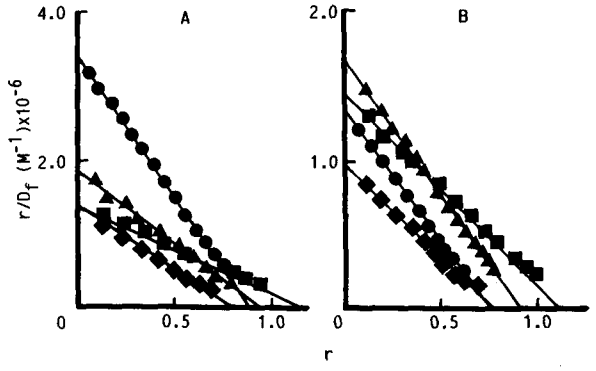


FIG. 1. Scatchard plots for binding of phenothiazine derivatives to AGP (A) and asialoAGP (B) systems, from fluorescence quenching method. ●, chlorpromazine; ▲, methoxypromazine; ■, perphenazine; ◆, promethazine.

Table 3. Binding parameters of chlorpromazine and methoxypromazine to AGP determined by three different methods. n = number of binding sites, K = binding constant, as determined from Scatchard plots.

Compound	Method	n*	K ($M^{-1} \times 10^{-6}$)
Chlorpromazine	Quenching	0·9	3·9±0·13
	E D	1·4	1·1±0·05
	C D	0·9	1·8±0·04
Mehoxypromazine	Quenching	0·9	2·0±0·17
	E D	0·9	0·9±0·02
	C D	0·9	0·8±0·06

ED: equilibrium dialysis. CD: circular dichroism. Quenching: fluorescence quenching. * s.d. of all n values were below ±0·02.

Table 4. Binding parameters of phenothiazine derivatives to AGP and asialoAGP as measured by fluorescence quenching at 25°C.

	AGP ($M^{-1} \times 10^{-6}$)		asialoAGP	
Compound	n*	K	n*	K ($M^{-1} \times 10^{-6}$)
Promazine	1·0	1·9±0·10	0·8	1·7±0·08
Chlorpromazine	0·9	3·9±0·13	0·7	1·2±0·24
Triflupromazine	1·0	2·5±0·18	1·1	1·9±0·07
Methoxypromazine	0·9	2·0±0·17	0·9	1·9±0·08
Perazine	0·9	1·3±0·09	0·8	1·2±0·14
Prochlorperazine	1·1	1·1±0·15	0·8	1·2±0·10
Trifluoperazine	0·8	1·8±0·21	0·8	1·0±0·15
Perphenazine	0·8	1·7±0·15	0·7	0·9±0·18
Fluphenazine	0·9	1·2±0·11	0·8	1·5±0·11
Promethazine	1·1	1·2±0·20	1·1	1·2±0·13
Propericiazine	0·8	1·3±0·03	0·8	1·1±0·13
Opromazine	1·0	0·7±0·07	1·0	0·8±0·05

All values are mean of three determinations ± s.d. n = number of binding sites, K = binding constant, as determined from Scatchard plots. * s.d. of all n values were below ±0·02.

Discussion

Comparison of methods

For quantitative correlation studies, all binding constants must be measured by the same method with high accuracy. Initially, we attempted to estimate binding constants by different methods. Table 3 shows the binding parameters of chlorpromazine and methoxypromazine to AGP estimated by fluorescence quenching, circular dichroism and equilibrium dialysis using Scatchard analysis (Fig. 1). Phenothiazine neuroleptics, including chlorpromazine, quenched the intrinsic fluorescence of AGP which arises from the tryptophan residue, since the binding sites for the drugs lie near the tryptophan residue in AGP (Friedman et al 1985). The amount of quenching directly reflected the amount of the drug bound to AGP. The binding of phenothiazines to AGP induced a large Cotton effect sufficient for quantitative study, as reported by El-Gamel et al (1983). For each drug, the number of binding sites was approximately one, and binding constants were consistent for all methods. However, the fluorescence quenching method was used for further studies because of its simplicity and high sensitivity.

Comparison of binding to AGP and asialoAGP

Scatchard plots from experiments with phenothiazine derivatives and AGP and asialoAGP gave straight lines and the number of binding sites was approximately one (Fig. 1,

Table 5. Thermodynamic parameters of binding of phenothiazine derivatives to AGP and asialoAGP systems.

	AGP			asialoAGP		
Compound	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (J Kmol^{-1})	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (J Kmol^{-1})
Promazine	−35·8	−21·5	45·0	−34·1	−19·6	48·7
Perazine	−33·9	−13·7	67·9	−33·9	−12·8	70·8
Opromazine	−32·7	−22·0	36·2	−33·2	−11·0	74·5

Table 4). The formation of a 1 : 1 complex of chlorpromazine with AGP and with asialoAGP was also confirmed by Job's plots (not shown). The binding parameters obtained for the AGP system were in reasonable agreement with the literature values (Brinkschulte & Breyer-Pfaff 1980; El-Gamel et al 1983). It is also evident from Table 4 that the AGP-phenothiazine system gave higher binding affinities than the asialoAGP-phenothiazine system, particularly for chlorpromazine. A similar large difference in binding constants of chlorpromazine to AGP and asialoAGP was also found by Friedman et al (1986). As the isoelectric points of AGP and asialoAGP were about 2·9 and 4·5, respectively, at pH 7·4, AGP would contain more negative charges than asialoAGP (Kremer et al 1988). Therefore, the above differences in binding data for AGP and asialoAGP systems suggest that the interaction between the positive charge of the phenothiazine derivatives and the negative charges of AGP was greater than that between phenothiazine derivatives and asialoAGP. The removal of sialic acid was not accompanied by the conformational change in the protein (Aubert & Loucheux-Lefebvre 1976).

Investigation of binding mode

To elucidate the binding mode of phenothiazines to AGP and asialoAGP, quantitative relationships between binding parameters of phenothiazines and their physicochemical properties were investigated using multiple regression analysis. As the number of binding sites varied from 0·7–1·1, the total binding affinity (nK) was used in the following analysis based upon 1 : 1 complex formation.

Hydrophobicity

Fig. 2 shows relationships between partition coefficients of phenothiazine derivatives and their binding affinities (nK) to AGP and asialoAGP. The fitted straight lines gave

for AGP: $\log nK = 0{\cdot}167 \log PC + 5{\cdot}39$
$(n = 12, r = 0{\cdot}80)$ (6)

for asialoAGP: $\log nK = 0{\cdot}083 \log PC + 5{\cdot}71$
$(n = 12, r = 0{\cdot}72)$ (7)

Partition coefficients, as a measure of hydrophobicity were correlated with binding affinities for AGP and asialoAGP with correlation coefficients of 0·80 and 0·72, respectively. The unexpectedly low binding constant of chlorpromazine with asialoAGP is emphasized in Fig. 2. When the chlorpromazine data were excluded, partition coefficients were correlated with the binding affinities to asialoAGP with an improved correlation coefficient of 0·78.

$\log nK = 0{\cdot}091 \log PC + 5{\cdot}68$ $(n = 11, r = 0{\cdot}78)$ (8)

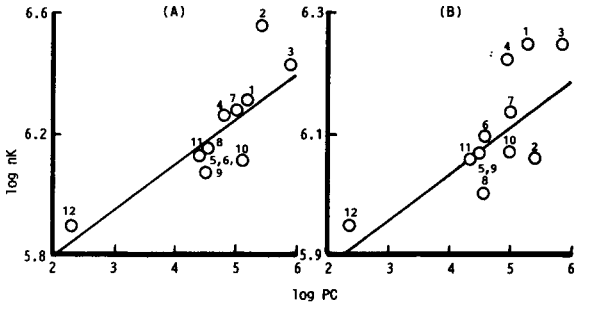


FIG. 2. Relationship between binding affinity and partition coefficient. A: AGP system, B: asialoAGP system. 1: promazine, 2: chlorpromazine, 3: triflupromazine, 4: methoxypromazine, 5: perazine, 6: prochlorperazine, 7: trifluoperazine, 8: perphenazine, 9: fluphenazine, 10: promethazine, 11: propericiazine, 12: opromazine.

These results suggested the contribution of a hydrophobic interaction in the binding of these drugs to the two proteins. However, the slope of the graph of log nK vs log PC for both AGP and asialoAGP was less than one, which suggests that phenothiazine derivatives were bound to hydrophobic surfaces on AGP and asialoAGP rather than to deeply buried sites in the two proteins (Helmer et al 1968). The intercept of the AGP plot was similar to that of the asialoAGP plot, suggesting that the binding sites of the two proteins may have almost the same hydrophobicity. However, considering the slopes for both systems the binding site on AGP seems to be the more sensitive to changes of hydrophobicity.

pK_a

The binding affinity also correlated with pK_a (not shown), which may reflect a correlation between log PC and pK_a (Table 2). Although Schley & Müller-Oerlinghausen (1986) reported that there was no correlation between association constants and ionization constants, their analysis included not only phenothiazine derivatives but also other tricyclic drugs. Phenothiazine derivatives in solution at pH 7·4 exist as an equilibrium mixture of the free base and the protonated species. The binding may be analysed by considering each molecular species separately using the following equations (Hulshoff & Perrin 1977):

$$DH^+ = D + H^+,\ K_a = [D]\cdot[H^+]/[DH^+] \quad (9)$$

$$D + P = D\,P,\ K_D = [DP]/[D]\cdot[P] \quad (10)$$

$$DH^+ + P = DH^+\,P,\ K_{DH^+} = [DH^+\cdot P]/[DH^+]\cdot[P] \quad (11)$$

where D and DH^+ are the free base and the protonated species, respectively, and P represents the protein (e.g. AGP

or asialoAGP). Binding constants, K_D and K_{DH^+} were calculated according to the following equations:

$$\log K_D = \log nK + \log (K_a + [H^+])/K_a \quad (12)$$

$$\log K_{DH^+} = \log nK + \log (K_a + [H^+])/[H^+] \quad (13)$$

If one molecular species only is bound to protein, it should be correlated to the partition coefficient:

$$\text{AGP: } \log K_D = 0{\cdot}471 \log PC + 6{\cdot}49 \ (n = 12, r = 0{\cdot}35) \quad (14)$$

$$\log K_{DH^+} = 0{\cdot}242 \log PC + 5{\cdot}71 \ (n = 12, r = 0{\cdot}75) \quad (15)$$

$$\text{asialoAGP: } \log K_D = 0{\cdot}184 \log PC + 6{\cdot}98 \ (n = 11, r = 0{\cdot}15) \quad (16)$$

$$\log K_{DH^+} = 0{\cdot}091 \log PC + 5{\cdot}96 \ (n = 11, r = 0{\cdot}49) \quad (17)$$

A good correlation was obtained only with equation 15, suggesting that the protonated species might be responsible for AGP binding.

Effect of van der Waals volume

We attempted to include the V_w parameter in the correlation of nK with PC. However, when attempts were made to combine these terms in equations 6 and 7, no improved correlation was found.

AGP:

$$\log nK = 0{\cdot}160 \log PC - 0{\cdot}75 \times 10^{-4} V_w + 6{\cdot}02 \ (n = 12, r = 0{\cdot}73) \quad (18)$$

asialoAGP:

$$\log nK = 0{\cdot}086 \log PC - 0{\cdot}71 \times 10^{-4} V_w + 5{\cdot}94 \ (n = 11, r = 0{\cdot}81) \quad (19)$$

The analysis suggests that the interaction between phenothiazine derivatives and AGP or asialoAGP is mainly hydrophobic, but for AGP the interaction might be modified slightly by the positive charge of the drugs.

Thermodynamic analysis

Table 5 shows the thermodynamic parameters of three phenothiazines bound to AGP and asialoAGP calculated according to the method of Cho et al (1971). The basis of the selection of these phenothiazines, as representative examples was dependent on the following two factors; (a) different types of groups attached at R_5 and R_{10} (Table 1), and (b) very small variation of s.d. of the binding constant (K), compared with other phenothiazines present in the same sub groups. From Table 5, it can be seen that ΔS° values were positive in all systems, which suggested that hydrophobic interaction played an important role in the binding process, since a positive entropy change is frequently taken as evidence for hydrophobic interaction. However, the values obtained in this work were somewhat different from those reported recently by Aki & Yamamoto (1989). Differences may be due to the different experimental conditions, including techniques and AGP concentration. However, since some other factors may be involved in the interaction of these systems, further investigations are needed into the mechanism of the binding. Interestingly, the contribution of ΔS° to ΔG° for asialoAGP systems was larger than for the AGP systems, suggesting a greater involvement of hydrophobic factors in the asialoAGP systems.

This hypothesis was supported by experiments using circular dichroism spectra of chlorpromazine bound to AGP and asialoAGP in the presence of fatty acids and neutral salt (Fig. 3). The induced circular dichroism intensity of chlorpromazine bound to AGP was decreased by the addition of caesium chloride or oleic acid; for asialoAGP, the observed ellipticity was found to be decreased only on the addition of oleic acid.

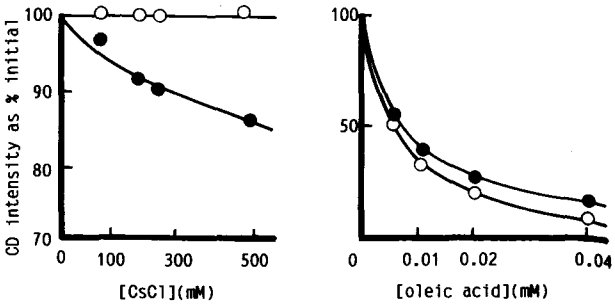


FIG. 3. Effects of caesium chloride (CsCl) and oleic acid on observed ellipticities of chlorpromazine-AGP (●) and asialoAGP (○). AGP and asialoAGP concentration of 10 μM and chlorpromazine concentration of 10 μM were used throughout. The observed ellipticities were measured at 258 nm.

In conclusion, we have shown that the total binding of phenothiazines to AGP is greater than to asialoAGP, and that hydrophobic interaction is the main contributor in both types of binding. The relative contribution of the hydrophobic interaction is higher for phenothiazines bound to asialoAGP than for phenothiazines bound to AGP, where electrostatic interaction also plays a role.

Acknowledgement

This work was supported by a Grant-in-Aid for Scientific Research (No. 02807196) from the Ministry of Education, Science and Culture, Japan.

References

Aki, H., Yamamoto, M. (1989) Thermodynamics of the binding of phenothiazines to human plasma, human serum albumin and α_1-acid glycoprotein: a calorimetric study. J. Pharm. Pharmacol. 41: 674–679

Aubert, J. P., Loucheux-Lefebvre, M. H. (1976) Conformational study of α_1-acid glycoprotein. Arch. Biochem. Biophys. 175: 400–409

Brinkschulte, M., Breyer-Pfaff, U. (1980) The contribution of α_1-acid glycoprotein, lipoproteins, and albumin to the plasma binding of perazine, amitriptyline, and nortriptyline in healthy man. Naunyn Schmiedebergs Arch. Pharmacol. 314: 61–66

Brunner, F., Müller, W. E. (1985) Prazosin binding to human α_1-acid glycoprotein (orosomucoid), human serum albumin, and human serum. Further characterization of the 'single drug binding site' of orosomucoid. J. Pharm. Pharmacol. 37: 305–309

Brunner, F., Müller, W. E. (1987) The stereoselectivity of the 'single drug binding site' of human α_1-acid glycoprotein (orosomucoid). Ibid. 39: 986–990

Cho, M. J., Mitchell, A. G., Pernarowski, M. (1971) Interaction of bishydroxycoumarin with human serum albumin. J. Pharm. Sci. 60: 196–200

El-Gamel, S., Wollert, U., Müller, W. E. (1982) Optical studies on the specific interaction of dipyridamole with α_1-acid glycoprotein (orosomucoid). J. Pharm. Pharmacol. 34: 152–157

El-Gamel, S., Wollent, U., Müller, W. E. (1983) Binding of several phenothiazine neuroleptics to a common binding site of α_1-acid glycoprotein, orosomucoid. J. Pharm. Sci. 72: 202–205

Friedman, M. L., Schlveter, K. T., Kirley, T. L., Halsall, H. B. (1985) Fluorescence quenching of human orosomucoid, accessibility to drugs and small quenching agents. Biochem. J. 232: 863–867

Friedman, M. L., Wermeling, J. R., Halsall, H. B. (1986) The influence of *N*-acetylneuraminic acid on the properties of human orosomucoid. Ibid. 236: 149–153

Fujita, T., Iwasa, J., Hansch, C. (1964) A new substituent constant, π, derived from partition coefficients. J. Am. Chem. Soc. 86: 5175–5180

Helmer, F., Kiehs, K., Hansch, C. (1968) The linear free-energy relationship between partition coefficients and the binding and conformation perturbation of macromolecules by small organic compounds. Biochemistry 7: 2858–2863

Hulshoff, A., Perrin, J. H. (1977) Quantitative correlations between albumin binding constants and chromatographic R_M values of phenothiazine derivatives. J. Med. Chem. 20: 430–439

Kremer, J. M. H., Willting, J., Janssen, J. H. M. (1988) Drug binding to human alpha-1-acid glycoprotein in health and disease. Pharmacol Rev. 40: 1–47

Maruyama, T., Otagiri, M., Takadate, A. (1990) Characterization of drug binding sites on α_1-acid glycoprotein. Chem. Pharm. Bull. 38: 1688–1691

Moriguchi, I., Kanada, Y., Komatsu, K. (1976) Van der Waals volume and the related parameters for hydrophobicity in structure-activity studies. Chem. Pharm. Bull. 24: 1799–1806

Otagiri, M., Maruyama, T., Imai, T., Imamura, Y. (1987) Fluorescence investigation of binding of phenprocoumon to α_1-acid glycoprotein. J. Pharm. Sci. 76: 646–649

Otagiri, M., Yamamichi, R., Maruyama, T., Imai, T., Suenaga, A., Imamura, Y., Kimachi, K. (1990) Drug binding to α_1-acid glycoprotein studied by circular dichroism. Pharm. Res. 6: 156–159

Piafsky, K. M. (1980) Disease-induced change in the plasma binding of basic drugs. Clin. Pharmacokinet. 5: 246–262

Primazic, S., McNamara, P. J. (1985) Effect of the sialylation state of α_1-acid glycoprotein on propranolol binding. J. Pharm. Sci. 74: 473–475

Rosen, A. (1970) The measurement of binding constants using circular dichroism. Biochem. Pharmacol. 19: 2075–2081

Routledge, P. A. (1986) The plasma protein binding of basic drugs. Br. J. Clin. Pharmacol. 22: 499–506

Schley, J., Müller-Oerlinghausen, B. (1986) Investigation of the binding of various tricyclic neuroleptics and antidepressants to α_1-acid glycoprotein. J. Pharm. Pharmacol. 38: 102–106

Urien, S., Albengres, E., Zini, R., Tillment, J. P. (1982) Evidence for binding of certain acidic drugs to α_1-acid glycoprotein. Biochem. Pharmacol. 31: 3687–3689

Warren, L. (1959) The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234: 1971–1975

Weber, G., Young, L. B. (1964) Fragmentation of bovine serum albumin by pepsin. Ibid. 239: 1415–1423

Whelpton, R. (1989) Ionization constants, octanol partition coefficients and cholinesterase inhibitor constants for chlorpromazine and its metabolites. J. Pharm. Pharmacol. 41: 856–858