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BINDING OF PSYCHOTROPIC DRUGS TO ISOLATED α_1-ACID GLYCOPROTEIN*

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Abstract—Alpha$_1$-acid glycoprotein (α_1-AG) was purified from human sera, and its binding properties with respect to psychotropic drugs were examined by equilibrium dialysis methods in order to clarify the specificity of binding. Radioactive imipramine, a tricyclic antidepressant, was used as the primary ligand. Other drugs, representative of different classes, were tested as potential inhibitors of the α_1-AG–imipramine binding. The K_a for imipramine was 2.8×10^5 ($\pm$ 0.8) M^{-1} (mean $\pm$ S.D.). Chlorpromazine, fluphenazine, thioridazine, loxapine and thiothixene, which are antipsychotic drugs, were competitive inhibitors of imipramine binding, and their K_a values were in the same range. Propranolol, haloperidol and diazepam were also competitive inhibitors but their affinities were lower. Molindone, an indolic antipsychotic, when tested at the same concentrations as the other drugs, did not affect imipramine binding. Trihexyphenidyl, an anti-Parkinson drug, was a potent but noncompetitive inhibitor. These data identify the antidepressant and major tranquilizer drugs that exhibit high affinity for α_1-AG and indicate that α_1-AG may account for 40 per cent of total imipramine bound in serum. Since in psychiatric clinical practice two drugs are frequently administered together, possible competitive effects are discussed as well as the potential role of α_1-AG in psychiatric illness.

In recent years it has been recognized that, in addition to albumin, the serum protein α_1-acid glycoprotein (α_1-AG)‡ plays a significant role in the binding of drugs. Whereas albumin binds a wide range of neutral and acidic compounds, α_1-AG appears to be more specific for compounds that are cationic at physiologic pH [1]. Many of the clinically used psychotropic drugs are more than 90 per cent serum bound [2] and are also cationic; the role of α_1-AG, therefore, is of particular interest to the pharmacodynamics of these drugs. Several studies have established that there exists a significant inverse relationship between α_1-AG levels and the free portion of the following drugs: alprenolol, propranolol, imipramine and chlorpromazine [1, 3]. Because α_1-AG is not readily available commercially, few studies have dealt directly with the binding of the drugs to the purified protein. Borga *et al.* [4], however, have reported that alprenolol and imipramine bind to purified α_1-AG and that this binding is inhibited by Tris(2-butoxyethyl) phosphate, a plasticizer present in Vacutainers.

Alpha$_1$-AG is one of the acute phase proteins whose serum levels increase markedly in inflammation, infection [5] and cancer [6]. If this protein fluctuates markedly during psychiatric illness and if, indeed, it binds psychotropic drugs avidly, then it may be a significant factor in regulating the total plasma levels of certain drugs. To understand better which drugs bind the most to α_1-AG and, thus, would be most likely affected by protein changes, we have isolated α_1-AG from human serum and carried out binding studies with the purified protein. In these studies radioactive imipramine was used as the primary ligand, and other nonlabeled drugs, representative of phenothiazine, butyrophenone, dibenzoxazepine, thioxanthine and indolic classes, were used as potential inhibitors. The data were used to calculate the association constant for the interaction, the number of binding sites, and the type of inhibition. This report presents and discusses the data derived from this study.

MATERIALS AND METHODS

Materials. Imipramine[^{14}C] (sp. act. 10.5 mCi/mmole) was purchased from the Amersham Corp., Arlington Heights, IL, and unlabeled imipramine HCl and chlorpromazine from the Sigma Chemical Co., St. Louis, MO. The following unlabeled drugs were provided by the suppliers indicated: diazepam (Valium), Hoffman–La Roche, Nutley, NJ; haloperidol (Haldol), McNeil Laboratory Inc., Fort Washington, PA; molindone (Lidone), Abbott Laboratories, North Chicago, IL; loxapine (Loxitane) and trihexyphenidyl HCl (Artane), Lederle Laboratories, Pearl River, NY; propranolol (Inderal), Ayerst Laboratories, New York, NY; thiothixene (Navane), Chas. Pfizer & Co., New York, NY; fluphenazine HCl (Permitil), Schering Corp., Kenilworth, NJ; and thioridazine HCl (Mellaril), Sandoz Pharmaceuticals, East Hanover, NJ. Proteins were obtained from the following sources: human serum albumin, crystallized and lyophilized

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‡ Abbreviations: α_1-AG, α_1-acid glycoprotein; RID, radial immunodiffusion; and α_1-AT, α_1-antitrypsin.

(Lot No. 126C-8070), and α_1-antitrypsin from human plasma (Lot No. 39C-0096), Sigma Chemical Co.; and Pentex human glycoprotein, Fraction VI, Miles Laboratories, Inc. Elkhart, IN. Charcoal (Carbon Decolorizing Neutral Norit) was purchased from the Fisher Scientific Co., Springfield, NJ, and Radial Immunodiffusion (RID) plates and standards (M-Partigen Kits), one specific for α_1-AG and the other specific for albumin, from CalBiochem-Behring, LaJolla, CA.

Purification of α_1-AG. One unit of fresh human blood (citrate phosphate dextrose anticoagulant) was obtained from a patient with hemochromatosis. After centrifugation at 1000 *g* for 20 min, 265 ml plasma was obtained. Purification of α_1-AG was carried out according to the general method of Hardwicke and de Vaux St. Cyr [7]. Since the original procedure was described using urine as the starting material, several modifications were introduced to adapt it for plasma and for larger amounts of material. The modified procedure is presented below.

The pH of the fresh plasma was adjusted to 4.0 with 6 N HCl. The suspension was stirred for 15 min and the precipitate was removed by centrifugation at 20,000 *g* for 15 min. This procedure, as well as the subsequent purification steps, was carried out at 5° unless indicated otherwise. To the supernatant solution solid ammonium sulfate was gradually added to 45% saturation (277 g/l). The suspension was stirred for an additional 30 min and centrifuged at 20,000 *g* for 15 min. The supernatant solution containing α_1-AG was saved. The precipitate was resuspended in 100 ml of 45% ammonium sulfate and recentrifuged. Both supernatant solutions were combined and dialyzed against 4 liters of 0.02 M sodium acetate buffer, pH 5.8, for 24 hr. The dialysate was changed four times during this time period. The dialyzed solution was concentrated to 41 ml by filtration through Amicon PM 10 membrane (Amicon Corp., Lexington, MA), 76 mm diameter, under nitrogen pressure of 20 psi at 25°.

Thirteen milliliters of the concentrated solution, representing 84 ml of plasma, was applied to a column (2.5 × 35 cm) of DEAE-Sephadex (A-50; Pharmacia, Piscataway, NJ) that had been equilibrated with 0.02 sodium acetate buffer, pH 5.8. The column was eluted with a linear gradient of NaCl and decreasing pH; the mixing vessel contained 300 ml of 0.02 M sodium acetate buffer, pH 5.8, and the reservoir contained 300 ml of 0.1 M sodium acetate–0.4 M NaCl, pH 4.0. The flow rate was 70 ml/hr and the fraction volume was 2.2 ml. The protein content of the effluent fractions was determined by absorbance at 280 nm, and sodium concentration was assayed by flame photometry (model 343, Instrumentation Laboratory, Lexington, MA). Three protein peaks were eluted from the column. The first two, A_1 and A_2, were only partially resolved, while the third, peak B (α_1-AG), emerged separately. Fractions constituting peaks A (both A_1 and A_2) and B were pooled, dialyzed extensively against water, and lyophilized. The protein preparations were stored at −20° until further analyses. The yield of α_1-AG was 48 mg. The remaining 28 ml of the concentrated serum supernatant solution, which had been stored at −20°, was chromatographed on two separate columns. An additional 88 mg of α_1-AG was obtained, thus resulting in a total yield of 136 mg from 265 ml of fresh plasma.

Disc gel electrophoresis. Analytical disc gel electrophoresis at pH 8.7 was carried out according to the general procedure of Davis [8]. The proteins were dissolved in 40% sucrose, and 7% polyacrylamide was used. Electrophoresis at pH 4.5 was carried out according to the procedure of Reisfeld *et al.* [9]. The gels were stained with Coomassie blue and destained as described by Weber and Osborn [10].

Charcoal treatment of proteins. This procedure was carried out according to the method of Evenson and Deutsch [11], with the exceptions that protein concentration was 0.1% and 6 N HCl was used for pH adjustment. As determined by the assay of Lowry *et al.* [12] there was approximately a 10 per cent protein loss after charcoal treatment. No correction for this loss was made in the calculations.

Equilibrium dialysis. Dialysis was carried out in an Equilibrium Dialyzer (Spectrum, Los Angeles, CA) equipped with Teflon cells. Spectrapor 1 membranes (Spectrum; exclusion limit 12,000–14,000) were sandwiched between the two halves of each cell after they had been soaked for 20 min in each of the following solvents: distilled-deionized water, 30% ethanol, running distilled-deionized water, and 0.1 M sodium phosphate buffer, pH 7.4. The same buffer was used to dissolve drugs and proteins. The cells were assembled and placed in the carrier. Imipramine was pipetted into one-half of each cell and protein solution, 0.5 mg/ml, into the other half. The volume of each compartment was 1 ml. The final concentration of imipramine was in the range of 2–15 μM, and it was made up of [^{14}C]imipramine and additional nonlabeled imipramine to achieve the desired concentration. Each drug concentration was run in duplicate. Other unlabeled drugs, tested as binding inhibitors, were included in the imipramine compartment at the start of the experiment. The cell carrier was placed in the drive unit and rotated (setting at 15) for 4.5 hr at 25°. In control experiments, it was ascertained that this time period was sufficient for imipramine to equilibrate across the dialysis membrane. The contents of each cell half were drained into 4-ml polystyrene tubes and a 0.1 ml aliquot was used for the assay of radioactivity.

The free imipramine concentration was calculated by dividing the amount of radioactivity present on the non-protein side of the cell by the specific activity of imipramine. The amount of bound imipramine was obtained by subtracting the free from the total drug present on the protein side of the cell. The reciprocal $1/D$ of free imipramine concentration, D, expressed in μM, was plotted on the x-axis against the corresponding reciprocal value, $1/r$, of bound imipramine, r (μM imipramine bound per μM protein), on the y-axis. The K_a was calculated from the following expression for a straight line:

$$\frac{1}{r} = \frac{1}{N K_a D} + \frac{1}{N} \qquad [13]$$

In this expression N is the number of binding sites,

$\frac{1}{N}$ is the y-intercept, and $\frac{1}{N K_a}$ is the slope of the plot. The dissociation constant (K_d) of an unlabeled inhibitor was calculated from the following expression: $m_1 = m(1 + \frac{z}{K_z})$ [14]. In this expression m_1 is the slope of the imipramine plot in the presence of the inhibitor, m is the slope without the inhibitor, z is the concentration of the inhibitor used in the experiment, and K_z is the K_d of the inhibitor. These calculations are based on the slope increase in the presence of the inhibitor and do not take into account the small changes in the y-intercept that were observed. The molecular weight of α_1-AG was taken as 40,000 and that of human serum albumin as 66,300 [15].

Assay of radioactivity. To 0.1 ml of radioactive solution in a polypropylene minivial, 4 ml of Aquasol (New England Nuclear Corp. Boston, MA) was added. Radioactivity was assayed in an Isocap/300 (Searle Analytic, Chicago, IL) liquid scintillation spectrometer. Counts per minute were corrected to disintegrations per minute by the external standard ratio technique.

RESULTS

Purification of α_1-AG. Purification of α_1-AG from human serum was achieved by precipitation with ammonium sulfate at pH 4.0 and chromatography of the supernatant solution on a column of DEAE-Sephadex (A-50). Alpha$_1$-AG (peak B) was obtained as the last protein peak as the column was eluted with a linear gradient of NaCl (Fig. 1); it emerged in tubes 180–200, at approximately 0.2 M sodium ion concentration. The identity and purity of α_1-AG were established on the basis of the following experiments. The material obtained from peak B formed a precipitin ring on RID plates monospecific for α_1-AG. It migrated as a single band on disc gel electrophoresis at pH 8.7 (Fig. 2). In this system it migrated faster than human albumin toward the anode, consistent with its lower isoelectric point of 2.7 [16]. A single band was also obtained on disc gels at pH 4.5; at this pH, α_1-AG still migrated toward the anode, in contrast to albumin and other serum proteins that had acquired a positive charge. In both electrophoretic systems, the isolated protein migrated just like the main component of Pentex Fraction VI, which contains predominantly α_1-AG.

Since α_1-AG was preceded by albumin in the elution profile, high concentrations (10 mg/ml) of the isolated protein were also analyzed for the presence of albumin by the RID technique. It was found that not more than 2.5 per cent of albumin was present and therefore the α_1-AG preparation was judged to be sufficiently pure for binding experiments. No other contaminating serum proteins were detected by immunoelectrophoresis.

Drug binding to α_1-AG. The binding of [^{14}C]imipramine to α_1-AG was examined by equilibrium dialysis methods. Before use the protein was defatted by treatment with charcoal at pH 3.0. This was done to achieve a more homogenous protein preparation. Previous reports have indicated that isolated proteins may contain various amounts of fatty acids depending upon the method of purification [11]. The binding data, plotted as a double reciprocal plot, gave a straight line with a slope of 1.9 ± 0.4 (mean ± S.D.; five experiments; Fig. 3). The K_a for imipramine was calculated to be $2.8 (\pm 0.7) \times 10^5\ M^{-1}$. The y-intercept was 1.9 ± 0.4 (reciprocal of the number of binding sites), indicating that, maximally, 0.5 moles of imipramine was bound per 1 mole of the protein. These data were derived from experiments in which 2–15 μM imipramine was used. At higher concentrations of the drug (25–80 μM) the plot assumed a downward curve indicating the generation of additional binding sites. These binding data were reproducible with α_1-AG samples that had been stored at −20° for up to 4 months, and with samples derived from different batches of fresh plasma from the same individual. If the plasma was stored for several months at −20°

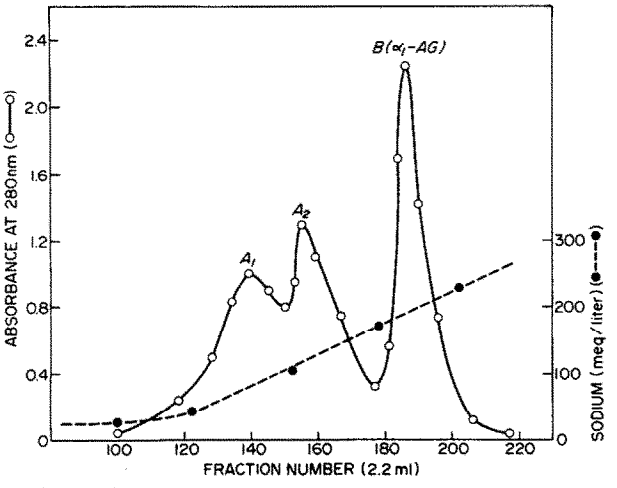

Fig. 1. Purification of α_1-acid glycoprotein by chromatography on a column (2.5 × 35 cm) of DEAE-Sephadex (A-50). The supernatant solution, obtained after fresh human serum was precipitated with ammonium sulfate at pH 4, was concentrated and applied to the column. Elution was carried out with a gradient of NaCl in sodium acetate buffer (see Materials and Methods for further details).

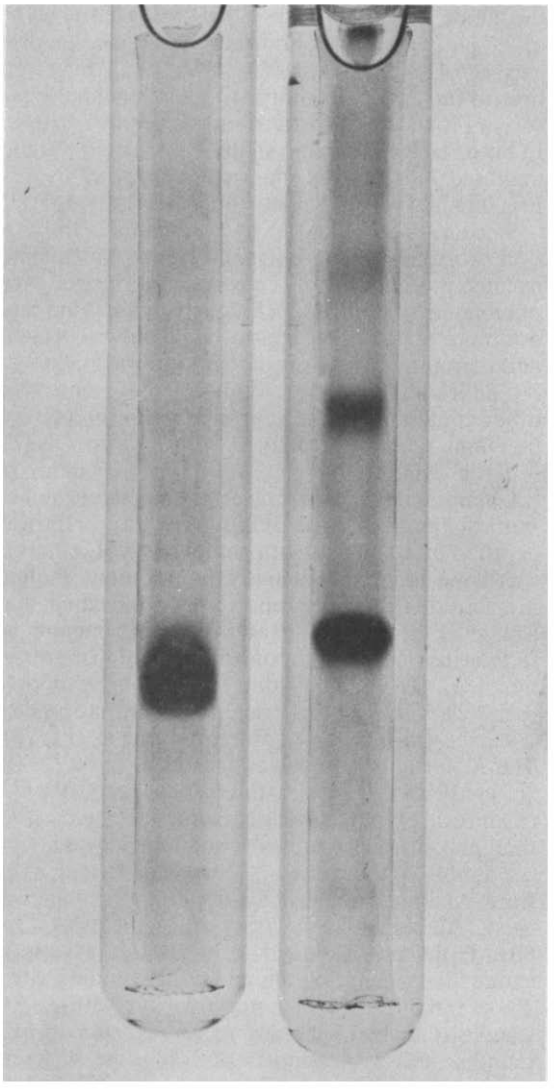

Fig. 2. Disc gel electrophoresis of purified α_1-acid glycoprotein (left) and commercial human serum albumin (right). Electrophoresis was carried out on 7% polyacrylamide gels at pH 8.7 and the proteins were stained with Coomassie blue. The anode was at the bottom and the cathode was at the top.

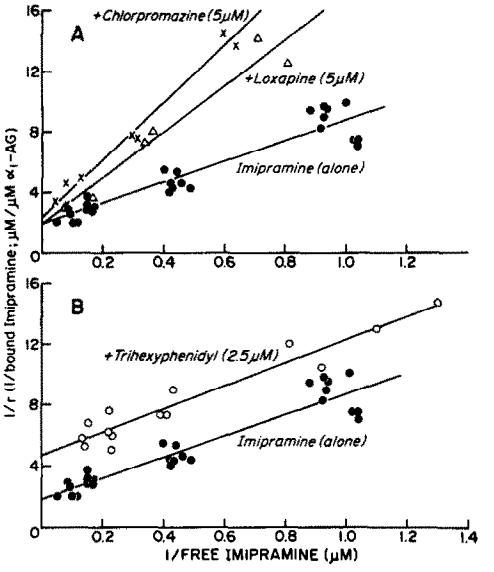

Fig. 3. Double reciprocal plot of [^{14}C]imipramine binding to α_1-acid glycoprotein in the absence and presence of inhibitors. Binding was measured by equilibrium dialysis methods. One side of the dialysis cell contained [^{14}C]imipramine and, if indicated, an unlabeled inhibitor in 1 ml of 0.1 M sodium phosphate buffer, pH 7.4; the other side contained α_1-AG, 0.5 mg/ml, in the same buffer. Panel A: imipramine binding in the absence of an inhibitor (●——●) ($m = 6.8 \pm 0.4$; $b = 1.9 \pm 0.4$; five experiments); in the presence of 5 μM loxapine (△——△) ($m = 15.1$; $b = 1.9$); and in the presence of 5 μM chlorpromazine (×——×) ($m = 18.6$; $b = 2.4$). Panel B: imipramine binding in the absence of an inhibitor (●——●); and in the presence of 2.5 μM trihexyphenidyl (○——○) ($m = 7.6$; $b = 4.7$).

before it was subjected to the purification procedure, it yielded α_1-AG with a lower affinity for imipramine than obtained from fresh plasma.

Several drugs markedly decreased imipramine binding to α_1-AG. As illustrated by chlorpromazine and loxapine (Fig. 3A), the double reciprocal plots in the presence of these drugs had markedly greater slopes while the y-intercepts were the same as that of the original imipramine plot. This pattern is characteristic of competitive inhibition and is observed when the inhibitor and the original ligand compete for the same binding site. Competitive inhibition was also observed with the following drugs, used at the indicated concentrations: fluphenazine, 10 μM; thioridazine, 5 μM; thiothixene, 2.5 μM; haloperidol, 25 μM; diazepam, 15 μM; and propranolol, 15 μM. The concentration used in the experiment depended on the effectiveness of the compound as a binding inhibitor. The y-intercepts for these plots ranged between 1.3 and 2.7, as compared with the value of 1.9 ± 0.4 obtained for imipramine without inhibitors. Molindone, an indolic compound, did not affect imipramine binding at a concentration of 30 μM. Trihexyphenidyl, an anti-Parkinson drug, decreased imipramine binding, but its pattern of inhibition was different from that observed with the other drugs (Fig. 3B). In this case the y-intercept was markedly increased, while the slope remained virtually unchanged. This type of graph is characteristic of non-competitive inhibition.

The K_a values for α_1-AG and each of the drugs were calculated on the basis of their inhibitory activities (Table 1). The highest binding affinity was exhibited by the three phenothiazine drugs and by imipramine, loxapine and thiothixene. Expressed as the more commonly used K_d values, denoting drug concentration at which protein binding sites are half-saturated, they were in the range of 1.2 to 4.2 μM. Propranolol, haloperidol and diazepam had lower affinities for α_1-AG and their K_d values were in the range of 10–16 μM. The data for trihexyphenidyl, calculated on the basis of its increased y-intercept, indicated a K_d of 2 μM.

Table 1. Psychotropic drug binding to α_1-acid glycoprotein*

Drug category	Drug name	$K_a \times 10^5\ M^{-1}$	$K_d \times 10^{-6}$ M
Antidepressant	Imipramine	2.8	3.6
Antipsychotic			
Phenothiazine			
Aliphatic side chain	Chlorpromazine	3.4	2.9
Piperazine side chain	Fluphenazine	2.6	3.9
Piperadine side chain	Thioridazine	8.0	1.2
Dibenzoxazepine	Loxapine	2.4	4.1
Thioxanthine	Thiothixene	2.4	4.2
Butyrophenone	Haloperidol	0.6	16.0
Benzodiazepine	Diazepam	0.4	28.0
Beta-adrenergic antagonist	Propranolol	1.0	9.8

* Imipramine binding was measured by equilibrium dialysis methods. The other drugs were competitive inhibitors of imipramine and their binding constants were calculated from the inhibition data. For further details see Materials and Methods.

Imipramine binding to other serum proteins. In addition to α_1-AG, the binding properties of serum albumin and α_1-antitrypsin (α_1-AT) were examined, to enable comparison with those of α_1-AG. These two proteins were of particular interest because albumin is known to be an avid binder of a great variety of drugs, and α_1-AT is a protein similar in several characteristics to α_1-AG. Both α_1-AT and α_1-AG contain large amounts of carbohydrate, have low isoelectric points, are classified as α_1-globulins, and increase in certain disease states and stress [17]. In initial experiments, it was found that a commercial sample of α_1-AT bound imipramine and diazepam to an appreciable extent. This activity was found to be due to the presence of albumin, and it was virtually eliminated after albumin was removed by passage through a column of Affi-Gel Blue (Bio-Rad Laboratories, Richmond, CA). From RID assays it was estimated that the commercial α_1-AT preparation contained as much as 40% albumin. The binding of imipramine and diazepam by the albumin-free α_1-AT was judged to be physiologically insignificant.

Imipramine binding to albumin was examined with and without prior treatment of the protein with charcoal. Experiments with charcoal-treated albumin yielded scattered binding data from which an adequate binding plot could not be constructed. Consistent data were obtained with non-treated albumin, from which it was calculated that K_a was $1.1 \times 10^5\ M^{-1}$ and N was 0.2. To enable comparison with α_1-AG, imipramine binding to non-treated α_1-AG was determined. The K_a for this interaction was $1.5 \times 10^5\ M^{-1}$ with 0.5 binding sites. These data indicate that α_1-AG was more effective than albumin as a binder of imipramine. They also show that charcoal treatment of α_1-AG enhances its affinity for imipramine, whereas the same treatment of albumin impaired its ability to bind imipramine.

DISCUSSION

In the present study, α_1-AG was isolated from human serum and its binding properties with respect to several psychotropic drugs were examined. Imipramine, a tricyclic antidepressant, used as the primary ligand, was found to have a high affinity for α_1-AG. Its K_a was $2.8 \times 10^5\ M^{-1}$ for the charcoal-treated protein and $1.5 \times 10^5\ M^{-1}$ for the non-treated protein. In both cases the number of binding sites was 0.5 per mole of protein. To evaluate the relative significance of α_1-AG binding compared to that of albumin, the data for both proteins were examined. The K_a of albumin was $1.1 \times 10^5\ M^{-1}$ with 0.2 binding sites, but its molar concentration in normal serum is approximately 30-fold higher than that of α_1-AG. The total binding capacity of each protein was calculated from the following expression [18]: per cent drug bound $= \dfrac{100 \times NP}{NP + 1/K_a + D}$, where N is the number of binding sites and P is the total protein concentration in moles. The term D, which denotes free drug concentration, has a small effect because an excess of protein is present. A value of 0.1×10^{-6} M was used for imipramine as it represents one-tenth of the total drug concentration reported in serum [19]. The calculations indicated that α_1-AG was capable of binding 55–69 per cent of serum imipramine, depending upon which K_a value was used. The total albumin capacity was 92 per cent. Thus, considering solely these two binding proteins, it might be expected that α_1-AG accounts for approximately 40 per cent, and albumin for 60 per cent, of serum-bound imipramine. The bound drug distribution would also be affected by the presence of other hematologic binding sites, by other ligands, and by the actual protein concentration present in serum. The relative importance of α_1-AG may be enhanced by the fact that it has a higher ligand specificity than albumin, and that its serum levels increase markedly in stress situations. The normal α_1-AG plasma concentration of 66 mg/dl [3] has been reported to double in certain diseases [5, 6].

Other drugs, which exhibited high affinity for α_1-AG and markedly inhibited imipramine binding, were chlorpromazine, fluphenazine, thioridazine, loxapine, thiothixene and trihexyphenidyl. The last drug, an anti-Parkinson agent, was a non-competitive inhibitor in the system, whereas all the other drugs were competitive inhibitors. Within the phenothiazine group, thioridazine had the highest K_a. This indicates that the binding was influenced to some extent by the substituents on the phenothiazine

nucleus. The K_d values for these compounds were 1.2 to 4.2 μM. After administration to patients, the serum level of chlorpromazine reaches 64–320 ng/ml or 0.2 to 1.0 μM [20], imipramine, 300 ng/ml or 1 μM [19], and thioridazine, 3.9 μg/ml or 12 μM [21]. The fact that the K_d range is close to the serum concentration emphasizes the potential physiologic significance of α_1-AG as a drug binder. The extent of chlorpromazine binding, like that of imipramine, has been found to have a positive correlation with serum α_1-AG levels [1, 3]. In addition, chlorpromazine has been reported to bind to albumin and to plasma lipoproteins [22, 23].

Propranolol, haloperidol and diazepam had lower affinities for α_1-AG than the phenothiazine compounds. The K_a for diazepam was determined to be $0.4 \times 10^5\,M^{-1}$. This value is considerably less than that reported for albumin, $2.1 \times 10^5\,M^{-1}$ [24]. This difference supports previous conclusions that albumin is the primary plasma binder of diazepam. Molindone, an indolic antipsychotic drug, was not active as an inhibitor of imipramine.

Clinical situations frequently involve the concurrent administration of two drugs. A recent Veterans Administration Hospital survey shows that of hospitalized psychiatric patients receiving one major tranquilizer, 28 per cent also had orders for an anti-Parkinson drug, 13 per cent for a second major tranquilizer, and 11 per cent for an antidepressant drug [25]. As demonstrated in the present study, the presence of two competing drugs may lead to the displacement of the lesser bound drug from its protein-binding site. The displaced drug, depending upon its properties, may penetrate into other body compartments or it may be redistributed among other hematologic binding sites. In addition to serum proteins, red cell membranes [22, 23] and platelets [26] have been reported to bind certain psychotropic drugs. A specific clinical situation in which the effect of one drug on the plasma level of another has been examined involves the concurrent administration of trihexyphenidyl and chlorpromazine. A study by Kolakowska *et al.* [27] reported that in chronic schizophrenic patients plasma chlorpromazine levels rose after the administration of trihexyphenidyl. Other studies contradict these results. Reports by Rivera-Calimlim *et al.* [20, 28] indicate that plasma chlorpromazine levels decrease in the presence of trihexyphenidyl. This effect was explained on the basis of the trihexyphenidyl anticholinergic property of delaying gastric emptying with a concomitant decrease in chlorpromazine absorption. An alternative explanation for a diminished plasma chlorpromazine concentration during concurrent trihexyphenidyl treatment is suggested by our data. If trihexyphenidyl displaces chlorpromazine from serum binding sites, it may hasten its efflux from the circulation and its adsorption by tissues, thus causing lower plasma chlorpromazine levels. In our experiments, trihexyphenidyl markedly displaced imipramine and showed high affinity for α_1-AG. Its noncompetitive relationship to imipramine, and by analogy to chlorpromazine, indicates that it probably exerts its effect via separate binding sites.

Numerous studies have documented the increase of α_1-AG, along with other acute phase proteins, in a variety of pathological conditions. It is less clear whether it changes in patients with psychiatric illness. One study, reporting no difference in α_1-AG levels in schizophrenics as compared with normals, identifies neither the phase of illness nor the diagnostic criteria applied for the selection of patients [29]. Another study, by Bertilsson *et al.* [30] indicates that serum α_1-AG levels of depressed patients treated for 3 weeks with an antidepressant are in the same range as those of normals. These data are based on a single blood sample from each patient. It is well-known that the dose of antipsychotic and antidepressant medication used in the treatment of acute psychiatric illness is generally twice as high as that needed for maintenance [31]. It is attractive to speculate that if α_1-AG transiently increases during the "acute" phase of a psychiatric illness, it may account for the difference in drug dosage. This hypothesis remains to be examined by further experimentation.

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