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Investigation of the binding of various tricyclic neuroleptics and antidepressants to α_1-acid glycoprotein†

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Recent studies have shown that the interaction of various tricyclic neuroleptics and antidepressants with isolated α_1-acid glycoprotein occurs at one common binding site and with relatively high association constants. The aim of the present study was to find differences in the binding of some phenothiazines, thioxanthenes, and several other drugs reported to bind α_1-AGP. The findings suggest that the affinity of the phenothiazines and thioxanthenes depends primarily on the existence of the tricyclic skeleton and is generally increased by the basic side chain. Substituents at position 3 of the phenothiazine nucleus influence the affinity in a variable way. The losses of radioactivity by non-specific absorptions to the dialysing chambers were considered for the calculation of the association constants. No correlation between association constants and the antipsychotic potency of neuroleptic drugs could be detected.

During the course of investigations on the different response of psychiatric patients to psychotropic drugs, the binding of such drugs to serum proteins has been examined, and its possible clinical relevance discussed (Casper et al 1980; Freedberg et al 1979; Gram 1980; Kragh-Sørensen 1980; Preskorn & Madakasiva 1980). As with other drugs, most studies on the tricyclics have concerned their binding to albumin. The association constants for most tricyclics with albumin are around 10^3 M^{-1}, most measurements having been conducted with dilute albumin solutions (for ref. see Vallner 1977). Some tricyclics were found to bind to α_1-acid glycoprotein (α_1-AGP) with a binding constant of 10^5 M^{-1} (Kornguth et al 1981; Schley & Müller-Oerlinghausen 1983). In this context disease-related variations of the α_1-AGP concentration in human serum have been observed (Piafsky et al 1978; Piafsky 1980; Routledge et al 1981).

In schizophrenic patients treated with perazine, a significant negative correlation was found between the free fraction of perazine and the serum concentration of α_1-AGP, a finding which is in contrast to results of corresponding experiments with albumin (Brinkschulte et al 1982; Schley et al 1980). The purpose of the studies now described was to determine to what extent the binding constants of antidepressants and neuroleptics with α_1-AGP vary and whether a correlation exists between the antipsychotic potency of neuroleptics, and their capacity to bind α_1-AGP. In addition, the nature of the interaction of the many drugs recently reported to bind to α_1-AGP (Piafsky & Borgå 1977; Romach et al 1981; Wood & Wood 1981) appears to be of interest in this context.

† Dedicated to Prof. Dr H. Coper (Head), Institute of Neuropharmacology, Free University of Berlin, on the occasion of his 60th birthday.

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MATERIALS AND METHODS

Materials

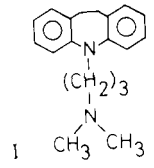
α_1-Acid glycoprotein was obtained from Behring-Werke (Marburg, FRG) (electrophoretic purity 99%). The synthesis of [^{3}H]perazine has been described previously (Schley et al 1979). Radiochemical purity of [^{3}H]perazine was checked by TLC according to Breyer & Villumsen (1976) and was found to be 99%. [^{3}H]Imipramine (20 Ci $mmol^{-1}$) was delivered by Amersham (Braunschweig, FRG). Unlabelled perazine was donated by Promonta (Hamburg, FRG). All other drugs were also generous gifts of the manufacturers.

Methods

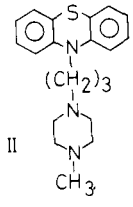
The binding of the investigated compounds to α_1-AGP was measured by means of equilibrium dialysis, using a Dianorm apparatus with micro-Teflon chambers (2 × 200 µl; Bachofer, Reutlingen, FRG). The chambers were separated by Visking cellulose membranes (Serva, FRG). For the displacement experiments, three concentrations of [^{3}H]perazine ($0{\cdot}5 - 2{\cdot}5 \times 10^{-6}$ M) were combined

with four concentrations of the displacers ($0 - 12 \times 10^{-5}$ M).

In another part of the study, presented in Table 2, displacement of [^{3}H]perazine (I) and [^{3}H]imipramine



I



II

(II) by several drugs reported to be bound to α_1-AGP was also determined. In the latter experiments, the concentrations of the radiolabelled drugs were $5{\cdot}0 \times 10^{-6}$ M, the concentration of the unlabelled displacers 25×10^{-6} M. In all experiments α_1-AGP was used in a concentration of $1{\cdot}75 \times 10^{-5}$ M. The assays were incubated in test-tubes at room temperature. Ethanolic solutions of the [^{3}H]perazine and the displacers were pipetted into the tubes, evaporated under N_2 stream to approx. 1% of the initial volume, and redissolved with buffer solution consisting of 66 mmol litre^{-1} sodium phosphate in 50 mmol litre^{-1} sodium chloride, the pH being adjusted to 7·4. The sample was then transferred into one chamber of the Dianorm apparatus. The other chamber contained buffer solution. A dialysing time of 6 h for 1 ml cells of this apparatus was used by Brinkschulte and Breyer-Pfaff (1979). However, for the microchambers used in the present study a shorter time for dialysis was sufficient due to the greater dialysing surface in relation to the volume. A dialysing time of 4 h with 12 rev min^{-1} proved to be adequate for achieving a stable equilibrium. The radioactivity was determined by liquid scintillation spectrometry with a LS 7000 scintillation counter (Beckman). 5 ml Ready-Solv MP (Beckman) were added to 150 µl of both the protein or the buffer-containing sides of the dialysis cells as well as to an equal amount of the incubated solution before dialysis to quantify the losses during the dialyses. Balance studies disclosed that about 10% of the radioactivity disappeared during the experiments; this was due to drug adsorption, mostly to the Teflon material and to a smaller extent to the membrane (Schley 1983).

For the quantification of the adsorption to the Teflon chambers, the recovery of [^{3}H]perazine in the range $0{\cdot}5$–$5{\cdot}0 \times 10^{-6}$ was calculated as follows: incubated [^{3}H]perazine/α_1-AGPmixtures were dialysed against α_1-AGP (assay A), and [^{3}H]perazine/buffer against buffer solutions (assay B). The different recoveries of radioactivity (assay A: 95 ± 1%, assay B: 75 ± 11%; n = 12) by unspecific adsorption were considered in the calculation of the association constants. The total losses assessed for each incubation assay were equated with the summed up losses in assays A and B (30%), and therefore distributed by the ratio 17:83 to the protein and buffer side, respectively.

Association constants were calculated according to Wombacher & Körber (1972) in analogy to a Dixon plot. All assays were performed in duplicate, sometimes in triplicate. The intra-assay difference between the individual association constants thus obtained never exceeded 2·8% of the mean value.

RESULTS

Fig. 1 shows a typical example of our findings obtained with the displacement method used in this study and also illustrates the usual small scattering of the results. The association constants of various drugs, particularly neuroleptics, are presented in

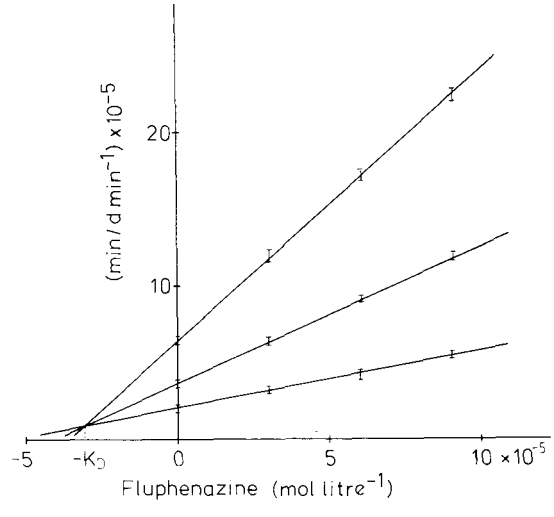


FIG. 1. Determination of the association constant of fluphenazine ($3{\cdot}2 \times 10^4$ M^{-1}) by displacement of [^{3}H]perazine from the α_1-AGP binding site using three different concentrations of [^{3}H]perazine (2·13; 4·26; 10·65 µmol litre^{-1}). All assessments performed in duplicate. The vertical bars represent the lower and upper value.

Table 1. Association constants of different tricyclics with α_1-acid glycoprotein.

Phenothiazine derivatives	
A. Alkylamines	K[M^{-1}]
Promazine	$3{\cdot}8 \times 10^4$
Chlorpromazine	$1{\cdot}1 \times 10^5$
Laevomepromazine	$0{\cdot}9 \times 10^5$
B. Piperazines	
Perazine	$3{\cdot}8 \times 10^5$
Desmethylperazine	$1{\cdot}5 \times 10^5$
Butaperazine	$2{\cdot}7 \times 10^5$
Trifluoperazine	$4{\cdot}4 \times 10^4$
Perphenazine	$3{\cdot}3 \times 10^4$
Fluphenazine	$3{\cdot}2 \times 10^4$
Thioxanthene derivatives	
A. Alkylamines	K[M^{-1}]
Chlorprothixene	$2{\cdot}6 \times 10^4$
B. Piperazines	
Clopenthixole	$3{\cdot}7 \times 10^4$
Flupenthixole	$2{\cdot}8 \times 10^4$
Other tricyclics	
Imipramine	$2{\cdot}2 \times 10^5$
Amitriptyline	$2{\cdot}0 \times 10^5$
Dibenzepine	$2{\cdot}9 \times 10^4$
Clozapine	$2{\cdot}3 \times 10^4$
Dimethacrine	$2{\cdot}0 \times 10^4$

Table 1. Differences in affinities amount to about one order of magnitude. However, no relationship seems to exist between the association constants and the known antipsychotic activity of individual drugs, e.g. fluphenazine or perphenazine show a considerably lower association constant than chlorpromazine or levomepromazine, although they possess a definitely stronger antipsychotic potency (Schmutz & Picard 1980; Bürki et al 1983).

In Table 2, drugs are listed which could be shown to possess the same binding site as the displaced drug [^{3}H]perazine. For most of the drugs reported in the literature to be bound to α_1-AGP, we determined the extent to which they could displace either [^{3}H]perazine or [^{3}H]imipramine from its binding to α_1-AGP. The data presented in Table 2 show the increase of the free fraction of the two radiolabelled drugs after addition of the unlabelled displacers. The data indicate that these drugs bind to a common binding site with variable affinities.

Fig. 2 illustrates that no correlation exists between the association constants of the investigated compounds and their ionization constants as has been reported in the literature.

Table 2. Displacement of [^{3}H]perazine and [^{3}H]imipramine (5·0 μmol litre^{-1}) by several drugs (final concentration 25 μmol litre^{-1}) reported in the literature to be bound to α_1-AGP ($1{\cdot}75 \times 10^{-5}$ M) expressed as the increase of the free fraction ×100 of the two radioactively labelled compounds (mean of experiments in triplicate). The free fraction ×100 of the two marker ligands were: [^{3}H]perazine = 10·1 ± 0·9%; [^{3}H]imipramine = 37·9 ± 0·8%.

Displacing drug	Increase of free fraction × 100 [^{3}H]Perazine	[^{3}H]Imipramine
Dipyridamole	42	45
Perazine	36	42
Warfarin	34	39
(±)-Propranolol	29	38
Aprinidine	26	37
Quinidine	24	34
Imipramine	22	28
Maprotiline	17	25
Progesterone	12	19
Phenylbutazone	10	17
Pipamperone	10	15
Morphine	9	15
Codeine	7	13
Indomethacin	7	16
Carbochromen	6	16
Naproxen	6	12

DISCUSSION

Müller & Stillbauer (1983) investigated the binding of different basic drugs to α_1-AGP and found that the drugs were bound exclusively to the same single binding site of the protein, which they called 'basic drug binding site'. At the same time we could verify these findings by displacement experiments with radiolabelled psychotropic drugs of chemically different structure, i.e. perazine, imipramine, diazepam, haloperidol, phenytoin, phenobarbitone (Schley & Müller-Oerlinghausen 1983). The radiolabelled drugs could be displaced from their binding to α_1-AGP by each of six unlabelled compounds.

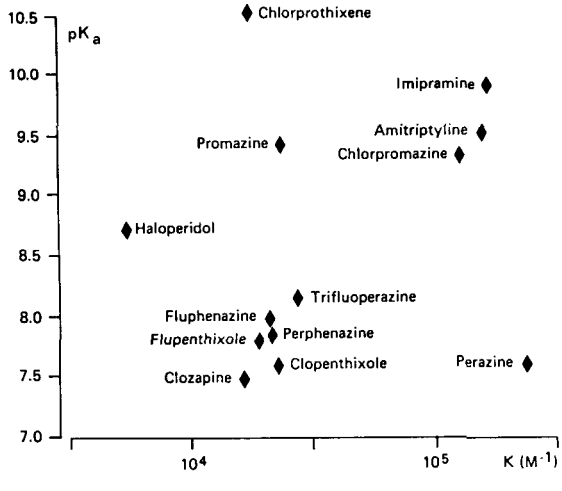


FIG. 2. Binding affinities towards α_1-AGP and ionization constants (pK_a) of neuroleptic and antidepressant drugs (the pK_a-values were taken from the following sources: Leysen 1984; J. Peeters, Beerse, personal communication).

Thus, all six drugs are presumably competing for a common binding site. Therefore, one can accept perazine as a marker ligand for the binding site, since the displacement of a labelled drug by an unlabelled drug must occur at an identical site (Petcher 1980). The method described here allows the determination of affinities at the perazine or the mentioned 'basic drug binding site' by other psychotropic basic drugs with a similar tricyclic structure. While it is not possible to assess the number of binding sites in these kinds of studies, it is clear from the displacement experiments that the tricyclic compounds clozapine, dibenzepine, and dimethacrine bind to the same site as perazine. The association constant for chlorpromazine is in rather good agreement with that found by Kornguth et al (1981), $3{\cdot}4 \times 10^5$ M^{-1}, but differs, however, from that reported by Müller & Stillbauer (1983). Also Verbeeck et al (1983) found a somewhat higher affinity. This discrepancy might be explained by special methodological aspects of equilibrium dialysis.

While adsorption of drugs to macromolecules and surfaces has been reported by some authors, its relevance for the evaluation of binding experiments is discussed controversially (Krieglstein et al 1972; Kinget et al 1979).

Kurz et al (1977) described an equilibrium dialysis using Visking cellulose bags which were put into glass tubes containing the buffer. Glass was preferred to plastic material, because the adsorption of drugs to glass vessels appeared negligible (Kurz et al 1977). In the case of the widely used equilibrium dialysis with Teflon-chambers (Dianorm apparatus), the losses of tricyclics (e.g. [^{3}H]perazine and [^{3}H]imipramine) due to drug adsorption during dialysis are measurable but do not differ considerably among individual compounds. Kurz et al (1977) suggested that adsorption of drugs does not affect the calculation of binding parameters, if both the free concentration in the protein-free solution and the total concentration in the plasma are determined. On the other hand, we reported recently that whether a displacement method or the conventional method described by Scatchard is used, equal association constants for perazine are obtained, if the losses by adsorption are adequately considered (Schley 1983). We consider this as sufficient evidence that losses due to drug adsorption during dialysis must be accounted for when calculating association constants, since it can be assumed that the drug in the chambers is in equilibrium between solution and surface of the chamber. Particularly at low concentrations, losses due to adsorption were found to be high. We suggest, therefore, that, if association constants are calculated by Scatchard plots, the corrected concentrations and not the actual concentrations should be considered. Calculations based on actual concentrations may result in too steep binding curves. This error possibly explains the high association constants for chlorpromazine ($3{\cdot}45 \times 10^6$) and (±)-propranolol ($1{\cdot}13 \times 10^6$) reported by Müller & Stillbauer (1983), whereas most authors have found association constants to α_1-AGP around 10^5 M^{-1}.

The affinity of the tricyclics for α_1-AGP can primarily be ascribed to their phenothiazine and thioxanthene ring systems as indicated by the high affinity ($1{\cdot}2 \times 10^4$) of the tricyclic phenothiazine in comparison to the other compounds examined here. In particular, the sulphur atom of the ring system, with its free electron pair, seems to play an important role. This last point is illustrated by the ten-times lower affinity of perazine-sulphoxide in which the sulphur electrons are withdrawn from the π-orbital, in comparison with perazine (Schley 1983). The affinity to phenothiazine is increased by the propylamine- and even further by the piperazinyl-side chain. On the other hand, cleavage of the piperazine ring into PPED (*N*-γ(phenothiazinyl)-10-propyl ethylenediamine) leads to no increase in affinity (Schley 1983). Substituted phenothiazine derivatives of the alkylamine class have a higher affinity, which appears to be due to the electro-negative substituents at the tricyclic skeleton (Schmutz 1975; Rodgers et al 1976). In contrast, substituents at the tricyclics in the piperazine class of compounds result in a reduction in affinity, presumably due to steric hindrance. However, the presence of a methyl-group at position 4 in the piperazine ring leads to an increase in affinity when compared to the metabolite desmethylperazine.

It may be argued that the binding affinity of these drugs should be related to their physicochemical properties rather than to the various chemical moieties. Fig. 2, however, shows clearly that no correlation exists between the binding affinities and the ionization constants of 11 neuroleptic drugs including haloperidol ($r = -0{\cdot}21$; n.s.). Also no relationship could be shown with the octanol–water partition coefficient logP ($r = 0{\cdot}14$; $N = 13$; n.s.). The latter parameter expressing the lipophilic nature of a drug has been found to be related to the histamine releasing activity of tricyclic neuroleptics and antidepressants (Frisk-Holmberg & van der Kleijn 1972).

Furthermore, it could be demonstrated that some

compounds without a tricyclic system listed in Table 2 can also bind to the perazine or the so-called 'basic drug binding site'.

A generalized relationship between the association constants of the neuroleptic compounds investigated and their antipsychotic potency could not be established. Whether a relationship exists to other pharmacological or clinical characteristics, remains to be examined.

Recently we reported on a mathematical re-evaluation of clinical and laboratory data providing evidence for a relationship between the total serum concentration of perazine and α_1-AGP on one side, and clinical data on the other. This points to the possibility that in certain cases perazine treatment itself, and/or the course of the disease may influence the amount of drug bound to α_1-AGP (Reuss et al 1985).

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