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DIFFERENCES IN THE SERUM PROTEIN BINDING OF PRAZOSIN IN MAN AND RAT

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Abstract—The serum protein binding of prazosin in man and rat has been studied *in vitro* by equilibrium dialysis. Prazosin was more extensively bound in human serum than in rat serum with binding ratios (B/F) of 14.3 ± 3.4 and 4.4 ± 0.2 (corresponding to 93.4 and 81.4% bound), respectively. This difference in binding between the species was partly due to qualitative differences between human and rat serum albumin, but also to the lower concentration of albumin in rat serum. Rat serum albumin (RSA) apparently showed two different classes of binding sites for prazosin, one with high ($K_D = 5.78 \times 10^{-6}$ M) and one with low ($K_D = 1.1 \times 10^{-4}$ M) affinity; the former is suggested as representing α_1-acid glycoprotein (α_1-AGP) with one binding site for prazosin per molecule, the latter as representing RSA with 0.28 binding sites per molecule. Human serum albumin (HSA) and human α_1-AGP both showed one class of binding sites with K_D values of 2.7×10^{-5} and 1.95×10^{-6} M, respectively. HSA possessed 0.5 and human α_1-AGP 1 binding site for prazosin per molecule. The binding parameters obtained for the isolated serum proteins overestimated to some degree the total serum protein binding of prazosin in man. This was explained by a specific deviation from the law of mass action. HSA was the major binding protein in human serum at therapeutic concentrations, with *ca.* 60% of the total binding, the remaining 40% being bound to α_1-AGP. Anticipating that the high affinity binding site on the RSA preparation represents the binding of prazosin to α_1-AGP, then this protein accounts for 70% of the binding in rat serum, while rat serum albumin accounts for approximately 23%. The binding of prazosin to lipoproteins was insignificant in both species. The observed differences between man and rat in the serum protein binding of prazosin implicate differences in the two species with respect to prazosin pharmacokinetics and the pharmacological effect.

The serum protein binding of several drugs has been shown to vary considerably from species to species, and is one of the factors accounting for interspecies differences in the pharmacokinetics and the pharmacological effects of drugs [1, 2].

Drugs are often bound to several proteins in serum, and the determination of the binding constants, the dissociation constant (K_D) and the number of binding sites (n) for the drug–protein complex is essential in order to evaluate the significance of each protein for the total binding in serum. Prazosin is reported to be extensively bound (92–97%) [3, 4] in human serum and has been shown to be bound to albumin (HSA)* and to α_1-acid glycoprotein (α_1-AGP), with higher affinity for α_1-AGP [4]. Albumin is, nevertheless, suggested to be the major binding protein [4, 5].

The aim of this study was to characterize the binding of prazosin to isolated proteins from human and rat serum, to evaluate their significance for the total binding in whole serum, and to discuss the relevance of our observations for pharmacological effects of prazosin in humans and rats.

* Abbreviations: HSA, human serum albumin; RSA, rat serum albumin; α_1-AGP, α_1-acid glycoprotein; K_D, dissociation constant; n, number of binding sites; B, molar concentration of bound prazosin; F, molar concentration of unbound prazosin; P, molar concentration of protein.

MATERIALS AND METHODS

Collection and treatment of serum. Human serum was obtained from nine healthy volunteers of both sexes, of age ranging from 23 to 38 years. Rat serum was obtained from nine male Sprague–Dawley rats weighing *ca.* 250 g. Humans and rats were not fasted and the blood was collected in the morning. Blood samples were allowed to clot for 2 hr, and serum was separated by centrifugation at 1100 *g* for 10 min. Serum was stored at −20° for up to 8 weeks, which did not alter the binding ability of the proteins.

Separation of lipoproteins. Lipoproteins were separated from unfrozen serum and were not stored for more than 2 days at +4°. Potassium bromide (324.7 mg/ml) was added to achieve a density of 1.210 g/ml. Serum was centrifuged at 105,000 *g* at 4° for 45 hr in a Kontron TGA 50 ultracentrifuge with a TY-40 rotor. The floating lipoproteins were withdrawn and dialysed against Krebs–Ringer phosphate buffer, pH 7.35. The lipoproteins were finally diluted in the same buffer to the initial concentration in serum.

Determination of serum protein binding. The binding of prazosin to serum and isolated serum proteins was determined by equilibrium dialysis using Plexi glass cells with two compartments of 1 ml, separated by a semipermeable membrane (Dialysis tubing, Medicall, U.K.). The dialysis was run for 18 hr at 23 ± 1°. The cells were gently shaken according to a standard procedure. Five hundred μl of serum or

protein solution, earlier dialysed against Krebs–Ringer phosphate buffer, pH 7.35, was added to one compartment of the cell. Five hundred μl of Krebs–Ringer phosphate buffer, pH 7.35, containing prazosin and trace amounts of [^{3}H]prazosin was added to the other compartment of the cell.

Equilibrium between the two cell compartments was achieved within 10 hr and was stable for up to 30 hr. pH and protein concentrations were determined before and after equilibrium dialysis. Usually a drop in pH from 7.35 to 7.25 and a dilution of proteins up to 18% were observed. The drop in pH did not influence the binding of prazosin and corrections were made for the dilution of proteins. Corrections were also made for the varying recovery of radioactivity (75–95%). Recovery was dependent on the concentration of protein in the dialysis cells; low protein concentrations resulted in the lowest recovery, probably due to an increased binding to the cell walls or the dialysis membrane. No signs of precipitation were observed. After achievement of equilibrium, duplicate aliquots of 100 μl were taken from each compartment and 5 ml of scintillation fluid (Unisolve 294, Koch Light, U.K.) was added. The counting efficiency of the Packard Tri Carb liquid scintillation spectrophotometer operated at 4° was *ca.* 38% for [^{3}H]prazosin both in the buffer and in the protein solutions. The binding of prazosin was calculated from the distribution of labelled compound, the recovery and the added amount of drug. Thin-layer chromatography on silica gel 60 plates (Merck 5553, F.R.G.) in two different solvent systems (ethyl acetate–diethyl amine, 95:5 and ethyl acetate–methanol–diethylamine, 80:20:1) demonstrated the identity between prazosin and [^{3}H]prazosin and that prazosin and [^{3}H]prazosin were stable during equilibrium dialysis. The isotope had a purity of more than 97.5%. The radioactive impurities behaved almost identically to the drug isotope during equilibrium dialysis.

Calculation of binding. The protein binding of prazosin was expressed as the binding ratio, B/F, where B and F represent the molar concentrations of bound and unbound prazosin, respectively, as described previously [6].

The dissociation constant, K_D, was determined from the reciprocal of the slope of the computerized straight line achieved by plotting B/P (ordinate) against B (abscissa) as described by Romer and Bickel [7] according to their modification of the Scatchard relationship [8]:

$$B/P = \frac{1}{K_D}(B_{max} - B) \quad (1)$$

where B_{max} is the maximum concentration of the bound drug and P is the molar concentration of the protein. This method requires a constant concentration of drug in serum whereas the concentration of protein is varied. The concentration of HSA and α_1-AGP in the present investigation ranged from 10 to 800 and from 15 to 70 μmole/l., respectively. As the total concentration of prazosin in the protein compartment decreased with decreasing concentration of protein, a mean drug concentration of 90 nmole/l was used for binding calculations. This correction was proved valid by control experiments where prazosin was added in different concentrations producing a constant concentration of drug in the protein compartments after equilibrium was achieved in the cells. The number of binding sites (n) available for prazosin on isolated proteins was calculated from the law of mass action [9]:

$$B/F = \frac{n \times P}{K_D + F} \quad (2)$$

If K_D is known and $K_D \gg F$, eqn (2) may be transformed as follows:

$$B/F = \frac{n \times P}{K_D} \quad (3)$$

This equation describes a linear relationship between B/F and P where n/K_D represents the slope of the computerized straight line through the origin. A high degree of reproducibility of binding constants was found if the protein concentration in the cells exceeded 10 μmole/l as demonstrated by the values (mean ± S.D.) from three separate experiments with human serum albumin (Sigma); $K_D = 2.8 \times 10^{-5} \pm 0.3$ M and $n = 0.53 \pm 0.06$.

The dissociation constants for two independent classes of binding sites on the same protein were determined by resolving the curved line into two straight lines as described previously [10]. The algebraic relationship between the curved line and its asymptotes is given by modifying the equation of Rosenthal [11] according to Romer and Bickel [7].

$$B/P = \frac{B_{max_1}}{P + K_{D_1}} + \frac{B_{max_2}}{P + K_{D_2}} \quad (4)$$

The validity of resolving the curved line (Fig. 3) into two straight lines was tested by regenerating the curve according to eqn (4), which gave a curve identical to the experimental one.

Determination of protein. The concentration of total protein was determined as described by Lowry *et al.* [12] using bovine albumin (fraction V) as standard. The concentrations of HSA and α_1-AGP in human serum were determined by a gel immunodiffusion technique using Norpartigen plates from Behring Werke (F.R.G.). The concentration of albumin in rat serum was determined as described by Pinnel and Northam [13] using rat serum albumin (fraction V) as standard.

Chemicals. Human serum albumin (A 2386, not free of fatty acids) was obtained from Sigma Chemical Co. (St. Louis, MO) and Kabi (Sweden), rat albumin (A 6272) from Sigma Chemical Co. (St. Louis, MO) and human α_1-AGP from Behring Werke (F.R.G.). Prazosin HCl was supplied by Pfizer (Norway) and ^{3}H-labelled prazosin (23 Ci/mmole with a purity of 97–98%) was purchased from Amersham (Bucks., U.K.).

Statistics. All lines were drawn by a computer (Hewlett Packard 85). Non-linear least-squares regression analysis was used for the curved line while linear least-squares regression analysis was used for the straight lines. The equations describing the lines are given in the figure legends, and the fit to experimental coordinates is given as r^2 values. The significance of the lines for the relationship between x and

Table 1. Intra- and interspecies variations in the serum protein binding* of prazosin in man and rat

	Total protein g/l	Binding ratio* (B/F)
Man	56.7 ±4.0	14.3 ± 3.4
Rat	54.1 ± 1.5	4.4 ± 0.2

* Given as the mean value ± S.D. of nine individuals. Each individual value represents the mean of two parallel experiments, each determined in duplicate.

† Prazosin was added to the buffer compartment at an initial concentration of 130 nmole/l.

Table 2. The binding* of prazosin to pooled serum and serum fractions from man and rat

		Binding ratio (B/F)† I	II
Serum	man	15.9	16.5
	rat	5.0	4.7
$d > 1.21$	man	14.2	17.5
	rat	5.0	4.7
$d < 1.21$	man	0.6	0.5
	rat	0.3	0.5

* Mean values of two parallel experiments, each determined in duplicate.

† Prazosin was added to the buffer compartment at initial concentrations of 3250 nmole/l (I) and 130 nmole/l (II).

$d < 1.21$ represents lipoproteins with a density less than 1.21 g/ml and $d > 1.21$ represents serum devoid of lipoproteins. Both fractions were diluted to their original concentration in serum.

y is given considering P values less than 0.05 as significant.

RESULTS

The intra- and interspecies variations in the binding of prazosin in serum from man and rats are given in Table 1. A considerable interspecies difference can be seen in the binding of prazosin, and also a high degree of variability within the human sera with binding ratios of 14.3 ± 3.4 and 4.4 ± 0.2 (corresponding to 93.4 and 81.4% bound) for man and rat, respectively. There was no sex-related difference in the binding of prazosin in humans. The observed difference in binding between man and rat was not related to the total concentration of protein according to Table 1.

Figure 1a compares the binding of prazosin to human and rat serum, while Fig. 1b compares the binding to isolated human serum albumin (HSA) and rat serum albumin (RSA) at various protein concentrations. Human serum bound quantitatively more prazosin than rat serum at equal concentrations of total protein in the range 1–70 g/l. This was also true when the binding was compared at equal concentrations of isolated albumin, indicating also qualitative differences in the protein binding of prazosin between the two species. The difference in binding between HSA and RSA, however, was smaller than for the corresponding whole sera.

Table 2 shows the binding to pooled serum and serum fractions from man and rat at high and low concentrations of prazosin. Only minor differences appeared in the binding by increasing the concentration of prazosin from 130 to 3250 nmole/l. For both man and rat the lipoproteins proved to be of minor importance as prazosin binding proteins in serum.

The binding characteristics for the interaction between prazosin and isolated proteins are derived from Figs. 2, 3 and 4 and are summarized in Table 3. Figures 2a, 3 and 4a are plotted according to Romer and Bickel [7]. Figures 2b and 4b are plotted according to eqn (3). It should be noted that the lines computed for the calculation of the number of binding sites on HSA and human α_1-AGP do not run through the origin. Identical binding parameters were obtained for two different preparations of HSA (Sigma and Kabi), as shown in Table 3, with K_D ranging from 2.6 to 2.8 × 10^{-5} M and n ranging from 0.51 to 0.54. RSA showed a binding pattern for prazosin different from that of HSA with apparently two classes of independent binding sites, as can be seen in Fig. 3. According to the method of Rosenthal [11], the high affinity class of binding sites possessed a K_D value for prazosin of 5.78 × 10^{-6} M and 0.036 binding sites per molecule, whereas the respective binding parameters for the low affinity class of binding sites were 1.1 × 10^{-4} M and 0.28. The affinity of prazosin for isolated human serum α_1-AGP, with a K_D of 1.95 × 10^{-6} M, was 14 times higher than that

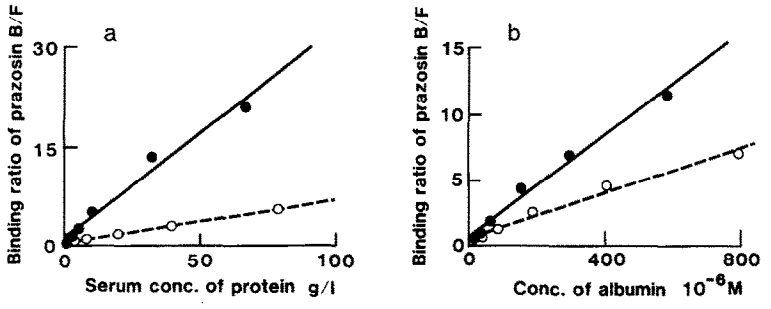

Fig. 1. The binding of prazosin to (a) serum and (b) albumin from man (●—●) and rat (○—○) at variable concentrations of protein. Serum and albumin concentrations ranged from 1 to 70 g/l and 10 to 800 μmole/l, respectively. Prazosin was added to the buffer compartment at an initial concentration of 130 nmole/l. Each point represents the mean of duplicate determinations from two parallel experiments.

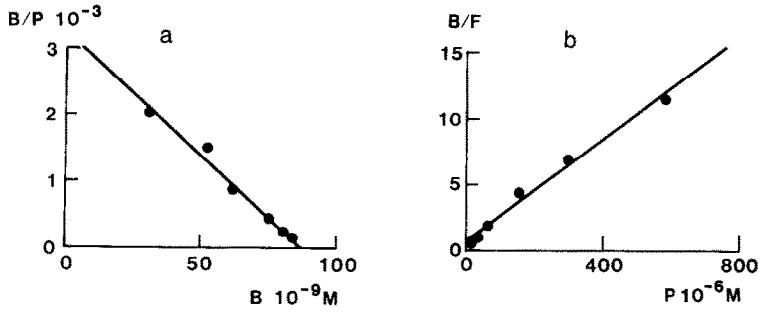

Fig. 2. The binding of prazosin to human serum albumin (HSA) (a) plotted according to Romer and Bickel using the Scatchard relationship. $y = 3.136 - 0.036x$, $r^2 = 0.999$, $P < 0.01$. (b) plotted according to a modification of the law of mass action. $y = 0.703 + 0.019x$, $r^2 = 0.984$, $P < 0.01$. B, F and P represent the molar concentrations of bound prazosin, unbound (free) prazosin and albumin, respectively. The concentration of albumin ranged from 10 to 800 μmole/l. Prazosin was added to the buffer compartment at an initial concentration of 130 nmole/l. Each point represents the mean of duplicate determinations of two parallel experiments.

for HSA, α_1-AGP also possessing one binding site ($n = 1.12$) for prazosin per molecule which is twice the number of binding sites observed for prazosin on the albumin molecule.

Table 4 presents the measured (experimental) binding of prazosin to human and rat serum and the calculated binding to HSA, human serum α_1-AGP and RSA in whole serum, the latter based upon the law of mass action and the calculated binding parameters given in Table 3. The concentration of unbound prazosin in serum was determined by equilibrium dialysis. According to Table 4, HSA binds more prazosin than α_1-AGP in normal human serum. The total binding of prazosin in human serum can be entirely explained by the binding to these two proteins. In the rat serum, however, the calculated binding to the RSA preparation was responsible for 92% of the total binding of prazosin. The corrected values in Table 4 are based on adjustments for deviation from the law of mass action (see Discussion).

DISCUSSION

The present study has demonstrated significant differences in the serum protein binding of prazosin in humans and rats. Human serum binds quantitatively more prazosin than rat serum, and qualitative differences exist in the binding of prazosin to HSA and RSA. The high affinity class of binding sites ($n = 0.036$) found on RSA may, however, be due to the presence of α_1-AGP in the RSA preparation studied [14]. Assuming that rat α_1-AGP possesses one binding site for prazosin, a ratio of 0.036 is obtained between the concentration of α_1-AGP and albumin in the RSA preparation. This ratio is in agreement with the reported normal serum concentration of rat α_1-AGP of *ca.* 16 μmole/l [15] and the concentration of albumin in serum found in this study of 526 μmole. The hypothesis of the presence of α^1-AGP or another binding protein in the RSA preparation is strengthened further by the fact that RSA alone is not likely to bind all prazosin in rat serum as indicated in Table 4. We therefore suggest that the high affinity class of binding sites in the investigated RSA preparation represents α_1-AGP with a K_D of 5.78×10^{-6} M and one binding site, while the low affinity class of binding sites represents albumin with a K_D of 1.1×10^{-4} M and 0.28 binding sites for prazosin.

Lipoproteins have been shown to be an important group of binding proteins for other basic drugs [16, 17]. It is obvious, however, that these proteins do not contribute significantly to the total binding of prazosin in serum, and that no differences exist between man and rat, indicating that variations in the serum concentration of cholesterol, triglycerides

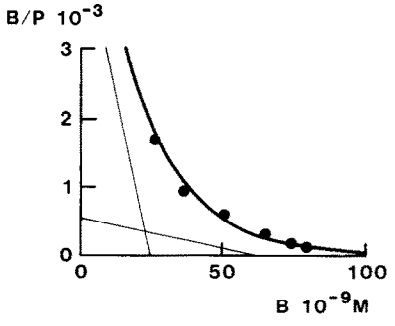

Fig. 3. The binding of prazosin to rat serum albumin (RSA). Conditions and designations are identical to those presented in Fig. 2. $y = 4.35 - 0.173x$, $r^2 = 0.999$, $P < 0.01$ for the high affinity class of binding sites. $y = 0.551 - 0.009x$, $r^2 = 0.996$, $P < 0.01$ for the low affinity class of binding sites.

Table 3. Apparent binding constants for the interaction of prazosin with HSA, RSA and human α_1-AGP

Protein		Dissociation constant (K_D 10^{-6} M)	Number of binding sites (n)
HSA	Kabi	26.3	0.51
HSA	Sigma	27.8	0.54
RSA	high affinity	5.78	0.036
	low affinity	111.0	0.28
Human	α_1-AGP	1.95	1.12

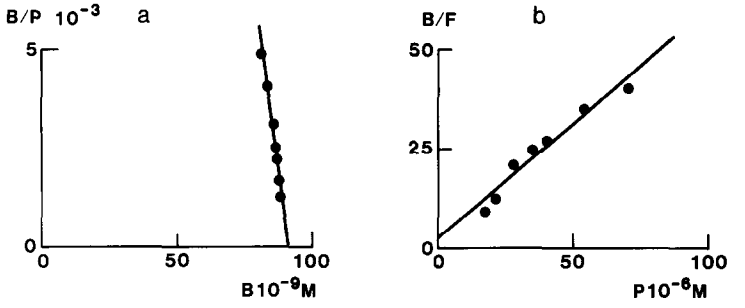

Fig. 4. The binding of prazosin to human serum α_1-AGP. The concentration of α_1-AGP ranged from 15 to 70 μmole/l. Designations for α_1-AGP are identical to those given for albumin in Fig. 2. (a) $y = 46.743 - 0.514\,x$, $r^2 = 0.952$, $P < 0.01$; (b) $y = 2.555 + 0.574\,x$, $r^2 = 0.948$, $P < 0.01$. Each point represents the mean of duplicate determinations of two parallel experiments.

and phospholipids will not greatly influence the binding of prazosin in serum.

The binding of prazosin in human and rat sera was constant when the concentration of prazosin increased from therapeutic concentrations of 130 nmole/l [18] to concentrations of more than 2350 nmole/l, a value reported in connection with intoxications [19]. This is an observation also documented by others for the binding of prazosin to isolated human α_1-AGP and HSA [4]. For this reason, the dissociation constants were determined according to the method of Romer and Bickel [7]. Our calculations of the dissociation constants for the interaction of prazosin with HSA and α_1-AGP (2.7×10^{-5} and 1.95×10^{-6} M, respectively) compare favourable with those reported by Rubin and Blaschke [4]. The number of binding sites available for prazosin on the proteins, however, has not been previously reported. The observed number of binding sites for prazosin on the albumin molecule of less than one 0.51–0.54) may be due to the presence of free fatty acids in the investigated albumin preparations probably reducing the ability of albumin to bind prazosin [17, 20, 21] and thereby reflecting the native binding of albumin in whole serum more clearly. Isolated α_1-AGP, on the other hand, shows one binding site for prazosin on each molecule. This is in agreement with earlier suggestions that α_1-AGP in man contains one binding site for the basic drugs quinidine and propranolol [22, 23], indicating that these basic drugs may have a common binding site on α_1-AGP [24]. However, low concentrations of propranolol do not influence the serum protein binding of prazosin [3].

The calculated values for the binding of prazosin in both human and rat sera are based upon the assumption that the binding parameters obtained for the isolated proteins are representative for the proteins when present as a mixture of proteins as in serum [17]. The uncorrected calculated binding of prazosin to HSA and human α_1-AGP based upon the binding parameters in Table 3, however, seems to overestimate the binding of prazosin in serum. This may indicate other properties of the isolated proteins, other than the native ones, caused by the isolation procedure [25]. The binding of prazosin in serum may also be reduced by the presence of endogenous substances inhibiting the drug–protein complexes [25]. However, all the calculations are based upon the law of mass action stating that n and K_D are constant values. This is shown not to be true when binding experiments are carried out over a range of protein concentrations [26, 27], and is also reflected in our experiments in Figs. 2a and 4a where the straight lines do not run through the origin as predicted. The intercept of the lines with the ordinate represents an overestimating of the ability of the proteins to bind prazosin which can be corrected for by reducing the actual protein concentrations with the values represented by the intercepts of the

Table 4. The apparent contribution of albumin (man, rat) and α_1-AGP (man) to the total binding of prazosin* in pooled serum (man, rat)

Protein	Concentration of protein in serum‡ (10^{-6} M)	Free prazosin in serum‡ (10^{-9} M)	Bound prazosin in serum (10^{-9} M)	
			Experimental	Calculated
Serum (man)†		5.6 ± 0.2	106.4 ± 2.2	
Albumin	629 ± 15	5.6		68.5 [64.5]
α_1-AGP	17.7 ± 0.7	5.6		56.0 [42.5]
Serum (rat)‡		17.4 ± 0.8	77.0 ± 0.8	
Albumin, high affinity	526 ± 16	17.4		57.0 [53.3]
Albumin, low affinity	526 ± 16	17.4		23.1 [17.5]

* Prazosin was added to the buffer compartment at an initial concentration of 130 nmole/l.
† Measured after equilibrium dialysis.
‡ Values are given as means ± S.D., $n = 6$.
Values corrected for deviations from the law of mass action are given in brackets.

straight lines with the abscissa (37 and 4.5 μmole/l for HSA and α_1-AGP, respectively). The corrections introduced for the two classes of binding sites in the RSA preparation were 33.8 and 127.3 μmole/l for the high and low affinity site, respectively.

The large differences observed in the binding of prazosin between man and rat will probably cause significant interspecies differences in the pharmacokinetics of prazosin. This has been earlier documented for both restrictively and non-restrictively eliminated drugs [1, 2]. Another obvious implication is that an equal total serum concentration of prazosin in man and rat will create a stronger pharmacological effect of prazosin in rat than in man. Interspecies differences in the serum protein binding of drugs should therefore be included in pharmacological studies when results from animals are extrapolated to man.

The considerable variation observed in the serum protein binding of prazosin in man may have pharmacokinetic implications for the individual patient. The inter-individual differences in the protein binding of propranolol similar to the differences found for prazosin in this study produced, for instance, a variation in the propranolol elimination half-life in man of more than 40% after intravenous administration [1].

The serum protein binding of basic drugs bound to α_1-AGP will increase when the concentration of α_1-AGP in serum increases, which often takes place in patients suffering from chronic diseases and acute inflammations [28] as well as post-operatively [29] and after trauma [30]. The concentration of the other major binding protein for prazosin, albumin, will usually decrease in connection with diseases [28] and thus possibly counteract the effect of increased concentrations of α_1-AGP. It is therefore difficult to predict how disease may change the total serum protein binding of prazosin in man.

In conclusion, our study has shown that human serum binds prazosin more extensively than rat serum. The difference is due to different binding characteristics of prazosin to albumin in man and rat, and their concentration in serum. α_1-AGP is suggested to be of higher importance for the total binding of prazosin in rat serum than in human serum. The observed inter- and intraspecies variations in the binding of prazosin may have pharmacokinetic implications.

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