Binding of prazosin and propranolol at variable α_1 -acid glycoprotein and albumin concentrations

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- 1 The effect of variable α_1 -acid glycoprotein (AAG) and albumin (HSA) concentrations on the binding of prazosin and propranolol was assessed in plasma after surgery and in mixtures of AAG/HSA with concentrations mimicking those found *in vivo*.
- 2 On the pre-operative day the binding of prazosin and propranolol was 94.8% and 89.0%, respectively and 97.3% and 93.2%, respectively, 5 days after surgery.
- 3 In solutions containing mixtures of highly purified AAG and HSA representing the pre-operative state, 88.6% and 83.9% binding of prazosin and propranolol was observed, whereas for solutions mimicking post-operative plasma, the equivalent values were 94.6% and 91.4%, respectively.
- 4 The ratios between bound and unbound concentrations of both drugs were closely correlated to the concentrations of AAG, but not to the concentrations of HSA.
- 5 The present study demonstrates that AAG is responsible for the binding variability of prazosin and propranolol in plasma from post-operative patients.

Keywords α_1 -acid glycoprotein protein binding prazosin propranolol albumin

Introduction

The non-selective β -adrenoceptor blocker propranolol is highly bound in human plasma/serum at 37° C (Piafsky *et al.*, 1978; Glasson *et al.*, 1980; Paxton & Calder, 1983; Verbeeck *et al.*, 1983; Bendayan *et al.*, 1984). The binding of the α_1 -adrenoceptor blocker prazosin is even higher (Rubin & Blasche, 1980; Schulz *et al.*, 1983; Brunner & Müller, 1985). α_1 -acid glycoprotein (AAG) binds propranolol (Müller & Stillbauer, 1983; Gillis *et al.*, 1985; Bree *et al.*, 1986) and prazosin (Rubin & Blasche, 1980; Dale & Nilsen, 1984; Brunner & Müller, 1985; Ferry *et al.*, 1986) with high affinity and limited capacity. In addition, propranolol shows non-saturable binding to isolated human serum albumin (HSA) (Glasson *et al.*, 1980; Belpaire *et al.*, 1984), whereas high affinity saturable binding has been reported for prazosin (Rubin & Blasche, 1980; Dale & Nilsen, 1984).

In human plasma after surgery, an increased

binding of propranolol has been reported previously (Farndon *et al.*, 1982), but the post-operative variability of prazosin binding has not been established. In the present study the binding variability of propranolol and prazosin was measured in plasma after surgery. The purpose of the present work was to determine the relative roles of AAG and HSA in accounting for the variability in binding of prazosin and propranolol *in vivo* and in an experimental model wherein the concentrations of the purified proteins were varied independently.

Methods

Chemicals

The following radiolabelled substances were used: [³H]-(±)-propranolol hydrochloride

(specific activity 20 Ci mmol⁻¹) and [³H]-(±)-prazosin hydrochloride (specific activity 24.4 Ci mmol⁻¹) from Amersham International, Bucks., UK. All other reagents were of analytical grade.

Incubation buffer

This comprised NaCl 122 mM, KCl 4.9 mM, MgSO₄ 1.2 mM, CaCl₂ 1.3 mM and Na₂HPO₄ 15.9 mM, pH 7.38.

Blood samples

Blood was collected from five females (67–73 years) before and after major hip surgery and placed in heparinized vacuum tubes (Vacutainer, Grenoble, France). The samples were taken on the pre-operative day and on each of the five first post-operative days. After 20 min centrifugation at 1000 g at 4° C, the plasma was aspirated and dialysed against the incubation buffer at 4° C for 48 h. The dialysis was performed to achieve a stable pH and to remove substances with affinity for AAG. The samples were stored at –20° C until use. The degree of dilution was determined by the change in total protein concentration before and after the dialysis.

Purification of AAG and HSA

Electrophoretically pure AAG and highly purified AAG-free HSA were prepared as described previously (Sager *et al.*, 1987).

Equilibrium dialysis

The binding of propranolol and prazosin in plasma or to mixtures of purified plasma proteins was measured by equilibrium dialysis at 37° C, in the dark. Triplicates of each plasma sample (500 µl) containing propranolol (5 nM) and prazosin (4 nM) were dialyzed against the buffer (500 µl). The plasma proteins were separated from the buffer by a semipermeable membrane (Spectrapor membrane tubing 1, Spectrummedical Instruments Inc., Los Angeles, CA, USA) in Perspex® cells. Equilibrium was achieved within 6 h. After termination of dialysis, duplicate samples (50 µl) of plasma or buffer were mixed with 3 ml scintillation liquid (Dilusolve, Packard, Groningen, The Netherlands). Radioactivity was determined in a Packard Tri-Carb Scintillation Spectrometer, Model 3320. Binding was calculated from the distribution of radioactivity between plasma and buffer. The ratio between bound and unbound concentrations (B/F) during the dialysis was corrected for the effect of protein dilution.

Concentrations of plasma proteins

The protein concentrations were measured after dialysis. AAG concentrations were measured in diluted plasma (9% (v/v)) spectrophotometrically (340 nm) after precipitation with anti-AAG (Orion Diagnostica, Espoo, Finland) in 5% (v/v) polyethylene glycol 6000 (E. Merck, Darmstadt, FRG). Standards (Standard-Human serum, Behring Werke AG, Marburg, FRG) were prepared in parallel. HSA concentrations were measured by the bromocresol-green method according to the Boehringer Mannheim automated analysis for the BM/Hitachi system 737 (December 1984 edition). Total protein concentrations were measured by the Biuret reaction according to the Boehringer Mannheim automated analysis for the BM/Hitachi system 737 (July 1984 edition).

Results

Drug binding and protein concentrations in vivo

The binding of prazosin in plasma was higher than that of propranolol. On the pre-operative day, the binding was 94.8% and 89.0% for prazosin and propranolol, respectively. In the post-operative period, the binding of the ligands reached a maximum at day 5 (Figure 1). The

Figure 1 The plasma protein binding of prazosin (○) and propranolol (●) on the pre-operative day (sample 1) and on the five post-operative days (samples 2–6). The results are presented as mean value ± s.d., *n* = 5.

maximal binding was 97.3% and 93.2% for prazosin and propranolol, respectively. A considerable interindividual variability in the binding was found. However, in the plasma from each subject the binding of prazosin was constantly greater than for propranolol and the binding of both ligands increased post-operatively.

In the post-operative period, the concentration of AAG increased progressively and the concentrations of HSA and total proteins were reduced (Table 1). The maximum AAG-concentration was observed at the 5th day. The concentrations of HSA and total proteins were lowest on the second day.

Drug binding and protein concentrations in vitro

Mixed solutions of highly purified AAG and HSA were prepared in order to mimic the changes in the levels of these two proteins in the acute phase plasma. The concentrations of AAG were elevated whereas the HSA concentrations were lowered (Table 2). Under these conditions the binding increased from 88.6% to 94.6% for prazosin and from 83.9% to 91.4% for propranolol (Figure 2). The binding of both ligands increased in parallel with the increasing AAG concentrations (Table 2). The changes of HSA concentrations did not affect the binding.

Figure 2 The protein binding of prazosin (○) and propranolol (●) in mixtures of purified AAG and HSA. The protein concentrations are given in Table 2. The results are presented as mean value $\pm$ s.d., $n = 3$.

Table 1 Concentrations of α_1 -acid glycoprotein (AAG), albumin (HSA) and total proteins (TP) the day before (sample 1) and the 5 days after surgery (samples 2–6) in five patients

	Sample					
	1	2	3	4	5	6
<i>AAG (μM)</i>						
Mean	18.0	22.9	28.5	31.3	33.1	34.5
s.d.	4.2	4.4	4.4	5.6	5.8	8.0
<i>HSA (μM)</i>						
Mean	595	392	442	448	431	454
s.d.	51	64	23	40	30	29
<i>TP (mg ml⁻¹)</i>						
Mean	72.0	48.3	54.4	57.0	55.6	59.7
s.d.	8.3	3.8	2.8	4.5	6.5	5.2

Table 2 Protein concentrations in dialysis chambers after addition of α_1 -acid glycoprotein (AAG) and serum albumin (HSA)

	Sample					
	1	2	3	4	5	6
<i>AAG (μM)</i>	11.6	15.5	19.3	23.2	27.1	31.0
<i>HSA (μM)</i>	556	371	417	417	417	417

Correlation between drug binding and protein concentrations

When the unbound concentration (F) is much lower than the dissociation constant (K_d), the law of mass action can be expressed as $B/F = 1/K_d \times P$ where B and P represent the bound concentration and the concentration of the binding protein (AAG), respectively. In the present experimental model the values of K_d and F were approximately $1 \mu\text{M}$ and 0.5 nM , respectively.

The concentration ratio between bound and unbound propranolol correlated well with the concentrations of AAG *in vivo* ($r = 0.87 \pm 0.04$) and *in vitro* ($r = 0.96 \pm 0.04$). The slopes of the regression lines were $0.35 \pm 0.06 \mu\text{M}^{-1}$ and $0.30 \pm 0.05 \mu\text{M}^{-1}$. The propranolol y-axis intercepts were 2.1 ± 0.3 and 1.5 ± 0.3 , which represent a binding of $68 \pm 3\%$ (range: 63–71%) and $60 \pm 5\%$ (range: 50–64%) *in vivo* and *in vitro*, respectively.

The more extensive binding of prazosin in plasma appeared to be a result of higher affinity for the AAG binding site, reflected by the slopes of the regression lines being $0.99 \pm 0.14 \mu\text{M}^{-1}$ and $0.51 \pm 0.05 \mu\text{M}^{-1}$ *in vivo* and *in vitro*. The prazosin y-axis intercepts were 3.1 ± 1.2 and 1.9 ± 0.3 , which represent a binding of $74 \pm 7\%$ (range: 67–83%) and $67 \pm 5\%$ (range: 58–70%) *in vivo* and *in vitro*, respectively. The B/F-ratios for prazosin showed a highly significant correlation with the concentrations of AAG *in vivo* ($r = 0.94 \pm 0.02$) and *in vitro* ($r = 0.98 \pm 0.05$).

Neither propranolol nor prazosin B/F-ratios correlated positively with HSA concentrations (results not presented).

Discussion

Many drugs bind to more than one class of plasma protein. AAG is the main binding protein for basic drugs, but other proteins such as HSA and lipoproteins may also transport these drugs in plasma. In order to establish the relative importance of drug binding proteins, the extent of binding to separate protein fractions has been determined. It was reported that $10 \mu\text{M}$ AAG and $600 \mu\text{M}$ HSA bound 29% and 84% of prazosin, respectively (Brunner & Müller, 1985). In the case of propranolol, $22.5 \mu\text{M}$ AAG and $580 \mu\text{M}$ HSA bound 60% and 50%, respectively (Glasson *et al.*, 1980). However, it is difficult to predict *in vivo* binding from data derived from the use of separate protein fractions because the proteins may influence the binding properties of each other (Chauvelot-Moachon *et*

al., 1985). On the other hand, during the development of an acute phase reaction, it is possible to assess the relative importance of AAG and HSA *in vivo* due to the fact that the concentrations of these proteins change in opposite directions (Aronsen *et al.*, 1972).

The binding of prazosin and propranolol was measured in acute phase plasma after surgery in the present study. The observed dependency between bound propranolol and the concentrations of AAG confirmed a previous report (Farndon *et al.*, 1982). Such a change is expected for propranolol because AAG binds propranolol in a saturable mode with high affinity (K_d : $1\text{--}10 \mu\text{M}$) whereas the interaction with HSA is non-saturable (Glasson *et al.*, 1980; Belpaire *et al.*, 1984). The observed correlation between the propranolol B/F-ratio in plasma and the concentrations of AAG, in the present study, is in agreement with previous reports (Piafsky *et al.*, 1978; Alván *et al.*, 1983; Paxton & Calder, 1983; Verbeeck *et al.*, 1983; Bendayan *et al.*, 1984; Benedek *et al.*, 1984). Assuming that AAG possesses a single drug binding site, the present results show that propranolol binds to AAG in plasma with a K_d -value of $2\text{--}3 \mu\text{M}$.

Also the binding of prazosin increased during the development of an acute phase reaction, an observation compatible with the previously reported prazosin high affinity binding site on AAG ($K_d \approx 1 \mu\text{M}$) (Rubin & Blaschke, 1980; Dale & Nilsen, 1984; Brunner & Müller, 1985; Ferry *et al.*, 1986). The B/F-ratios of prazosin showed a positive correlation with the concentrations of AAG in plasma. The present results indicated a K_d -value of $1 \mu\text{M}$ for the interaction between prazosin and AAG in plasma, assuming that AAG possesses a single binding site.

However, the binding pattern of prazosin in acute phase plasma was unexpected since HSA has been reported to bind prazosin with relative high affinity ($K_d \approx 10 \mu\text{M}$) (Rubin & Blaschke, 1980; Dale & Nilsen, 1984). Furthermore, it has been suggested that HSA is responsible for approximately 84% of the binding of prazosin in human serum at therapeutic concentrations (Brunner & Müller, 1985). Because the concentration of HSA in plasma is much higher than that of AAG, an initial fall of prazosin binding would be expected in the acute phase plasma.

It is difficult to explain the increase of prazosin binding in acute phase plasma in the light of its extensive binding to HSA (Brunner & Müller, 1985). One possibility is that the binding to HSA has been overestimated due to the fact that commercial HSA preparations may be contaminated by AAG (Lima & Salzer, 1981). In the present study the y-axis intercepts are indi-

cative of binding at zero AAG concentration, but still in the presence of albumin. The extent of binding was comparable with the previously reported values (Brunner & Müller, 1985). However, an alternative explanation is that AAG and HSA interact and thereby suppress binding to HSA in a mixture of the proteins (Chauvelot-Moachon *et al.*, 1985).

Owing to the fact that HSA modifies the binding properties of AAG, it has been recommended to determine binding of basic drugs in a mixture of AAG and HSA (Chauvelot-Moachon *et al.*, 1985). In the present study, AAG and HSA were mixed to mimic the protein concentrations present in the acute phase plasma. The results obtained *in vitro* were nearly identical with those *in vivo*. The binding of propranolol and prazosin increased in parallel with the increasing AAG concentrations. The estimated dissociation constants were similar to those obtained in plasma, approximately 2 μM for prazosin and 3 μM for propranolol. The

binding of both ligands was somewhat higher in the plasma samples compared with the corresponding *in vitro* mixtures. This observation can be explained by the difference in AAG concentrations.

The acute phase reaction after surgical trauma lowered the free fraction of prazosin from 0.074 to 0.027 and the free fraction of propranolol from 0.11 to 0.068. These changes will alter the disposition both of propranolol (Evans *et al.*, 1973) and prazosin (Øie *et al.*, 1987). In addition it has been shown that the free concentration is closely correlated to the β -adrenoceptor blocking effect of propranolol (McDevitt *et al.*, 1976). Such changes in protein binding for prazosin and propranolol may thus be of clinical relevance.

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