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*Report*

# Protein Binding of Cocaine in Human Serum

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The protein binding characteristics of cocaine have not been extensively studied. Since cocaine is related to other local anesthetic compounds which are highly protein bound, we examined the binding of cocaine in human serum using an ultrafiltration method. The free fraction averaged 0.083 ± 0.018 in the serum of 12 healthy volunteers. Binding was studied at concentrations ranging from 0.1 to 500 μg/ml and was concentration dependent, with increases being most pronounced at concentrations above 5 μg/ml. Two classes of binding sites were identified with affinity and capacity constants consistent with binding to alpha-1-acid glycoprotein (AAG) and albumin. The addition of AAG to serum resulted in a decrease in the free fraction from 0.079 to 0.041, while tris(butoxyethyl)phosphate increased the free fraction to 0.233. The binding ratio was found to be highly correlated with the AAG concentration ($r$ = 0.89). In addition, the predicted free fraction in the absence of AAG (0.67) was in good agreement with the observed value of 0.647 in a solution of human serum albumin (4.5 g/dl). Of the metabolites of cocaine, only norcocaine displaced the parent drug from serum binding sites. These results indicate that cocaine is highly bound to serum proteins, primarily albumin and AAG. The significance of concentration-dependent binding to cocaine toxicity remains to be established.

KEY WORDS: cocaine; protein binding; albumin; alpha-1-acid glycoprotein.

## INTRODUCTION

Despite the illicit use of cocaine for many years, the health hazards of cocaine abuse have only recently become widely recognized, with the introduction of dosage forms (such as "crack") which result in significantly more abuse, addiction, and toxicity than traditional methods of ingestion. This has resulted in a renewed interest in understanding the pharmacologic and pharmacokinetic properties of this drug. Previous studies have suggested that the effects of cocaine may be related to both the serum concentration and the rate of increase in the serum concentration (1–3). However, based on the current data, it is not possible to predict the magnitude of effect produced by a given dose or serum concentration of cocaine. Furthermore, the relationship among cocaine concentrations, seizures, myocardial infarction, and death remains undefined, with concentrations associated with cocaine-related death ranging from less than 1 to greater than 10 μg/ml (4–7).

Studies with other basic drugs have demonstrated that the free drug concentration correlates better with the intensity or magnitude of the effect than does the total concentration (8–10). One might expect, therefore, that variability in the degree of protein binding may partially account for clinical observations of wide interindividual sensitivity to the effects of cocaine. Cocaine is structurally and chemically related to lidocaine and bupivicaine, compounds which are known to be highly bound to serum proteins, with both albumin and alpha-1-acid glycoprotein (AAG) contributing to binding (11–13). However, there appears to be only one report in the literature which refers to the protein binding of cocaine and these investigators were unable to obtain satisfactory results due to technical problems associated with the methods used (14). The purpose of this investigation, therefore, was to determine the extent to which cocaine is bound to proteins in human serum, identify those proteins responsible for binding, assess the effect of cocaine metabolites on the binding of the parent compound, and determine if binding is saturable at concentrations observed in patients experiencing cocaine toxicity.

## MATERIALS AND METHODS

[$^3$H]Cocaine (28.2 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, Mass.). The purity of this product was confirmed to be greater than 97% using high-performance liquid chromatography (HPLC) (14) coupled with liquid scintillation counting of the eluent.

Serum protein binding was measured using ultrafiltration since significant degradation of cocaine has been reported under conditions similar to those required for equilibrium dialysis (14). This was confirmed by incubating [$^3$H]cocaine in serum at 37°C for 4 hr. Preliminary studies in our laboratory indicated that only 72% of the drug could be accounted for as parent compound when cocaine was incubated in serum at 37°C for 4 hr. Aliquots (500 μl) of serum containing cocaine [unlabeled as well as a trace amount (1 ng/ml) of labeled drug] were placed in ultrafiltration devices

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(Centrifree, Amicon Corp. Danvers, Mass.) and immediately centrifuged at maximum speed (3825 rpm, 1097 rcf) for 10 min at room temperature. Cocaine concentrations in ultrafiltrate and serum were determined by liquid scintillation counting. All samples were assayed in triplicate and the coefficient of variation for this method was 2.8%. No significant degradation of cocaine or binding to the ultrafiltration device was observed. In addition, no differences in cocaine binding were found when ultrafiltration was performed at 37°C.

Venous blood was obtained from 12 drug-free, nonsmoking, healthy volunteers (6 female, 6 male) using glass syringes. Blood was transferred to glass tubes with Teflon-lined screw caps and allowed to clot. The serum was removed after centrifugation and stored at 4°C for no longer than 24 hr prior to analysis. The free fraction of cocaine was determined in the serum of each individual at a concentration of 25 ng/ml.

In order to study the potential role of AAG in cocaine protein binding, fresh serum was obtained from six healthy volunteers (some of which had given serum for the previous experiment), and each sample separated into three aliquots. Binding was assessed under control conditions, after the addition of AAG 75 mg/dl (Sigma Chemical Co., St. Louis, Mo.) and in the presence of tris(butoxyethyl)phosphate (TBEP), 75 μg/ml (Aldrich Chemical Co., Milwaukee, Wis.), at a cocaine concentration of 25 ng/ml. AAG concentrations were measured before and after the addition of AAG to serum by a radial immunodiffusion procedure (Calbiochem, San Diego, Calif.). In addition, binding was determined in a solution of human serum albumin 4.5 g/dl which was essentially fatty acid free (<0.005%) (Sigma Chemical Co., St. Louis, Mo, Cat. No. A3782).

The potential for saturable protein binding was examined by spiking serum (pooled from three healthy subjects) with cocaine over a wide range of concentrations (0.1–500 μg/ml). The degree of binding was measured and the results were plotted by the method of Rosenthal (15). Nonlinear least-squares regression analysis was used to estimate binding parameters. In addition, pooled serum obtained from two healthy drug-free individuals was spiked with benzoylecgonine, ecgonine, or norcocaine at a concentration of 10 μg/ml. The free fraction of cocaine (at a concentration of 25 ng/ml) was compared to that observed in normal serum.

## RESULTS AND DISCUSSION

Cocaine was found to be highly bound to serum proteins, as the free fraction in 12 healthy subjects averaged 0.083 ± 0.018 and ranged from 0.046 to 0.123 at a concentration of 25 ng/ml. This suggests that the binding of cocaine is similar to that reported for structurally and chemically related basic compounds such as lidocaine and bupivicaine (11–13) but is in contrast to the previous report by Garrett and Seyda (14), who were unable to demonstrate significant binding of cocaine to plasma proteins. However, these investigators found their method to be unsatisfactory due to extensive binding of cocaine to ultrafiltration cones. In our procedure, no significant binding of [$^3$H]cocaine to the membrane or any other part of the ultrafiltration device was observed.

In order to identify the specific proteins involved in cocaine binding, a number of experiments were performed. Table I illustrates the effect on the cocaine binding ratio of adding AAG and TBEP, a compound which has been previously shown to selectively displace basic drugs from biding sites on AAG (16), to serum obtained from six healthy volunteers. The mean free fraction in normal serum was 0.079 ± 0.025. This was decreased to an average value of 0.041 ± 0.011 by the addition of AAG (mean concentration increased from 68.4 to 143.5 mg/dl) and increased to 0.233 by the addition of TBEP. The relationship between the cocaine binding ratio and the AAG concentration in these samples is shown in Fig. 1. The binding ratio was found to be highly correlated with the AAG concentration ($r$ = 0.89). These data strongly suggest that AAG plays an important role in the protein binding of cocaine in serum and is consistent with previous studies which have shown this to be a major binding protein for related compounds such as lidocaine (11,12). The binding of cocaine was also studied in a solution of isolated human serum albumin (4.5 g/dl in phosphate buffer, pH 7.40) and a free fraction of 0.647 was observed. Data from Fig. 1 indicate that in the absence of AAG, a binding ratio of 0.478 ($y$-intercept value) corresponding to a free fraction of 0.67 is expected. The excellent agreement between the observed binding in an albumin solution and the expected binding in serum in the absence of AAG indicates that binding to albumin accounts for serum binding not associated with AAG.

Further confirmation of the identity of the proteins responsible for cocaine binding is provided by examining the effect of increasing concentration on cocaine binding. Analysis of these data by the method of Rosenthal (15) resulted in two distinct classes of binding sites (Fig. 2), with the first site characterized as having an approximately 250-fold higher affinity for cocaine and a 100-fold lower capacity. The values for the affinity and capacity constants observed are consistent with studies with other drugs bound to both AAG and albumin, where AAG typically shows a greater affinity but a lower capacity for binding basic drugs (17,18).

The identification of AAG as a binding protein for cocaine has a number of potential implications. Since AAG has a lower capacity than albumin for binding drugs, saturation of binding with a significant increase in the free fraction may occur at relatively low concentrations. Significant increases in free drug associated with greater pharmacologic effect

Table I. The Effect of Adding Tris(butoxyethyl)phosphate (TBEP; 75 μg/ml) and Alpha-1-Acid Glycoprotein (AAG) to Serum from Healthy Volunteers on the Free Fraction of Cocaine

| Subject No. | Cocaine free fraction | | |
|---|---|---|---|
| | Normal serum | AAG added | TBEP added |
| 1 | 0.085 | 0.050 | 0.277 |
| 2 | 0.123 | 0.056 | 0.275 |
| 3 | 0.077 | 0.041 | 0.234 |
| 4 | 0.072 | 0.035 | 0.230 |
| 5 | 0.068 | 0.032 | 0.214 |
| 6 | 0.046 | 0.029 | 0.168 |
| Mean | 0.079 | 0.041* | 0.233* |
| SD | 0.025 | 0.011 | 0.041 |

* Significantly different from normal serum ($P < 0.01$).

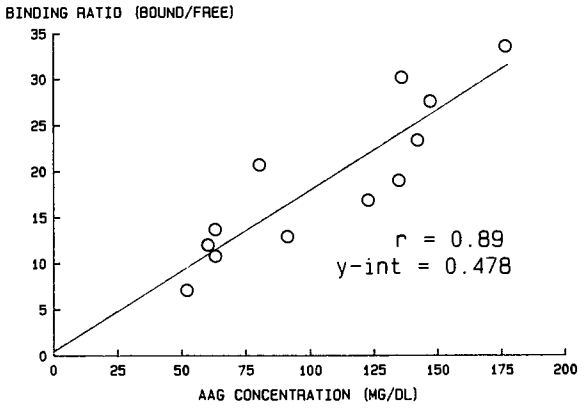


Fig. 1. The relationship between the binding ratio of cocaine and the alpha-1-acid glycoprotein concentration (AAG) in serum from healthy volunteers.

have been observed with disopyramide at concentrations as low as 1–2 μg/ml (9). In addition, high concentrations of structurally similar metabolites could compete for the limited number of binding sites available on AAG, resulting in displacement of the parent compound. In this study, we observed an increase in the free fraction from 0.084 at a cocaine concentration of 100 ng/ml to 0.276 at a concentration of 500 μg/ml. Changes in the free fraction were relatively modest up to a concentration of 5 μg/ml (0.102; 21.4% increase) but increased sharply to 0.129 at 10 μg/ml (53.6% increase) and 0.176 at 20 μg/ml (109.5% increase). Studies with the metabolites of cocaine indicated that the addition of benzoylecgonine and ecgonine to serum at a concentration of 10 μg/ml resulted in a cocaine free fraction of 0.104 and 0.100, respectively, as compared to a value of 0.101 in normal serum. However, the free fraction of cocaine was increased to 0.137 (35.5% increase) by the addition of norcocaine at the same concentration.

The clinical significance of these observations remains to be established. Although our data indicate that binding is concentration dependent and that norcocaine may compete with cocaine for serum protein binding sites, it is unclear whether or not this is likely to be significant in patients with cocaine-related toxicity. Cocaine concentrations associated with death have generally been in the range of 1–10 μg/ml (4–7), where significant saturation of binding sites appears to begin to occur. Norcocaine concentrations have not been measured in most reports of cocaine overdose but were far less than 1 μg/ml in one report (19). In addition, we know of no data concerning the relative concentration of binding proteins such as albumin and AAG in subjects who are drug abusers. Further studies are needed in patients who have actually ingested cocaine to determine if changes in the degree of serum protein binding or concentrations of binding proteins play a role in the clinical toxicology of this compound.

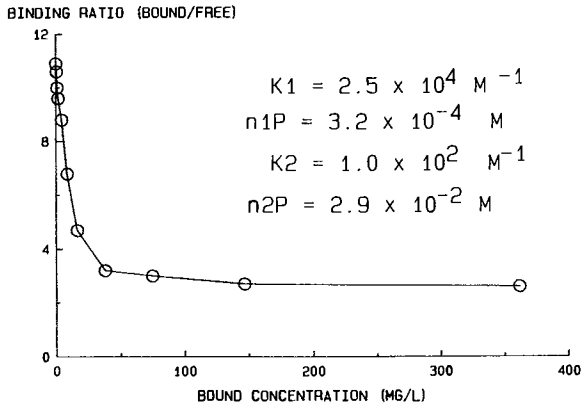


Fig. 2. The relationship between the binding ratio (bound/free) and the bound concentration of cocaine in pooled serum from healthy volunteers.

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