In Vitro Binding Studies of Drugs to Hair: Influence of Melanin and Lipids on Cocaine Binding to Caucasoid and Africoid Hair

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Abstract

Although the mechanism(s) of drug deposition in hair are unknown, there is evidence that suggests that the amount and type of melanin present are major factors in determining how much drug enters hair after exposure. The role of other hair components, such as lipids, has received less attention. We used in vitro binding techniques to evaluate the binding of radiolabeled cocaine to different types of treated and untreated hair specimens. Divided male and female Caucasoid (black/brown and blond colored) and Africoid (black colored) hair specimens ($N = 7$) were exhaustively extracted to remove lipid components (lipid-extracted hair). Separate portions were bleached to denature or alter melanin content. Experiments with radiolabeled cocaine were performed on untreated, lipid-extracted, and bleached portions of hair from different groups. Cocaine binding was significantly higher ($p < .01$) to male Africoid hair compared with other groups. The amount of drug binding was similar among female Africoid and male and female, black/brown Caucasoid specimens. The lowest amount of binding was observed with blond, female Caucasoid specimens. Binding experiments also revealed that specific cocaine binding generally did not differ significantly between lipid-extracted hair and untreated hair, but bleaching of most hair specimens resulted in significant ($p < .01$) decreases in specific binding compared with untreated hair. In separate experiments with cocaine-treated hair specimens, digested samples were evaluated to determine if removal of the insoluble melanin fraction from soluble hair components provided a means of normalization of drug content and elimination of color bias. Removal of the insoluble melanin fraction was not effective in removal of significant amounts of cocaine, which indicated that the digestion process released bound cocaine into the digest solution. Overall, these experiments suggested that lipids in hair play a minor role in drug binding, whereas melanin functions as a major binding site for cocaine. Natural (ethnic) or artificial (bleaching) differences in melanin content may determine the extent of cocaine entrapment in hair after drug exposure. Further, digestion of hair samples and removal of insoluble melanin failed to be effective in removal of hair color bias.

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Introduction

The interpretation of hair test results for drugs of abuse has been hindered by a limited understanding of how drugs are deposited and retained by hair. Cocaine, opioids, amphetamines, and many other drugs and metals have been detected and measured in hair (1–4), but only a few studies have attempted to delineate the mechanism(s) of drug entrapment. Hair is a complex structure that consists primarily of proteins, lipids, and melanin (5). Drugs and metals may bind to these hair components by weak electrostatic interactions, hydrophobic attractions, and ionic bonds. Melanin is considered to be composed of polymers of indole quinone units capable of binding many drugs (6). Lipids in hair contain polar groups, including unsaturated double bonds, alcohol groups, and ester groups, that could bind drugs through specific and nonspecific mechanisms (7,8). Proteins in hair also contain many constituent groups capable of binding drugs, including carboxyl, amine, hydroxyl, and sulfhydryl groups. The exact binding site(s) for drugs in hair has not been elucidated, and this has led to difficulties in evaluating hair test results because of the many different hair types found in different populations. The color and morphology of hair could have a substantial impact on the sequestration of drugs in hair.

The incorporation of drugs and metals into hair has been reported to differ between many hair types. Sky-Peck (4) described significant differences in the concentration of trace elements in hair because of hair color, age, and cosmetic hair treatment. Kidwell and Blank (9) investigated the in vitro binding of cocaine by Korean, African-American, Caucasoid, Hispanic, and Italian hair specimens that were treated with aqueous solutions of cocaine. The ratio of cocaine bound by Korean to Caucasoid hair was 6.8, and the ratio for Africoid to Caucasoid hair was 2.9. Differences were also reported by Reid et al. (10), who studied the binding of benzoylecgonine, a cocaine metabolite, to black, brown, and blond hair. The ratio of benzoylecgonine bound to black, brown, and blond hair was 7:3:1, respectively.

The mechanism of drug deposition in hair must be elucidated for interpretation of hair test results. As part of an

ongoing study of these mechanism(s) of drug deposition in hair, we developed an in vitro model for evaluation of drug binding to different hair components. This model was recently evaluated by comparing in vitro with in vivo incorporation of codeine and morphine into rat hair (11). In vivo and in vitro results were similar, thereby lending credence to the use of this model as a scientific tool for investigating the mechanism of drug binding to hair. This report details further studies on in vitro binding of cocaine to Caucasoid and Africoid hair specimens selectively treated to remove lipids and melanin components from hair.

Materials and Methods

Chemicals and reagents

Chemicals were obtained from the following sources: ^{3}H(*l*)-cocaine, 30.1 Ci/mmol (DuPont, New England Nuclear Division, Boston, MA); (*l*)-cocaine HCl, proteinase K (type 11) from *Tritirachium album*, dithiothreitol, and lauryl sulfate (Sigma Chemical Co., St. Louis, MO); Poly-fluor™ liquid scintillation cocktail (Packard Chemical Co., Meriden, CT); and Wellite® cream bleach kit and Welloxide® clear stabilized color developer (Wella®, Englewood, NJ). Solutions of ^{3}H(*l*)-cocaine were prepared by adding (*l*)-cocaine HCl to dilute the radioactivity of ^{3}H(*l*)-cocaine from 30.1 Ci/mmol to 1.0 Ci/mmol for all experiments. HPLC-grade trishydroxymethylaminomethane (Tris) was used to prepare 50mM Tris-HCl buffer with a pH of 7.0 and 50mM Tris-HCl buffer with a pH of 7.4. All other chemicals were reagent grade.

Hair collection and treatments

Africoid and Caucasoid hair specimens were collected from staff and from barber shops and styling salons in the Baltimore and Washington area. A history of cosmetic treatments for most hair specimens was not available. Individual hair specimens were placed in plastic Ziploc™ bags labeled by age, sex, and ethnicity and then stored at –30°C until the time of analysis. Hair specimens were initially analyzed by gas chromatography–mass spectrometry for cocaine and metabolite content according to a previously published procedure (12). Only drug-free specimens were used in binding assays. The color of all male and female Africoid hair specimens was determined to be black by visual inspection. Caucasoid male and female hair specimens were classified by visual inspection as brown/black and blond. Seven hair specimens were included in each of the following groups: male Africoid; female Africoid; black/brown, male Caucasoid; black/brown, female Caucasoid; and blond, female Caucasoid.

Hair specimens (300–500 mg) were placed in filtration columns, a vacuum was applied, and tap water (three 10-mL portions) was added to rinse the hair. Each hair specimen was then dried to a constant weight and divided into three portions (approximately 100 mg per portion). The first portion of each specimen was then stored for later analysis. The second portion of each specimen was bleached with Wellite bleach according to the manufacturer's instructions. Bleach solutions were mixed thoroughly with all hair specimens for the maximum time suggested (45 min). Bleached hair specimens were thoroughly rinsed with tap water to remove bleaching reagents and then dried to a constant weight.

Soxhlet extractions were performed on the third portion of hair specimens to remove lipids. Approximately 100 mg of hair was placed in a nitrocellulose filter in a Soxhlet extractor–condenser setup. Extraction solvent (20 mL) was added to 50-mL flasks that were placed in heating mantles. Lipids were initially extracted from hair with ether at 2–3 min/cycle (extractor fill time) for 1.25 h under vacuum (gauge pressure, 50 mm Hg), followed by methylene chloride at 4–5 min/cycle for 2 h under vacuum (gauge pressure, 300 mm Hg), and followed by ethanol at 12–15 min/cycle for 6 h under vacuum (gauge pressure, 575 mm Hg). Lipid-extracted hair specimens were then washed with 30 mL of tap water and dried to a constant weight.

Hair homogenization

Approximately 15–25 mg of untreated, bleached, and lipid-extracted hair was weighed separately into 5-mL Elkay mailing tubes. Hair strands were cut into 0.5-cm segments or smaller with surgical scissors. Kimble 2.5-mm borosilicate beads were added to the tubes until one-third of the volume of the tube was filled. Tubes containing hair and beads were capped and placed in a BioSpec Mini-Beadbeater-8™ cell disrupter (Bartlesville, OK) modified to accommodate 5-mL tubes. The dial on the homogenizer was set at 50% of maximum speed (approximately 1400 oscillations/min), and specimens were homogenized for 3 min. Tubes were removed and shaken manually to dislodge hair that occasionally became lodged in screw threads and cap inserts. Tubes were replaced in the homogenizer and homogenized again at the medium setting for 3 min. After homogenization was complete, 3.0 mL 50mM Tris-HCl buffer (pH 7.4) was added to tubes that were then placed in the homogenizer and shaken for 5 s. The suspension was pipetted into 50-mL Elkay tubes, and this procedure was repeated two additional times to dislodge hair fragments from the beads and sides of the mailing tubes. Hair suspensions were then diluted with 50mM Tris-HCl buffer (pH 7.4) to the desired concentration.

Binding assays

Elkay polypropylene tubes were prepared in duplicate to measure total binding of ^{3}H(*l*)-cocaine to hair fragments by adding 900 µL of a 0.7-mg/mL hair suspension, 50 µL of a 3.03-µg/mL ^{3}H(*l*)-cocaine solution, and 50 µL of 50mM Tris-HCl buffer (pH 7.4). The final volume (1.0 mL) in each tube contained 151.5 ng ^{3}H(*l*)-cocaine and 0.63 mg of hair. Tubes were prepared in duplicate to measure nonspecific binding of ^{3}H(*l*)-cocaine to hair fragments by adding 900 µL of a 0.7-mg/mL hair suspension, 50 µL of a 3.03-µg/mL ^{3}H(*l*)-cocaine solution, and 50 µL of a 606-µg/mL (*l*)-cocaine solution. The final volume (1.0 mL) in each tube for nonspecific binding measurement contained 151.5 ng ^{3}H(*l*)-cocaine, 30.3 µg (*l*)-cocaine, and 0.63 mg of hair. After preparation, specimens were vortex mixed for 10 s and placed in a 25°C water bath for 1 h as reported by Su et al. (13) for the attainment of binding equilibrium.

A 5.0-mL aliquot of 50mM Tris buffer (0°C) was added to each tube, and a Brandel M-48 cell harvester under vacuum was used to aspirate specimens onto Whatman GF/B filter paper. The filter paper was soaked in a 0.5% polyethylenimine solution

for 2 h before use. After aspirating specimens onto filter paper, two 5-mL portions of 50mM Tris-buffer (pH 7.4) were added to each tube and aspirated to wash unbound $^3H(l)$-cocaine from hair fragments. The time required for filtration was 10 s or less. Filter paper containing hair was collected and placed in 4.0-mL Pony™ vials. A 3.5-mL aliquot of Poly-fluor liquid scintillation cocktail was added to each Pony vial. After 5 h, total and nonspecific binding were measured in duplicate by determining the radioactivity of specimens. Specific binding was determined for each specimen by subtracting the average response for nonspecific binding from the average response for total binding.

Statistics

Hair specimens were grouped as follows: male Africoid hair; female Africoid hair; black/brown-colored, male Caucasoid hair; black/brown-colored, female Caucasoid hair; and blond-colored, female Caucasoid hair. Each specimen was divided; one portion remained untreated, one portion was bleached, and one portion was extracted to remove lipids (lipid-extracted hair). For each group, two-tailed, paired *t* tests were used to determine significant differences ($p < .01$) in $^3H(l)$-cocaine binding between bleached hair and untreated hair and between lipid-extracted hair and untreated hair specimens. Comparisons also were made in $^3H(l)$-cocaine binding between untreated hair specimens in each group. Group variances were initially compared using the *F* test for equality of variances ($p < .01$). In comparing groups with similar variances, two-tailed *t* tests for independent groups were used to determine significant differences in mean $^3H(l)$-cocaine binding between groups of untreated hair specimens. Mean responses between groups with unequal variances were analyzed by the Cochran–Cox two-tailed *t* test for independent groups, which compensates for unequal group variances.

Protease hair digestion assays

Africoid male hair specimens and blond, female Caucasoid hair specimens were used to prepare hair suspensions containing 2.5 mg of homogenized hair per milliliter of 50mM Tris buffer at pH 7.0. A 3.9-mL aliquot was added to centrifuge tubes. Two tubes designated for total binding measurement for each specimen were prepared with 50 µL of a 6.06-µg/mL $^3H(l)$-cocaine solution and 50 µL of 50mM Tris buffer (pH 7.0). Two tubes designated for nonspecific binding measurement for each specimen were prepared with 50 µL of a 6.06-µg/mL $^3H(l)$-cocaine solution and 50 µL of a 4.24-mg/mL (*l*)-cocaine solution. Specimens were placed in a 25°C water bath for 1 h and vortex mixed every 10 min. Specimens were then centrifuged at 3000 rpm for 5 min, and the supernatant was discarded. Tris buffer (4 mL) was added to each tube. Specimens were vortex mixed and centrifuged at 3000 rpm for 5 min, and the supernatant was discarded.

A 1-mL aliquot of digest solution was added to tubes that contained hair homogenates after removal of the supernatant. The digest solution was prepared according to procedures described in a commercial patent (14). The solution contained 0.5 mg proteinase K, 60 mg dithiothreitol, 20 mg lauryl sulfate, and 10 mL 50mM Tris buffer (pH 7.0). Specimens were placed in a shaking water bath at 37°C for 1 h, and then they remained in the water bath (without shaking) for 14 h. Specimens were vortex mixed once every hour during the last 3 h. Specimens were then centrifuged at 4000 rpm for 5 min, and the supernatant (hair digest) was collected. Pony vials were prepared with 0.5–1 mL of hair digest, the total volume was adjusted to 3.5 mL with Poly-fluor, and specimens were analyzed after 5 h to determine radioactivity. The pellet (melanin fraction) was suspended in 3.0 mL Poly-fluor in centrifuge tubes and vortex mixed thoroughly. Pony vials were prepared with 2.5 mL Poly-fluor, 1 mL of the melanin fraction was added, and specimens were analyzed after 5 h to determine radioactivity.

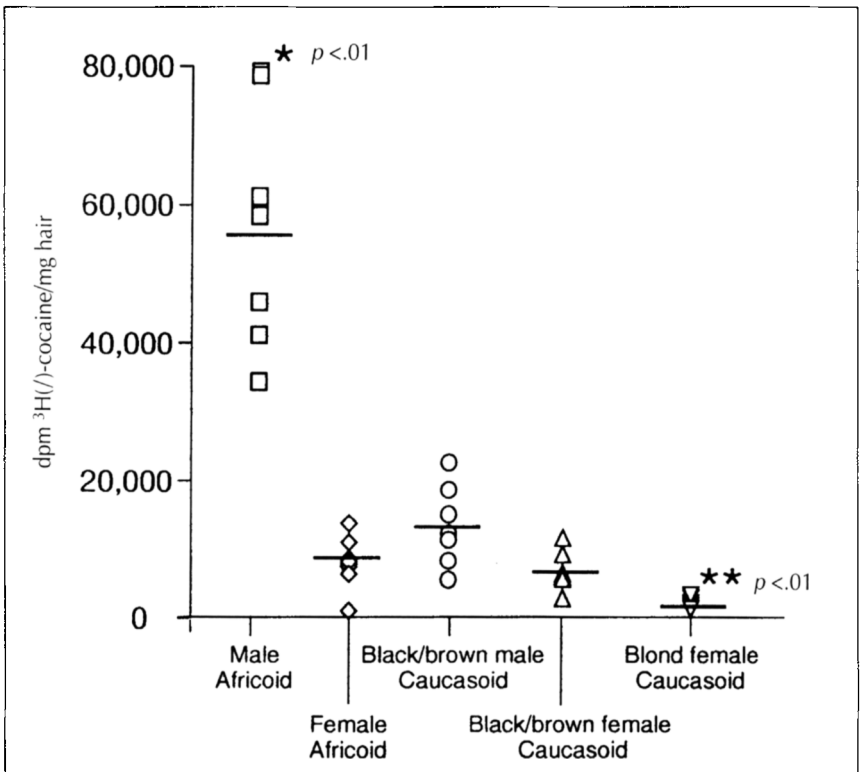

Figure 1. Total binding of cocaine to untreated hair types. Hair suspensions were prepared in duplicate to measure total binding with 151.5 ng $^3H(l)$-cocaine/mL and 0.63 mg of hair/mL. The asterisk indicates that male Africoid responses were significantly greater ($p < .01$) versus all other groups. The double asterisk indicates that blond, female Caucasoid responses were significantly less ($p < .01$) versus all other groups.

Instrumentation and quality control

The radioactivity of specimens was determined in disintegrations per minute (dpm) with a 6500 Beckman (Fullerton, CA) liquid scintillation counter. The counting efficiency of specimens typically ranged from 35% to 45% as determined by tritium single-label quench curves. Control specimens were used in homogenization procedures by preparing mailing tubes with borosilicate beads only.

These specimens were processed identically to hair specimens in the binding assays to determine the extent of binding of $^3H(l)$-cocaine to plastic and glass fragments generated during homogenization. Radioactivity of these control specimens was generally less than 50 dpm. Quality control specimens also were prepared by adding 50 µL of a 3.03-µg/mL $^3H(l)$-cocaine solution to Pony vials that contained 3.5 mL Poly-fluor liquid scintillation cocktail; specimens were analyzed to determine radioactivity. The between-run coefficient of variation was 3.5%.

Results

Differences in cocaine binding between untreated male and female Caucasoid and Africoid hair

Total binding of radiolabeled cocaine to untreated (N = 7) male and female Caucasoid and Africoid hair specimens is illustrated in Figure 1. Male Africoid hair bound significantly more ($p < .01$) cocaine than all other groups. The mean total binding for male Africoid hair was 34-fold greater compared with blond, female Caucasoid hair. Binding of cocaine to female Africoid hair and black/brown, female and male Caucasoid hair did not differ significantly. Total binding of cocaine to blond, female Caucasoid hair was significantly less ($p < .01$) than all other groups.

Specific and nonspecific cocaine binding for individual hair specimens from the different groups are illustrated in Figure 2, and the mean data are listed in Table I. Specific binding tended to be higher than nonspecific binding for all groups with the exception of blond, female Caucasoid hair, whose specific binding was lower than nonspecific binding. Specific binding for male Africoid hair (mean, 48,601 ± 15,673 dpm/mg) was significantly greater ($p < .01$) than all other groups . Specific binding was not significantly different but was highly variable for female Africoid (mean, 6282 ± 3225 dpm/mg), black/brown, female Caucasoid (mean, 5293 ± 2640 dpm/mg), and black/brown, male Caucasoid specimens (mean, 10,563 ± 5209 dpm/mg). Specific binding was significantly less ($p < .01$) for blond, female Caucasoid specimens (mean, 618 ± 458 dpm/mg) compared with all other groups.

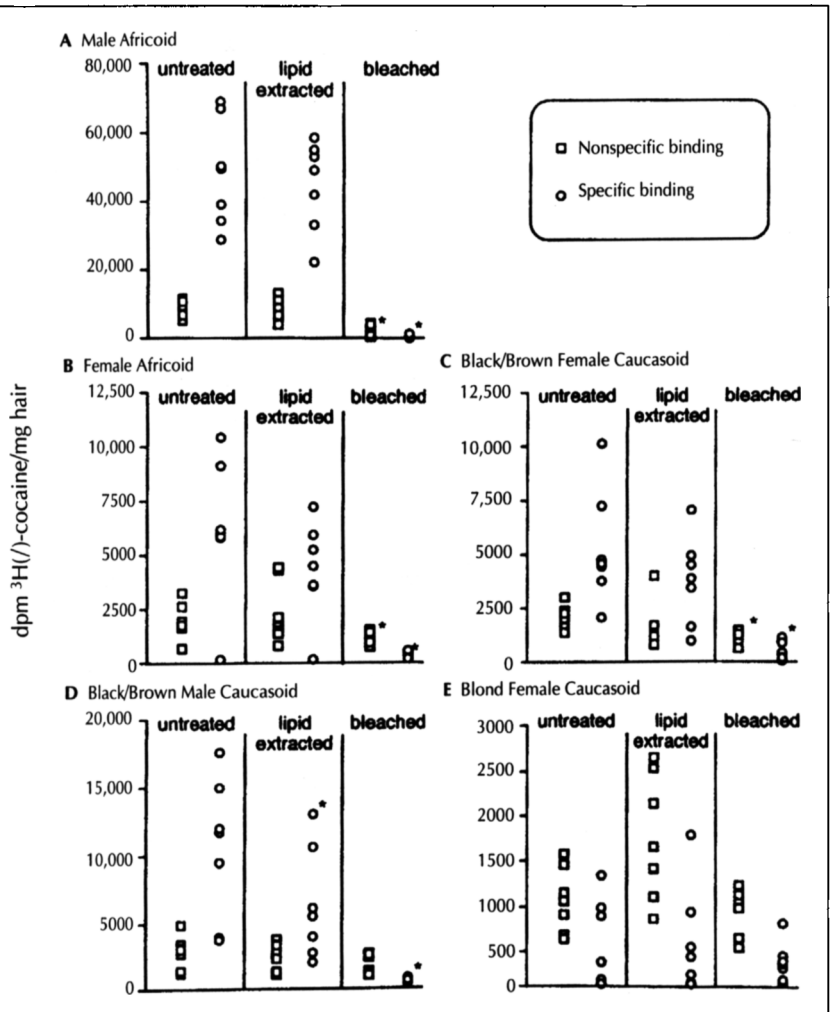

Figure 2. Nonspecific and specific binding of cocaine to hair. Individual specimens were divided into three portions, which were either bleached, extracted to remove lipids, or untreated. Hair suspensions were prepared with 151.5 ng $^3H(l)$-cocaine/mL and 0.63 mg of hair/mL. Nonspecific binding suspensions also contained 30.3 µg (l)-cocaine/mL. Specific binding represented the difference between total and nonspecific binding. Mean values are indicated with bars. The asterisks indicate that cocaine binding was significantly less ($p < .01$) in comparison with the untreated group for the hair type.

Differences in cocaine binding between untreated and lipid-extracted hair

Nonspecific and specific binding of cocaine to lipid-extracted hair did not differ significantly in comparison with untreated hair for all groups with the exception of black/brown, male Caucasoid specimens. Specific binding was significantly less ($p < .01$) for black/brown, male Caucasoid specimens than for untreated hair.

Differences in cocaine binding between untreated and bleached hair

Nonspecific and specific binding of cocaine to bleached hair was significantly ($p < .01$) less in comparison with untreated hair for each group with the exception of two hair types. For black/brown, male Caucasoid specimens, only specific binding was significantly ($p < .01$) less than for untreated hair. Neither nonspecific or specific binding differed significantly from untreated hair for blond, female Caucasoid hair specimens.

Distribution of cocaine in the insoluble melanin and soluble hair digest fractions after protease treatment

Male Africoid and blond, female Caucasoid hair specimens containing bound, radiolabeled cocaine were digested with proteinase K. Starting amounts of cocaine bound to male Africoid specimens in this experiment ranged from fivefold to 55-fold higher than that found for blond, female Caucasoid

specimens. After digestion, the supernatant (hair digest) and unwashed pellet (melanin fraction) for the different hair types were separated and analyzed. Table II lists the distribution of cocaine in the insoluble (melanin) and soluble hair fractions. More than 90% of the bound cocaine was present in the soluble fractions. In all cases, the amount of radiolabeled cocaine bound to melanin was less than 10% of the total bound cocaine.

Discussion

In vitro binding assays have been of major importance in investigations of the mechanisms for drug binding to proteins, lipids, and melanin (15–18). Generally, receptor binding assays attempt to minimize nonspecific binding and maximize specific binding. Nonspecific binding is considered to involve binding of drugs to lipids and tissue components, whereas specific binding involves binding to a selective binding site (e.g., receptors). In the present study, the binding of cocaine to hair was evaluated by comparing in vitro cocaine binding between untreated, bleached, and lipid-extracted male and female Africoid and Caucasoid hair specimens.

Although it was initially anticipated that removal of hair lipids would produce a decrease in nonspecific cocaine binding, exhaustive removal of lipids by solvent extraction did not significantly lower nonspecific binding in most cases. The effect of lipid extraction on cocaine binding was minor with Africoid hair specimens and black/brown and blond, female Caucasoid specimens. Only black/brown lipid-extracted male Caucasoid hair bound significantly less ($p < .01$) cocaine than untreated hair. Overall, the results demonstrated that extractable lipids in hair account for only a minor fraction of cocaine binding to black, brown, and blond hair specimens.

The role of melanin in cocaine binding was evaluated by bleaching hair specimens with a commercial hair treatment product. Specific binding of cocaine to bleached, black Africoid and bleached, brown/black Caucasoid hair specimens was significantly less ($p < .01$) in comparison with each respective untreated group. Bleaching also produced significant decreases ($p < .01$) in nonspecific cocaine binding to black and brown Africoid and Caucasoid specimens. The decrease in nonspecific and specific cocaine binding for most specimens was likely due to melanin denaturation. Bleaching of hair with hydrogen peroxide has been reported to result in a rapid solubilization of melanin (6). This was followed by a less rapid oxidative degradation of melanin to ultimately yield colorless products. The specific mechanism of bleaching is uncertain but may involve the generation of perhydroxy ions or hydroxy radicals, which react with the indole quinone ring structures thought to comprise the basic structure of melanin.

Specific and nonspecific binding of cocaine to blond, female Caucasoid hair after bleaching was not significantly affected ($p < .01$). A history of hair treatment was available for only three of the seven blond specimens. These three specimens had never been bleached or treated with chemicals with the exception of shampoo and conditioners. The similarity in binding results after bleaching and lipid extraction for these three specimens compared with the remaining blond specimens suggested that blond hair, regardless of treatment, binds very little cocaine. The lack of binding was probably due primarily to the small amount of melanin present in blond hair relative to brown and black hair (19). Hair color is determined largely by the concentration and type of melanin present. Eumelanin is a

Table I. Mean Nonspecific and Specific Binding (±SD*) of Radiolabeled Cocaine to Hair Types after Different Treatments†

Hair type	Hair color	Treatment	DPM‡ mg Hair: nonspecific binding ± SD	DPM‡ mg Hair: specific binding ± SD
Male Africoid	Black	Untreated	8678 ± 2333	48,601 ± 15,673
		Lipid-extracted	8717 ± 3025	45,061 ± 13,165
		Bleached	2356 ± 1518	875 ± 364
Female Africoid	Black	Untreated	1962 ± 828	6282 ± 3225
		Lipid-extracted	2422 ± 1464	4417 ± 2228
		Bleached	882 ± 306	212 ± 164
Male Caucasoid	Black/brown	Untreated	2961 ± 1231	10,563 ± 5209
		Lipid-extracted	2653 ± 995	6342 ± 4077
		Bleached	1639 ± 760	478 ± 261
Female Caucasoid	Black/brown	Untreated	2154 ± 516	5293 ± 2640
		Lipid-extracted	1759 ± 1072	2906 ± 1715
		Bleached	1289 ± 681	783 ± 641
Female Caucasoid	Blond	Untreated	1066 ± 363	618 ± 458
		Lipid-extracted	1748 ± 694	593 ± 591
		Bleached	887 ± 280	384 ± 218

* SD = Standard deviation.
† Hair suspensions (0.63 mg of hair per milliliter) were treated with 151.5 ng ^{3}H(l)-cocaine per milliliter. Nonspecific binding suspensions also contained 30.3 µg (l)-cocaine per milliliter.
‡ dpm = Disintegrations per minute.

black/brown melanin that is present in higher concentrations in black- and brown-colored hair than in blond hair. In the present study, bleaching affected cocaine-specific and nonspecific binding to most black and brown hair specimens but had no effect on binding to blond hair. Oxidative changes in amino acids can also occur after bleaching of hair (20); however, this should have not selectively affected cocaine binding to black and brown hair in comparison with blond hair.

The results from Figure 2 strongly suggest that melanin is the primary hair constituent responsible for both nonspecific and specific binding of cocaine. Similarly, other studies have demonstrated binding of drugs to melanin in natural tissues and to synthetic melanin (16,18,21,22). Patil (21) studied cocaine binding to pigmented and nonpigmented irises. Pigmented irises had an 18-fold greater binding capacity for cocaine in comparison with irises that did not contain melanin. Shimada et al. (18) and Baweja et al. (23) demonstrated the binding of cocaine and other amines to synthetic melanin. Larsson and Tjalve (24,25) studied the effect of metal ions on chlorpromazine binding to melanin and suggested that melanin bound drugs by accepting electrons and that nonelectrostatic forces such as van der Waals forces also may be involved.

A major finding in the present study was that untreated, male Africoid hair specimens bound significantly more ($p < .01$) cocaine than all other hair types evaluated. In stark contrast, cocaine binding to untreated, female Africoid hair specimens was similar to that observed with male and female, black/brown Caucasoid hair. Blond, female Caucasoid hair specimens bound the least amount of cocaine than all other hair types. Differences greater than 50-fold were observed in cocaine binding to Africoid male hair compared with blond, female Caucasoid hair specimens. Also, binding of cocaine to all groups of black and brown hair specimens was greater than to blond, female Caucasoid hair. These results are consistent with a study by Henderson et al. (26), who demonstrated greater in vivo concentrations of cocaine in the hair of Hispanics, Indians, and blacks in comparison with whites who received identical doses. Gygi et al. (11) also recently demonstrated significant differences in codeine and morphine binding in vivo and in vitro to rat hair; there was greater drug incorporation into black hair in comparison with brown hair, and brown hair bound more drug in comparison with white hair. Green and Wilson (27) reported greater in vivo incorporation of methadone into black rat hair in comparison with white rat hair. However, Mieczkowski and Newel (28) observed that, although black arrestees tested positive for cocaine at nearly twice the rates of white arrestees, the differences could be attributed to higher rates of use, based on self-reported data. They concluded that there was no evidence of racial bias in hair testing.

The differences in binding observed in the present study appear to be due to differences in melanin content between hair specimens. Although the black-colored, female Africoid hair specimens were similar in color to male Africoid hair specimens before homogenization, the color of most male Africoid hair suspensions was visually darker than that of suspensions of female Africoid hair. It seems likely that visual inspection of hair color is not a sensitive indicator of melanin content in dark hair specimens. Thody et al. (29) measured a fourfold difference in eumelanin content between two dark-brown Caucasoid hair specimens described by the investigators as similar in color based on visual inspection.

If significant bias in hair testing for cocaine occurs as a result of large variations in melanin between different ethnic groups, removal of melanin during analysis could potentially remove the source of bias and serve as a normalization step. Accordingly, an experiment was conducted that involved digestion of different hair samples containing bound cocaine. After protease digestion, the insoluble fraction (melanin) was separated before measurement of cocaine. This experiment was conducted with male Africoid and female, blond Caucasoid hair specimens because they represented extremes in cocaine binding. Removal of the insoluble melanin fraction eliminated less than 10% of the cocaine originally bound to hair; more than 90% of the cocaine remained in the soluble hair fraction after digestion for both hair types. The process of removal of the insoluble melanin fraction has been reported to eliminate ethnic bias and hair color bias that may result because of selective accumulation of drugs in hair specimens that differ in melanin content. DuPont and Baumgartner (30) reported that "any drugs contained in the melanin fraction are excluded from our analytical results and any bias due to hair color and race is thereby effectively avoided." Clearly, removal of the melanin fraction from the hair digest does not eliminate binding differences between different hair types. The

Table II. Distribution of ^{3}H(*l*)-Cocaine in Melanin Fraction and Hair Digest of Different Hair Types after Proteinase Digestion of Hair*

Hair type			% Distribution of total cocaine		
Subject	sex	color	insoluble	soluble	total DPM† in hair
Africoid					
A	Male	Black	5	95	200,478
B	Male	Black	6	94	177,318
C	Male	Black	6	94	236,445
D	Male	Black	7	93	217,772
E	Male	Black	5	95	109,985
F	Male	Black	5	95	170,039
Caucasoid					
G	Female	Blond	3	97	20,977
H	Female	Blond	4	96	9959
I	Female	Blond	6	94	9151
J	Female	Blond	5	95	3904
K	Female	Blond	4	96	10234
L	Female	Blond	9	91	6757

* Ground hair samples were treated with ^{3}H(*l*)-cocaine, incubated, washed, and separated. The specimens were digested, and the insoluble melanin fraction and soluble hair digest were analyzed as described in the Methods section.
† DPM = Disintegrations per minute.

reason for the failure in this process is probably due to the release of cocaine into the aqueous phase during digestion. Specific binding of cocaine to hair is a reversible process. In this study, specific binding typically accounted for more than 70% of cocaine binding to black- and brown-colored hair specimens. It is most probable that cocaine bound to melanin in hair would dissociate rapidly into the aqueous phase during digestion.

In summary, this study demonstrated significant in vitro differences ($p < .01$) in cocaine binding between male Africoid hair specimens and other hair types including female Africoid hair. These differences appear to be related to the melanin content of hair and not to differences in lipid content. Bleaching significantly lowered cocaine binding for all hair types with the exception of blond specimens. Consequently, it is expected that cosmetic treatments that involve bleaching or alteration of hair color can have a major impact on the outcome of hair test results. These results also indicated that removal of melanin before analysis of the soluble hair digest following enzyme digestion of hair did not eliminate cocaine binding differences between hair types. These findings add to mounting evidence that suggests that bias can exist in hair testing for drugs of abuse because of selective accumulation of drugs in male Africoid hair. This may predispose certain populations with dark-colored hair to test positive by hair analysis at a higher rate in comparison with other groups with brown and blond hair. Clinical studies are urgently needed to evaluate the possibility of bias due to ethnicity, hair color, and sex-related differences before the adoption of hair testing as a reliable drug-testing methodology.

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