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# ELEVATED CONCENTRATIONS OF DOPAMINE SULFATE IN PLASMA OF COCAINE ABUSERS*

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**Abstract**—This study investigated the effect of cocaine abuse on peripheral catecholamines. Specifically, we measured the concentration of free dopamine, dopamine sulfate, free norepinephrine, norepinephrine sulfate, free epinephrine and epinephrine sulfate in plasma samples obtained from the blood of a group of patients with cocaine addiction (N = 15). The concentrations of free and sulfoconjugated catecholamines in plasma were measured by a radioenzymatic technique. The results of this study revealed significant ($P < 0.0001$) elevation in plasma dopamine sulfate (8926 ± 1204 pg/mL) of cocaine addicts upon admission to an in-patient treatment facility when compared with the level of this dopamine metabolite in plasma of control subjects (2356 ± 121 pg/mL). Furthermore, there was a significant ($P < 0.0001$) relationship between elevation in plasma dopamine sulfate levels and severity of cocaine use among these patients, and in the majority of cases the plasma levels of dopamine sulfate declined appreciably in time with abstinence from cocaine. In contrast, no appreciable difference was observed in the concentrations of either free or sulfate-conjugated norepinephrine and epinephrine in plasma of cocaine addicts as compared with controls. Differences in plasma dopamine sulfate among these patients versus controls may be interpreted as a reflection of activation of extracellular dopamine metabolism associated with chronic cocaine exposure in humans.

Cocaine abuse has grown rapidly over the past several years, evolving into a major medical and social problem with far-reaching effects [1, 2]. Cocaine abuse causes a myriad of effects leading to cardiovascular and central nervous system complications. Symptoms of intoxication include euphoria, hypertension, hyperthermia, respiratory paralysis, and death.

Several pharmacological responses of cocaine [3] could be explained by its ability to block the uptake of the biogenic amines norepinephrine, dopamine, and 5-hydroxytryptamine into presynaptic neurons [4, 5]. This effect leads to acute increases in central nervous system and peripheral concentrations of catecholamines [6, 7]. Limited data are available describing concentrations of circulating catecholamines in chronic cocaine abusers. In view of the recent observation that the dopaminergic pathway may be implicated in the reinforcing properties of cocaine [8], we have taken the initiative in this investigation to study the plasma concentration of dopamine in patients with cocaine addiction. Because of the virtual absence of free dopamine in plasma [9, 10], we chose to study its sulfoconjugated metabolite dopamine sulfate, which represents more than 95% of total dopamine in circulation. Furthermore, the effect of cocaine abuse on plasma concentration of free and sulfate-conjugated norepinephrine and epinephrine was also examined.

Utilizing a newly developed radioenzymatic technique [9], we measured the concentration of dopamine sulfate, norepinephrine sulfate and epinephrine sulfate in plasma samples obtained from the blood of a group of patients with cocaine addiction and from controls. Furthermore, we sought an association between abnormalities in the concentration of plasma dopamine sulfate and degree of cocaine use.

## METHODS

*Subjects.* The cocaine addicts evaluated in this study consisted of ten male and five female adults. Of these addicts, nine were black and six were white. The patients were from fifteen counties in southeast Georgia who were admitted for in-patient treatment at the state-sponsored alcohol and drug treatment facilities in Waycross and Statesboro, Georgia. The cocaine addicts admitted to the study met the DSM-III R criteria for cocaine dependence [11]. Cocaine addicts who were co-dependent on alcohol and other substances of abuse were excluded from the study. Patients with active liver disease or primary psychiatric illness were excluded from the study. The control group consisted of fifteen subjects of similar age, sex, and race composition as the cocaine addicts. These were seen in The Emory Clinic for general medical evaluation. These individuals had

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Table 1. Concentrations of free dopamine (DA), dopamine sulfate (DA-S), free norepinephrine (NE), norepinephrine sulfate (NE-S), free epinephrine (E) and epinephrine sulfate (E-S) in plasma of cocaine addicts and controls

| Subjects | DA | DA-S | NE | NE-S | E | E-S |
|---|---|---|---|---|---|---|
| | (pg/mL) | | (pg/mL) | | (pg/mL) | |
| Controls (N = 15) | 10 ± 5 | 2356 ± 121 | 263 ± 95 | 720 ± 98 | 34 ± 20 | 170 ± 50 |
| Cocaine addicts (N = 15) | 12 ± 3 | 8926 ± 1204* | 212 ± 70 | 680 ± 120 | 34 ± 17 | 224 ± 70 |

Values are means ± SEM.
* Significantly different from control ($P < 0.0001$).

no history of alcoholism or cocaine addiction, had no physical or psychiatric illness, were on no medications, and had no history of any chronic medical problem. Informed consent, approved by the Human Research Review Board of the Georgia Department of Human Resources and by the Human Investigations Committee of Emory University, was obtained from all subjects prior to enrollment in the study. Blood samples were taken in the morning after an overnight fast. The majority of the subjects participating in the study received a normal diet that excluded the consumption of bananas, chocolate, soy sauce, and other food items that may affect *in vivo* concentration of dopamine.

*Sample collection.* Blood samples for the determination of sulfated catecholamines were drawn from a peripheral vein into evacuated tubes containing glutathione and [ethylene bis (oxyethylenenitrilo)] tetraacetic acid (EGTA,* Amersham Co., Arlington Heights, IL), chilled to 0°, and centrifuged immediately at 500 *g* for 10 min. The plasma was then removed and quickly frozen at −70° for later analysis.

*Radioenzymatic assay.* The levels of sulfated dopamine, norepinephrine and epinephrine were assayed by a radioenzymatic method according to modified procedures of Faraj *et al.* [9] and Johnson *et al.* [12]. Briefly, the assay procedure features the incubation of 50 μL of plasma in a total incubation volume of 100 μL consisting of 100 mM Tris, 30 mM $MgCl_2$, 10 mM EGTA, 5 μCi [$^3$H-*methyl*]*S*-adenosylmethionine (SAM) (sp. act. 9–12 Ci/mmol), 0.1 mM benzyloxyamine and catechol-*O*-methyltransferase (COMT) at a final pH of 8.1 to 8.3. A 500-pg dopamine, norepinephrine and epinephrine standard was included in every assay. A similar set of tubes was prepared in the presence of 0.05 U of the enzyme sulfatase (Arylsulfatase from Aerobacter; 10 U, Sigma Chemical Co., St. Louis, MO). The samples were incubated at 37° for 60 min. The reaction was stopped by the addition of 50 μL of a borate solution (pH 10.1) that contained 800 mM boric acid, 80 mM EDTA-$Na_2$, and 4 mM each of 3-methoxytyramine, normetanephrine and metanephrine. The extraction and isolation by thin-layer chromatography of the resulting [$^3$H-*O-methyl*]-catecholamines have been described [9, 12]. This assay enables determination of the sum of free and sulfate-conjugated catecholamine. Subtraction of free catecholamine from the sum offered the value of the sulfoconjugated catecholamine. Repeated assay of standard samples had a coefficient of variation of less than 10%. When known amounts (500 pg) of dopamine, norepinephrine and epinephrine were added to a series of plasma samples and the radioenzymatic assay was carried out in the presence and absence of sulfatase, the recovery of these catecholamines was quantitative (90–95%).

*Statistical analysis.* Correction analyses were done using Pearson product-moment estimates of linear association. Comparison of plasma dopamine sulfate, norepinephrine sulfate and epinephrine sulfate levels

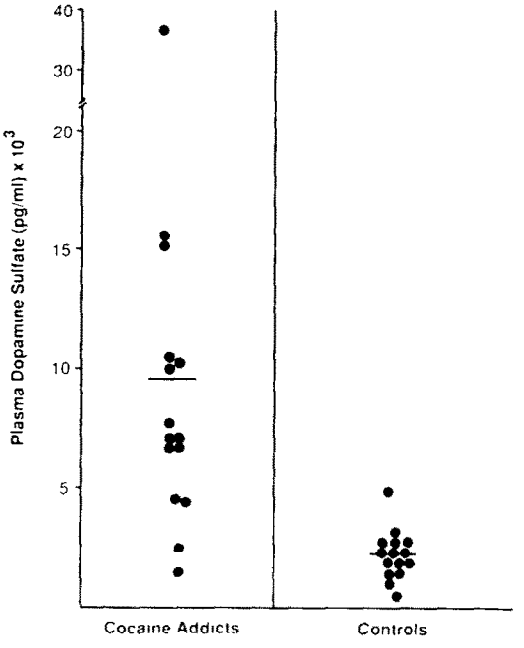


Fig. 1. Scattergram of plasma dopamine sulfate concentration levels in cocaine addicts and controls.

* Abbreviations: EGTA, [ethylene bis (oxyethylenenitrilo)] tetraacetic acid; SAM, [$^3$H-*methyl*]*S*-adenosylmethionine; COMT, catechol-*O*-methyltransferase; and PST, phenolsulfotransferase.

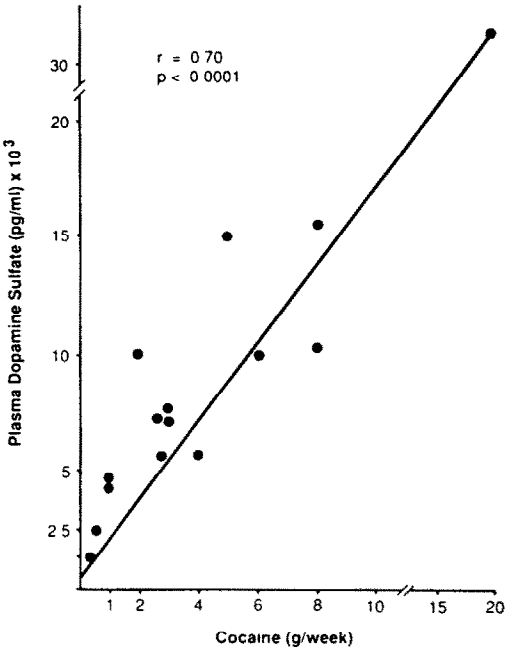


Fig. 2. Correlation between plasma dopamine sulfate and reported amount of cocaine used in cocaine addicts.

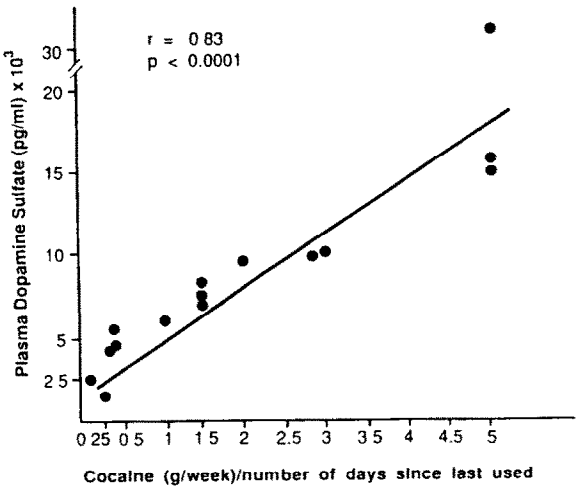


Fig. 3. Correlation between plasma dopamine sulfate and ratio of the amount of cocaine used (A) to that of the days since last use of cocaine (B) in cocaine addicts.

between cocaine addicts and controls was done using the Kruskal–Wallis test. Statistical significance was set at the 0.05 level.

## RESULTS

*Patient profile.* An overwhelming majority (94%) of the subjects evaluated in this study smoked crack cocaine. The use of cocaine intranasally was less common (6%). The average amount of cocaine used was 5 g/week. The reported presence of alcoholism and drug addiction among immediate family members of cocaine addicts studied here was about 61%.

*Concentrations of sulfoconjugated catecholamines in plasma of cocaine addicts and controls.* Patients with cocaine addiction exhibited significantly ($P < 0.0001$) elevated levels of plasma dopamine sulfate (8926 ± 1204 pg/mL, mean ± SEM; 1650–36,700 pg/mL, range) compared with controls (2356 ± 121 pg/mL, mean ± SEM; 1110–4915 pg/mL, range) (Fig. 1). However, no appreciable difference was observed in the concentrations of either free or sulfate-conjugated norepinephrine and epinephrine in plasma of cocaine addicts versus those of control subjects (Table 1).

*Correlation analysis.* Tests were done to determine whether there were correlations between the level of dopamine sulfate in plasma and age, sex, race, family history of alcoholism and/or drug addiction, reported amount of cocaine used (g/week), number of days since the last use of cocaine, and the ratio of the amount of cocaine used divided by the number of days since last used. A significant association was noted with the following. A significant correlation was noted between plasma dopamine sulfate versus amount of cocaine used ($r = 0.70$, $P < 0.0001$) and ratio of the amount of cocaine used divided by the number of days since last used ($r = 0.83$, $P < 0.0001$) (Figs. 2 and 3).

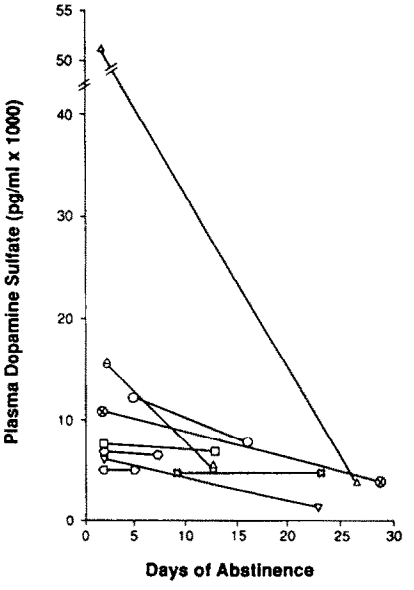


Fig. 4. Follow-up of plasma dopamine sulfate in nine patients with cocaine addiction at various time intervals with abstinence from cocaine during in-patient treatment.

An evaluation of plasma dopamine sulfate in nine of the patients demonstrated a variable decrease in dopamine sulfate with abstinence from cocaine during treatment (Fig. 4).

## DISCUSSION

The results presented in this paper emphasized

the importance of sulfoconjugation in the peripheral metabolism of dopamine. We noted that an overwhelming proportion of circulating catecholamines was represented by dopamine sulfate. In agreement with Wang *et al.* [13] and Kuchel *et al.* [14], dopamine sulfate constituted about 75% of the total catecholamines in normal human plasma and free dopamine amounted to less than 1% of total dopamine. The observed levels of sulfoconjugated dopamine, norepinephrine and epinephrine obtained in the plasma of control subjects were similar to the concentrations of these catecholamine metabolites reported by Kuchel *et al.* [14], Wang *et al.* [13] and Johnson *et al.* [12].

The source of the sulfated catecholamines in plasma remains puzzling. It is possible that amines that are released from peripheral neuroeffector junctions and overflow into circulation are sulfated in liver, kidneys, platelets and gastrointestinal tract, tissues rich in phenolsulfotransferase (PST; EC 2.8.2.1). Similar to O-methylation, sulfate conjugation of catecholamines occurs in extraneuronal tissues. The evidence supporting this hypothesis has been summarized and includes the following: (1) Tyce and Rorie [15] found a greater release of dopamine sulfate from rat brain slices when release was evoked by high $[K^+]$ than after release with phenylethylamine. Release by phenylethylamine primarily involves displacement of dopamine from vesicular storage sites into neuroplasm, whereas high $[K^+]$ release via an exocytotic mechanism into the synaptic cleft; (2) Tyce *et al.* [16] found elevated levels of dopamine sulfate in ventriculocisternal perfusates of green monkeys in the presence of nomifensine, a monoamine reuptake inhibitor; and (3) the administration of L-dopa to rats pretreated with an MAO inhibitor considerably and concomitantly augmented dopamine sulfate and 3-*O*-methyldopamine, both of which correlated with dopamine concentrations [17]. Interestingly, the increases in dopamine sulfate and 3-*O*-methyldopamine were not reduced prior to treatment of rats with the neurotoxin 6-hydroxydopamine. This suggested that dopamine sulfoconjugation, like its O-methylation, could occur outside catecholaminergic neurons.

Cocaine has been shown to block the reuptake of norepinephrine, dopamine and serotonin [4, 5]. Any one or a combination of these could markedly affect extracellular levels of these catecholamines and their metabolites in central and peripheral nervous systems. The main objective of this investigation, therefore, was to examine the impact of cocaine abuse on the concentrations of dopamine sulfate in plasma of human subjects. The results of this preliminary study revealed that cocaine addicts, upon initial presentation for in-patient treatment, had significant elevation in the plasma levels of dopamine sulfate as compared with controls. It is important to note only the sulfated dopamine that was increased in plasma of cocaine abusers since levels of both free and sulfate-conjugated norepinephrine and epinephrine obtained in plasma of these patients were comparable with those seen in controls. In spite of individual variability, we noted a significant correlation between elevation in plasma dopamine sulfate and the amount of cocaine used. This association was even stronger when levels of plasma dopamine sulfate were correlated with the amount of cocaine used divided by the number of days since last used. In an attempt to determine whether the time of abstinence from cocaine influenced the plasma dopamine sulfate concentrations, we measured the plasma level of this dopamine metabolite in nine patients at time of admission and at various time intervals following abstinence from cocaine. In four out of nine patients evaluated, there was a greater than 50% decline in dopamine sulfate levels after 11–27 days abstinence from cocaine. The noted observation that abstinence from cocaine resulted in a significant reduction in plasma dopamine sulfate levels in about 40% of the patients tested suggested to us that the effect of cocaine exposure on peripheral dopamine metabolism is partly reversible.

The effects of cocaine abuse on peripheral dopamine noted in this study may be mediated by direct and indirect mechanisms. The direct mechanism may involve blockage of neuronal dopamine reuptake by cocaine. Inhibition of neuronal uptake of released dopamine by cocaine exposes more of the amine to metabolism in non-neuronal tissues resulting in increased amounts of dopamine sulfate in plasma of patients with cocaine addiction. Interestingly, Di Giulio *et al.* [18] and Kuczenski and Segal [19] studied the effect of cocaine on the rate of formation of the O-methylated metabolite of dopamine, a pathway of catecholamine metabolism that occurs outside the neurons [20]. They found that amphetamine and dopamine uptake blockers, cocaine and nomifensine, promoted dose-dependent increases on caudate and accumbens dialysate concentrations of dopamine and its extracellular metabolite, 3-*O*-methyldopamine. The increase of extracellular dopamine levels in the brain following administration of cocaine has a time course that is in approximate agreement with the time course of euphoria reported by humans taking cocaine [21].

The preliminary observation presented here of elevated concentrations of dopamine sulfate in plasma of cocaine addicts may have significant implications in view of the recent report that the pleasurable effects of cocaine and the reinforcement that makes it habit-forming have been linked specifically to its inhibition of dopamine uptake [8]. This raises the possibility, therefore, that the measurement of plasma dopamine sulfate immediately after a binge, in association with an estimate of the elimination rate of plasma dopamine sulfate over days, may present us with a potential biological marker for assessing the severity of cocaine addiction in humans.

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