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Hair analysis for drugs of abuse

VII. The incorporation rates of cocaine, benzoylecgonine and ecgonine methyl ester into rat hair and hydrolysis of cocaine in rat hair

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Abstract. We studied the incorporation rates of cocaine and its major metabolites, benzoylecgonine (BE) and ecgonine methylester (EME), into hair from blood. It was demonstrated in our previous report (Nakahara et al. 1992a) that the incorporation rate of cocaine from blood into hair was much higher than those of BE and EME following administration of cocaine. In this study, the incorporation rates of these drugs into rat hair were investigated following independent administration of BE and EME at 10 mg/kg per day for 5 days, and co-administration of cocaine, BE-d_3 and EME-d_3 at 3, 0.5 and 0.5 mg/kg per day for 10 days. When BE and EME were administered to rats independently, levels of both these drugs were hardly detectable or quite low in hair, though their AUCs in plasma were very high. On co-administration of cocaine, BE-d_3 and EME-d_3, the deuterium labeled metabolites in hair were negligibly lower in comparison with the unlabeled ones and in particular the concentration of BE in rat hair was significantly higher than that of BE-d_3 although the AUC of BE-d_3 was higher than that of BE. It was concluded that the incorporation rates of BE and EME into hair were very low in comparison with that of cocaine and most of the BE detected in hair would be a hydrolytic product derived from cocaine in hair matrix after incorporation.

Key words: Cocaine – Benzoylecgonine – Ecgonine methyl ester – Hair – Gas chromatography/mass spectrometry

Introduction

In our previous report (Nakahara et al. 1992a), we highlighted the obvious differences in the incorporation rates of cocaine and its metabolites benzoylecgonine (BE) and ecgonine methylester (EME) from plasma into hair of rats administered cocaine. We demonstrated that cocaine was incorporated into hair much better than BE and EME although the area under the concentration versus time curve (AUC) of cocaine in plasma was much lower than that of the other two compounds. We concluded that the incorporation rate of cocaine into hair from plasma is much higher than that of the metabolites. It has also been demonstrated in other reports (Cone et al. 1991; Harkey et al. 1991; Ferko et al. 1992; Fritch et al. 1992; Henderson et al. 1992; Moller et al. 1992; Kidwell 1993) that cocaine is the main analyte in the hair of cocaine users. However, until recently it seemed that some reports using immunoassay had primarily targeted BE in the hair of humans and animals intoxicated with cocaine (Baumgartner et al. 1982; Brunner et al. 1988; Reuschel and Smith 1991). Some reports (Cone et al. 1991; Harkey et al. 1991; Moller et al. 1992) have described the ratios of cocaine to BE and cocaine to EME in cocaine users' hair. However, it is still unclear what the ratios of cocaine to its metabolites in hair mean and why these products of cocaine are not the main analytes in hair although they are found in the highest concentrations in blood.

In this study, which further investigates the incorporation of drugs into hair, animals were dosed with BE and EME independently and with cocaine and its deuterium labeled metabolites, BE-d_3 and EME-d_3, simultaneously. Their AUCs in plasma and concentrations in hair were then analyzed in order to elucidate the incorporation mechanism of cocaine and its metabolites into hair.

Materials and methods

Animals

Before drug administration, the back hair of rats was cut with an animal electric shaver. Male Dark-Agouti pigmented rats, aged 5 weeks, were intraperitoneally dosed once a day with cocaine (5 mg/kg), BE (10 mg/kg) or EME (10 mg/kg) for 5 days independently and the mixture containing cocaine, BE-d_3 and EME-d_3 (3:0.5:0.5 mg/kg) for

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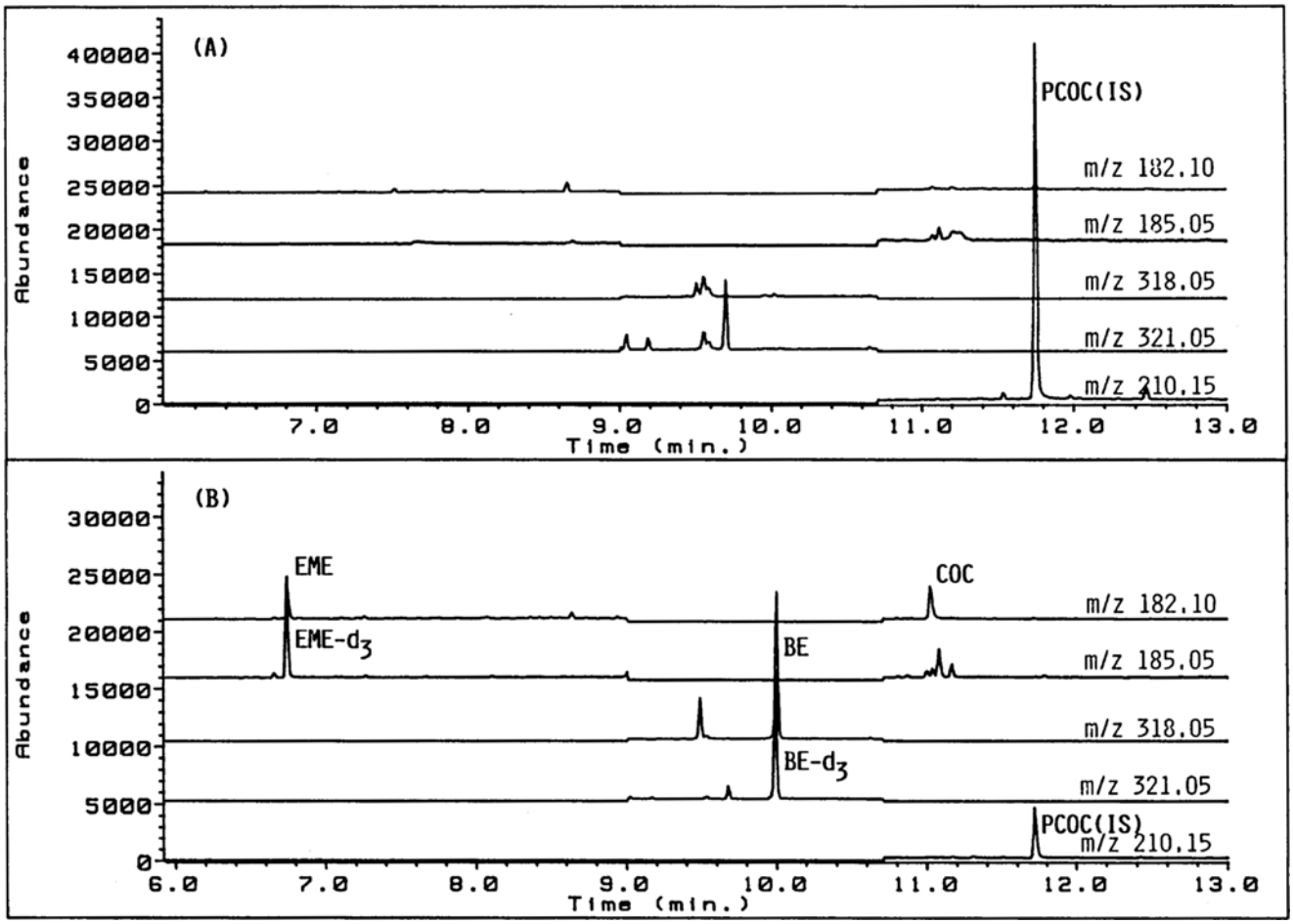

Fig. 1. Typical GC/MS-SIM chromatograms of **A** the extract from the rat control plasma and **B** the extract from the rat plasma 30 min after co-administration of cocaine, BE-d_3 and EME-d_3 at 3, 0.5 and 0.5 mg/kg

10 days (n = 3). The collection and pretreatment of hair samples and plasma samples were performed in the same manner as our previous report (Nakahara et al. 1992a).

Chemicals and materials

Cocaine hydrochloride was purchased from Takeda Pharmaceutical Co. (Osaka, Japan). BE hydrochloride and EME hydrochloride were obtained from Takeda Pharmaceutical Co. (Osaka, Japan). Cocaine-d_3, benzoylecgonine-d_3 and ecgonine methylester-d_3 were prepared as reported previously (Nakahara et al. 1992a). Pentafluoropropionic anhydride (PFPA) and hexafluoro-iso-propanol (HFIP) was purchased from Aldrich Chemical Co. (Milwaukee, Wis., USA). Bond Elut Certify, which contains a mixture of bonded silica gel and cation ion exchange, was purchased from Varian sample preparation products (Harbor City, Cal., USA).

Analytical procedures

Plasma. To 200 µl plasma was added 500 µl 0.1 M phosphate buffer (pH 6.0) and 100 µl of the internal standard (IS) solution containing cocaine-d_3, BE-d_3 and EME-d_3 at 1 µg/ml, except for propylcocaine (PCOC) as an internal standard for analysis of the deuterium compounds. The solution was applied to pre-activated Bond Elut Certify and the column was washed with water (1 ml), 0.1 M acetic acid (1 ml) and water (1 ml), successively. The column was dried under vacuum for 5 min. After the column was rinsed with methanol (1 ml) and dried under vacuum for 2 min, cocaine and the metabolites were eluted with methylenechloride-methanol (4:1) containing 2% concentrated ammonia (3 ml).

Hair. After hair samples were washed with 0.1% sodium dodecyl sulfate (SDS) and water, and dried, hair sample (30 mg) added with the internal standard solution (100 µl) was incubated with proteinase K as previously reported (Nakahara et al. 1992a). The incubated solution was extracted with Bond Elut Certify as mentioned above.

GC/MS measurement. The dried extracts from plasma and hair were heated at 60° C for 20 min in 100 µl PFPA and 50 µl HFIP. The derivatized drugs were analyzed by GC/MS (Hewlett-Packard Model 5890 MSD; Neutrabond-1 capillary column (0.25 mm × 25 m) in the reported manner (Nakahara et al. 1992a). The limits of detection were 0.2 ng/mg for BE and EME in hair, 0.4 ng/mg for cocaine in hair, 50 ng/ml for cocaine in plasma and 25 ng/ml for BE and EME in plasma in which the three major ions of the three drugs can be confirmed at a signal to noise ratio of more than 5. Typical chromatograms are shown in Figs. 1 and 2.

Stability test of cocaine during the procedure

The 300-mg rat control hair was placed in a 50-ml centrifuge tube and immersed in 4 ml methanol. To the solution containing the control hair was added 1 ml of a dichloromethane solution containing 300 ng free base cocaine. The solution was gently evaporated under nitrogen

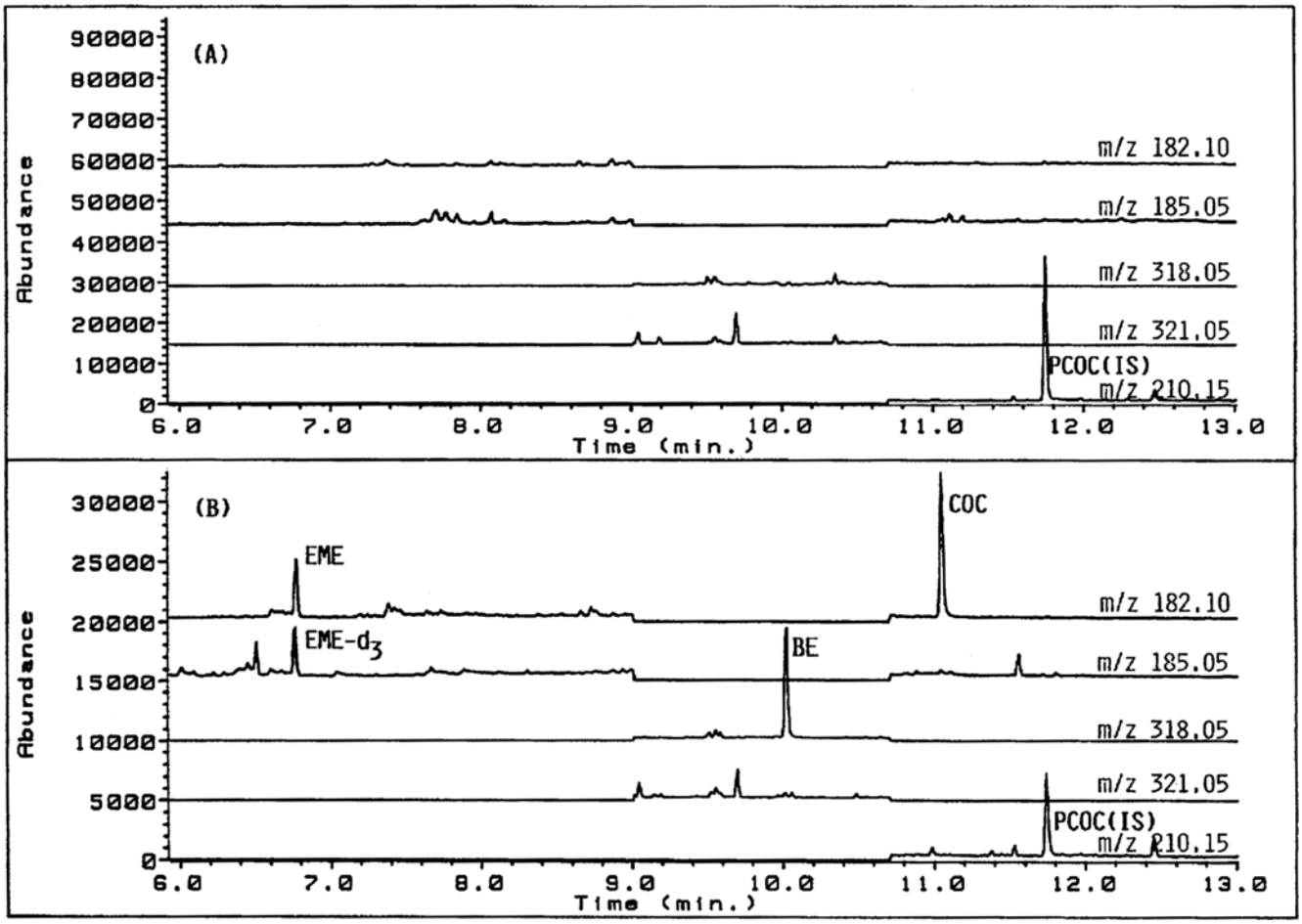

Fig. 2. Typical GC/MS-SIM chromatograms of **A** the extract from the rat control hair and **B** the extract from the rat hair 4 weeks after co-administration of cocaine, BE-d_3 and EME-d_3 at 3, 0.5 and 0.5 mg/kg

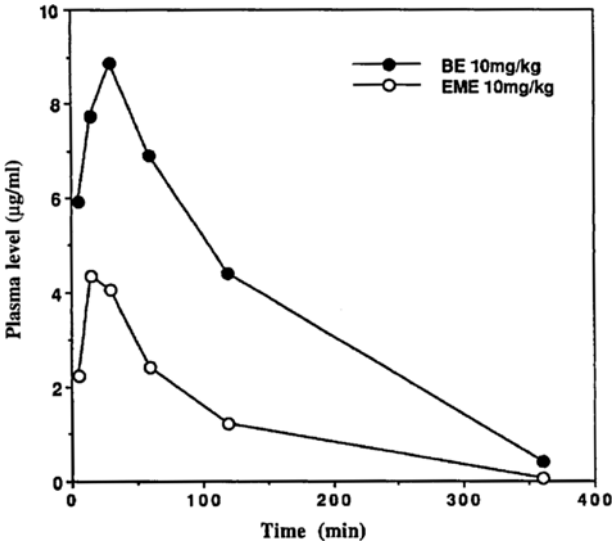

Fig. 3. Time courses of BE and EME in rat plasma after i.p. administration of BE and EME at 10 mg/kg, independently

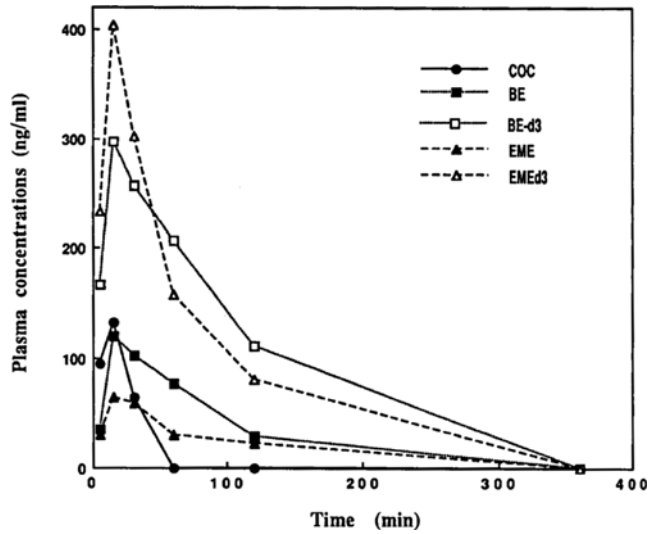

Fig. 4. Time courses of cocaine, BE, EME, BE-d_3 and EME-d_3 after i.p. co-administration at 3, 0.5 and 0.5 mg/kg, respectively

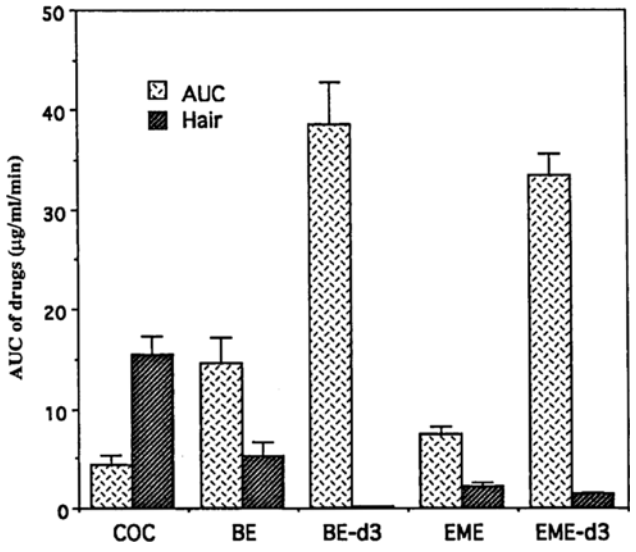

Fig. 5. Comparison of rat plasma AUC and concentration in rat hair between cocaine, BE, EME, BE-d_3 and EME-d_3 after i.p. co-administration of cocaine, BE-d_3 and EME-d_3 at 3, 0.5 and 0.5 mg/kg

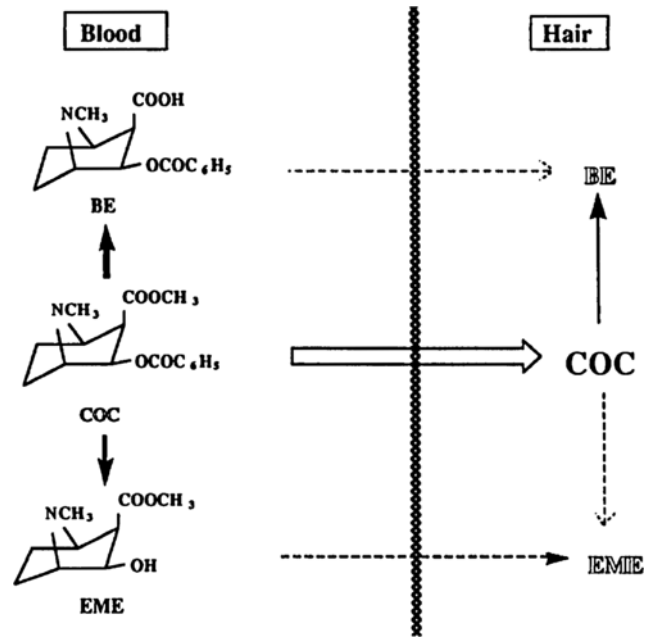

Fig. 6. Possible mechanism of the incorporation of cocaine and metabolites from blood to hair and the chemical conversion of cocaine in hair

stream with well mixing. The 30 mg of dried hair spiked with cocaine at 10 ng/mg was analyzed three times using the same procedure mentioned above.

Results

Stability test of cocaine during the procedure

In the analysis of the rat control hair spiked with cocaine, it was found that almost no cocaine was hydrolyzed to BE or EME. In the same way, cocaine-d_3 was not degraded to the metabolites during the whole procedures.

Cocaine administration

Following administration of cocaine at 5 mg/kg to rats, the average peak plasma concentrations were 415 ng/ml at 15 min for cocaine, 435 ng/ml at 30 min for BE and 188 ng/ml at 30 min for EME. The ratios of AUCs of cocaine, BE, and EME in rat plasma were 1:4.3:3.8. In contrast, the concentrations of cocaine, BE, and EME in the rat hair were 16.4, 1.7 and 0.8 ng/mg (20:2:1), respectively. In addition to our results, it has been reported (Cone et al. 1992) that the mean ratios of cocaine to BE and EME in the hair of cocaine users were approximately 10:1 and 20:1. These facts suggested that the incorporation rate of cocaine into hair was much higher than those of BE and EME. Ecgonine was also detected in hair at a level of 0.2 ng/mg.

BE or EME administration

On the administration of BE or EME to rats at 10 mg/kg per day for 5 days, both AUCs in plasma were very high (Fig. 3), 1323 μg/ml per min for BE and 411 μg/ml per min for EME. However, BE was hardly detected and EME was comparatively low (6 ng/mg) in hair despite their high AUCs.

Simultaneous administration of cocaine, BE-d_3 and EME-d_3

For the confirmation of the source of metabolites in hair, cocaine, BE-d_3 and EME-d_3 were simultaneously administered to rats at 3:0.5:0.5 mg/kg for 10 days, respec-

Table 1. Comparison between the AUCs in rat plasma and the drug concentrations in rat hair after cocaine, BE-d3 and EME-d3 i.p. administration at 3,0.5 and 0.5 mg/kg, respectively

	COC	BE	EME	BE-d3	EME-d3
AUC (∞) in plasma (μg/ml/min)*	4.3±1.1	14.6±2.6	7.3±0.9	38.5±4.3	33.5±2.1
Concentration in hair (ng/mg)*	15.4±4.8	2.2±0.7	0.6±0.2	0.1±0.01	1.4±0.1
Ratio in plasma (COC = 1)	1.0	3.40	1.7	9.0	7.8
Ratio in hair (COC = 1)	1.0	0.14	0.04	0.006	0.09
[Hair/Plasma]**	3.6	0.15	0.08	0.003	0.04

* Mean data from three experiments ± SEM are presented

** [Hair/Plasma) means the ratio of the drug concentration in hair to AUC in plasma

tively. The time courses of each drug in plasma are shown in Fig. 4 and the results regarding their AUCs and the concentrations in hair in Table 1. The concentration of cocaine in hair was also much higher than those of BE and EME, although the AUC of cocaine was lower than those of BE and EME. The AUCs of BE-d_3 and EME-d_3 in plasma were 3.1 and 4.6 times higher than those of BE and EME derived from cocaine. However, the concentrations of BE-d_3 and EME-d_3 in hair were much lower than those of BE and EME as illustrated in Fig. 5. In particular, the level of BE-d_3 was quite low in hair. It is concluded that most of the BE detected in hair was a hydrolytic product of cocaine incorporated into hair and the incorporation rates of BE and EME themselves into hair were very low.

Discussion

The rat control hair spiked with cocaine was analyzed three times by our method to test whether cocaine is hydrolyzed to BE or EME during the procedures. The results showed that no cocaine was hydrolyzed to BE or EME. In the same way, cocaine-d_3 was not degraded either to metabolites during the whole procedures. Harkey et al. (1991) have also described in their report, which used the almost same procedure as ours, that enzymatic digestion with proteinase K was the only procedure which did not degrade cocaine.

If the administration route is the same, the AUCs would be proportional to the doses within the usual range. There are some reports (Baumgartner et al. 1978, 1982; Nakahara et al. 1992b) which described the positive correlation between doses and drug levels in hair. The AUCs of cocaine and its metabolites in rat plasma were compared with each drug level in rat hair on the administration of cocaine to rats. When their AUCs were compared to each other, the AUCs of BE and EME were approximately 4 times larger than that of cocaine in plasma. In contrast, the concentrations of BE and EME in rat hair were, respectively, approximately 1/10 and 1/20 that of cocaine in rat hair. If the incorporation rate of drug from blood to hair was not different from the others, BE and EME concentrations in hair would certainly be higher than that of cocaine. Therefore, we concluded that the incorporation rate of cocaine into hair was much higher than that of BE and EME.

We had thought previously that no drugs incorporated into hair were chemically changed and therefore the metabolites of cocaine in hair came only from blood. When BE itself was administered to rats independently at 10 mg/kg almost no BE was found in the hair despite its high AUC in plasma. This result means that very little BE was incorporated into hair from blood. Following administration of EME, only a small amount of EME in comparison with its AUC in plasma was found in the hair.

If the incorporation rates are simply compared with the ratio of hair concentration to plasma AUC (hair/plasma), the hair/plasma ratios of cocaine, BE and EME are 1.15, 0.028 and 0.015 (77:2:1), if the three compounds are assumed to be stable in hair. In fact, it was shown that cocaine was more unstable than BE and EME so that a part of cocaine changed to BE even in hair. Therefore, it is concluded that most of the BE in hair came from cocaine incorporated into hair. The overwhelming accumulation of cocaine in hair might be because the permeability of cocaine from plasma into hair is much higher than that of BE and EME. This phenomenon is similar to the permeation of drugs through the blood-brain barrier.

On the co-administration of cocaine, BE-d_3 and EME-d_3, the AUCs of BE-d_3 and EME-d_3 in plasma were 2.6 and 4.6 times larger than those of BE and EME derived from cocaine. However, the concentrations of BE-d_3 and EME-d_3 in hair were much lower than those of BE and EME. In particular, the level of BE-d_3 was quite low in hair. This data also indicated that most of the BE detected in hair would be a hydrolytic product of cocaine incorporated into hair and the incorporation rates of BE itself into hair were very low. The hair/plasma ratio of EME-d_3 was 0.04 which was nearly same as the ratio (0.015) of EME following cocaine and EME administration. These data suggested that very little cocaine was converted to EME in hair. The hair/plasma ratio of EME was approximately 1/80 that of cocaine. It is presumed that the hydroxy group of EME might resist permeation between blood and hair. It is still unclear why on the co-administration of cocaine, BE-d_3 and EME-d_3, the hair/plasma ratios of BE and EME were considerably higher, compared with other experiments. The possible mechanism of the incorporation and chemical conversion of cocaine in hair is illustrated in Fig. 6. It could be concluded that only cocaine enters into hair from blood although the AUCs of the metabolites in plasma are much higher than that of cocaine, and some of cocaine incorporated into hair is hydrolyzed to BE.

Cone and coworkers (1991) found that cocaine is the principal analyte in cocaine abuser's hair and the mean ratio of cocaine to BE was 10.5, together with only traces of EME in the hair of cocaine abusers, mainly following i.v. injection. Henderson and coworkers (1992) reported that cocaine was present at concentrations approximately 5 times higher than BE and 12 times higher than EME in coca chewers' hair. Moller and coworkers (1992) reported that the mean ratio of cocaine to BE was 3 (range = 1.3–4.7) and that of cocaine to EME was 6 (range = 1–11) in coca chewers' hair. We also have some data showing that the mean ratio of cocaine to BE was 3 and that of cocaine to EME was 10 in smoking cocaine abusers' hair. It seems that the ratios of cocaine to BE and cocaine to EME in hair are similar among the different routes of administration.

We have also reported (Nakahara et al. 1992c) that the total morphine level in the hair of monkeys dosed with diacetylmorphine was much higher than that with morphine, and 6-acetylmorphine was incorporated in hair more easily than morphine when administered with heroin.

It is presumed that the incorporation rates of drug into hair might depend on physical properties of the drugs, such as their hydrophobicity, membrane permeability and affinity for melanin pigment compared with their congeners.

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