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HAIR ANALYSIS FOR DRUGS OF ABUSE XX. INCORPORATION AND BEHAVIORS OF SEVEN METHAMPHETAMINE HOMOLOGS IN THE RAT HAIR ROOT

Yuji Nakahara[1], Ruri Kikura and Kazunori Takahashi

National Institute of Health Sciences, Kamiyoga 1-18-1 Setagaya, Tokyo 158-8501, Japan

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Summary

To elucidate drug disposition in hair, the incorporation and retention behavior of 7 phenethylamines in the rat hair root were investigated: methamphetamine(MA), 3,4-methylenedioxymethamphetamine(MDMA), benzphetamine(BZP), ephedrine(EP), N,N-dimethylamphetamine(DMA), p-nitro-methamphetamine(NO2MA), and N-acetyl-methamphetamine(AcMA). On day 10 after shaving the hair on the back of the rats, drug was intraperitoneally administered at a single dose of 10 mg/kg to Long Evans rats (which were male and 6 weeks of age), possessing black and white hair, and the back hair that grew was collected by plucking with hair nippers at 0.083 h (5 min), 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 9 h, 24 h, 33 h and 48 h. After washing the plucked hairs three times with 0.1 % sodium dodecylsulfate, the amount of drug in each of the hair root samples was analyzed by a selected ion monitoring of gas chromatography mass spectrometry (GC-MS) analysis. The times at which the concentration of each drug in the hair root samples reached the peak concentration, ranged between 3.30 and 41.51 ng/mg. For each drug, the point of time at which the largest positive incremental change in drug concentration was seen, ranged between 5 min and 1 h, for all of the drugs except for AcMA which was hardly incorporated in the rat hair. The data showed that there are mainly 4 modes in which a drug becomes incorporated into the black hair root: rapid and prolonged incorporation (NO2MA, MDMA), rapid and short incorporation(MA, DMA), slow and prolonged incorporation(BZP, EP), slow and short incorporation, which includes hardly any incorporation (AcMA). As all seven drugs were hardly incorporated into the white hair, it was concluded that the combination of melanin and basic compounds is essential for a drug to become incorporated into hair. Our results suggest that a portion of the drugs in the hair root is accumulated in the hair shaft, and the remaining portion is redistributed outside the hair shaft. The second finding is that the concentration of drug incorporated into hair mainly depends on two processes - (1)the drug incorporation into hair and the drug retention in hair.

Key Words: **hair analysis, methamphetamine homologs, hair root, plasma, drug abuse**

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[1]Correspondent to; Yuji Nakahara, Ph.D., National Institute of Health Sciences, Kamiyoga 1-18-1 Setagaya-ku, Tokyo 158, Japan

Hair itself plays a role, not only as an excretory organ for drug, but also as an organ which accumulates the drug. Drug disposition in hair is quite different from that in blood and urine. Therefore, drug disposition in hair should be studied from the basic aspects to correctly evaluate the exposure of drugs to body for the past long duration. However, there are very few reports on the behavior of drugs in hair, especially in the hair root, although over 400 papers regarding analysis of drugs in the hair shaft have been published. Although it is commonly believed that a drug enters the hair very slowly from the blood, it has been shown that drugs are relatively rapidly incorporated into hair. Gygi et al. (1) have reported the rapid distribution of codeine in hair root after intraperitoneal administration. We also found (2-4) that, upon intraperitoneal administration of methamphetamine(MA), methylenedioxymethamphetamine (MDMA) or phencyclidine into rats the drug reached the rat hair root within 5 min of drug administration. Therefore, it would be possible to monitor drugs in the hair root shortly after a drug is administered, similar to drug monitoring in the plasma. Furthermore, it is expected that, since a drug remains in hair for a longer period of time than in plasma, more data can be obtained by studying drug disposition in hair. It is important to investigate the fate of a drug in hair, and compare it to that in plasma in order to elucidate drug disposition in hair and to correctly interpret the retrospective drug exposure.

In this study, seven methamphetamine homologs were administered to Long Evans rats; the black and white hairs were obtained over a 48-h period after the drug was administered. The black and white hair roots were analyzed by gas chromatography-mass spectrometry (GC-MS), and the behaviors of each drug in the hair root were compared.

Methods

Materials. The following seven phenethylamines were used in this study (Figure 1); methamphetamine(MA), methylenedioxymethamphetamine(MDMA), benzphetamine(BZP), ephedrine(EP), N-acetylmethamphetamine(AcMA), dimethylamphetamine(DMA), and p-nitromethamphetamine(NO2MA). MA and EP were purchased from Dainippon Pharmaceutical Co.(Osaka, Japan). BZP was obtained from Upjohn Company (Kalamazoo, MI). AcMA, DMA and MDMA were prepared by previously reported methods (5, 6). NO2MA was prepared by reductive condensation of p-nitrophenylacetone and methylamine, using sodium cyano-borohydride as the reductant. The structures of each compound was confirmed by both gas chromatography-mass spectrometry(GC-MS) and nuclear magnetic resonance. The purity of each drug, checked by high-performance liquid chromatography, was over 99.5% . The following compounds were used as the internal standard(IS) for GC-MS analysis of each compound: 2-methylamino-1-phenylpropane-2,3,3,3-d4(MA-d4), N-acetyl-MA-d4(AcMA-d4), 2-dimethylamino-d6-1-phenylpropane(DMA-d6), 2-benzyl, methylamino-1-phenylpropane-2,3,3,3-d4(BZP-d4), 2-methylamino-d3-1-phenylpropane-1-ol(EP-d3) and 3',4'-methylenedioxy-2-methylamino-1-phenylpropane-2,3,3,3-d4(MDMA-d4) . For GC-MS analysis of NO2MA analyses, 2-dimethylamino-d3-1-p-nitrophenylpropane(NO2DMA-d3) was used as the IS. All the deuterated drugs except for NO2DMA-d3, were prepared as previous described (4, 5). NO2DMA-d3 was prepared by performing N-deuterated methylation of NO2MA with formaldehyde-d2 and formic acid-d2.
Pentafluoropropionic anhydride (PFPA) was purchased from Wako Pure Chemical Industries, Ltd.(Tokyo, Japan). Bond Elut Certify was purchased from Varian Sample Preparation Products (Harbor City, CA).

Animal Experiments. A total of twenty-one male Long Evans(LE) rats(Charlesriver, Boston), which weighed between 120 and 130 g(age 5 weeks) were used. Each drug was administered to three rats; at each time point, the concentration of the drug in the hair root, and that in the plasma, were averaged. The animal experiments were conducted according to the guidelines of the Ethical Committee on Animal Experimentation of the National Institute of Health Sciences(Tokyo, Japan).

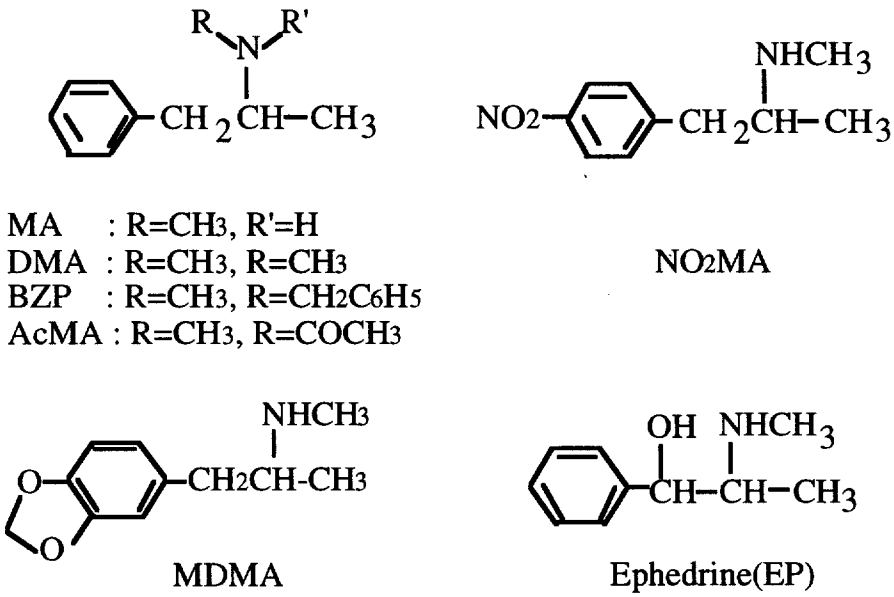

FIG. 1
Chemical structures of 7 drugs used in this study

Long Evans rats whose hair on the back had been shaved 10 days before drug administration, were injected intraperitoneally with a single dose(10 mg/kg) of one of the seven amphetamines in this study. Hair roots were plucked with hair nippers at 5, 15, 30, 60, 120, 240, 360, 480, 600 and 1440 min after drug administration. About 10 - 15 mg of hair roots was collected at each point. All data are presented as the mean ± S.E.M., of the results obtained from the three rats which received a particular amphetamine.

Washed and Unwashed Hair Samples. Fifty percent of the hair root samples obtained from each group of rats which received a particular amphetamine, were washed three times with 2 ml of 0.1 % sodium dodecylsulfate (SDS), followed by rinsing with 2 ml of distilled water; each of the three washings and the rinse were done by vortex mixing for a period of 1 min each. The hair sample was then dried in a desiccator under reduced pressure. The remaining hair root samples were not washed.

Extraction Procedures. Plasma; To 200 µl of plasma was added 500 µl of 0.1M phosphate buffer (pH 6.0) and 100 µl of the internal standard (IS) aqueous solution which contained one of the deuterated target drugs at the concentrations of 5 - 20 ng/ml. The solution was applied to a pre-activated Bond Elut Certify column, and sequentially washed with water (1 ml), 0.1M acetic acid (1 ml) and again 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, the drugs and its metabolites were eluted with 3 ml of methanol-5M hydrochloric acid (HCl) (20:1).

Hair; A volume of 100 µl of the methanol solution which contained 20-50 ng of IS, was added to approximately 10 strands of the washed and unwashed hair roots obtained from the group of rats that received the respective phenethylamine, in a 2.2 ml plastic tube. The samples were then extracted with 2 ml of methanol-5M HCL (20:1) by vortex mixing at room temperature for 14 h.

The hair roots were removed, and the solvent was evaporated to dryness under a stream of nitrogen.

Derivatization and GC/MS Analysis. For each amphetamine except for the tertiary amines, extracts of the drug obtained from the plasma, and those obtained from the hair root, were dissolved in 200 µl of PFPA/ethyl acetate (1:1); this was heated at 55℃ for 20 min. After allowing the solvent to evaporate, the residue was redissolved in 50 µl of ethyl acetate for GC-MS analysis. For the phenethylamines which are tertiary amines, the extracts mentioned above were dissolved in 50 µl of ethyl acetate-BSA (2:1); 2 µl was injected into the GC-MS column with an automatic injector.

GC-MS analysis was performed using a Hewlett Packard 5890 SERIES-II gas chromatograph equipped with a 7673A autosampler and MSD 5971. The gas chromatography(GC) was carried out with a 15 m x 0.25 mm I.D., 0.25 µm cross linked methylsilicone fused silica TC-1 (GL Sciences Inc., Tokyo, Japan). The injection port temperature was maintained at 250℃ (splitless mode) and helium was used as the carrier gas (4 psi head pressure). The oven temperature was held at 60℃ for 0.5min following injection and programmed to 280℃ at a rate of 20℃/min. The amount of drugs present in each biological specimens were investigated by the selected ion monitoring as follows; m/z 204, 160 and 118 for PFP-MA, m/z 204, 162 and 135 for PFP-MDMA, m/z 204 and 160 for PFP-EP, m/z 204 and 160 for PFP-NO2MA, m/z 148 and 130 for BZP, m/z 72 for DMA, and m/z 58 for Ac-MA; m/z 208 for PFP-MA-d4 and PFP-MDMA-d4, m/z 207 for PFP-EP-d3, m/z 153 for BZP-d5, m/z 76 for DMA-d4, m/z 75 for NO2DMA-d3 and m/z 62 for Ac-MA-d4. For a particular biological specimen, the selected ion monitoring of the respective drug and the deuterated drug, were used.

The limits of drug detection are 0.1 - 0.5 ng/mg for all of the drugs in hair. The presence of the 3 major ions on the GC-MS chromatograms, was confirmed if the ratio of signal:noise is greater than 5:1.

Pharmacokinetic Calculations. Plasma: For each phenethylamine, a graph of the concentration of the drug in the plasma, and that in the hair root, versus time, was drawn. The area under the plasma concentration vs. time curve(AUC)$_{[PL]}^{\infty}$ was calculated by the trapezoidal rule over the indicated time of measurement, and the remainder of the curve was estimated as ß·Clast where ß is the terminal rate constant and Clast is the concentration at the last time point observed. Results show means ± S.D.
Hair: AUC$_{[HR]}$ was calculated by the trapezoidal rule using 48-h measurement points. The results are shown as mean ± S.D.

Results

As shown in Fig. 2, the values of AUCs$_{[HR]}$0-6h of MA and AcMA show that the concentration of drug in the unwashed sample of black hair, is 3 to 5 times higher than that in the respective washed black samples. Trace amounts of AcMA were detected in the unwashed black hair sample, only immediately after drug administration (< 1h). Nearly all of the MA in the white hair, and nearly all of the AcMA in the black hair, were removed after three brief washings with SDS.

Figure 3 shows a graph of the concentration of each drug in the rat hair roots over the entire 48-h period. Compared to the other drugs, a relatively large amount of MDMA and NO2MA were incorporated into the hair. The concentration of each of these two drugs increased up through 10 h after drug administration; the drug was then retained well in the hair. MA was relatively rapidly incorporated into the hair root up through 4 h after administration, but, thereafter, its concentration

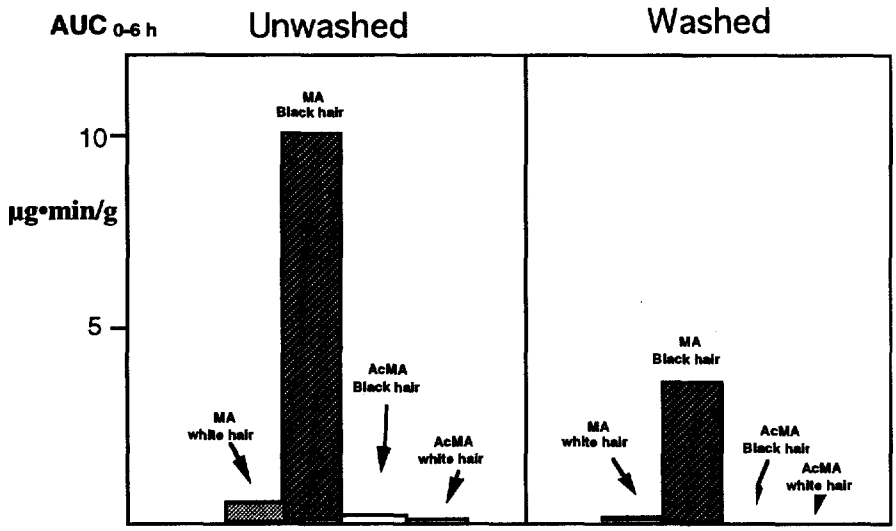

FIG. 2
The effect of washing on the value of AUC(0-6h) of MA and AcMA, in the black and white hair roots.

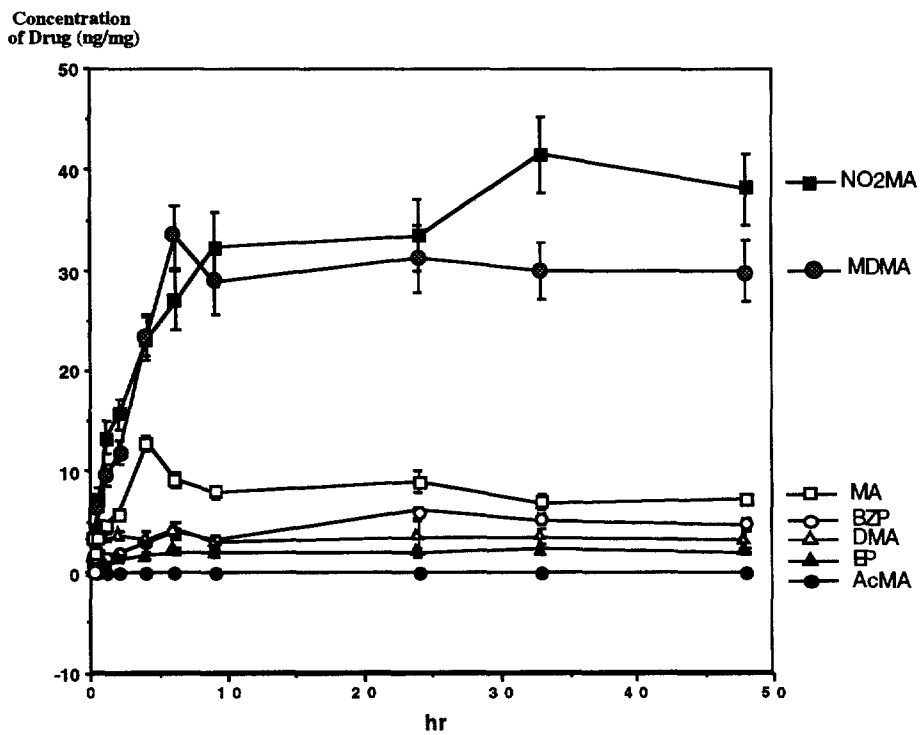

FIG.3
Time courses of the incorporation of 7 drugs in rat hair root over 48 h

in the hair root decreased until until 24 ~ 33 h after administration, and then became constant. DMA was very rapidly incorporated into the hair root during the first 15 min; thereafter, the concentration of this drug gradually decreased over 48 h. BZP and EP were gradually incorporated in the hair over the 48-h observation period. As described previously, the trace level of AcMA in the rat hair could not be detected.

The concentrations of NO2MA and MDMA in the hair root at 48 h (C_{48h}), had decreased only 5% from the respective peak concentration (C_{max}). The amount by which the concentration of each of these two drugs, decreased, is very small, compared to the other 4 drugs, which, at 48 h, had decreased 30 to 60% from the respective peak concentration.

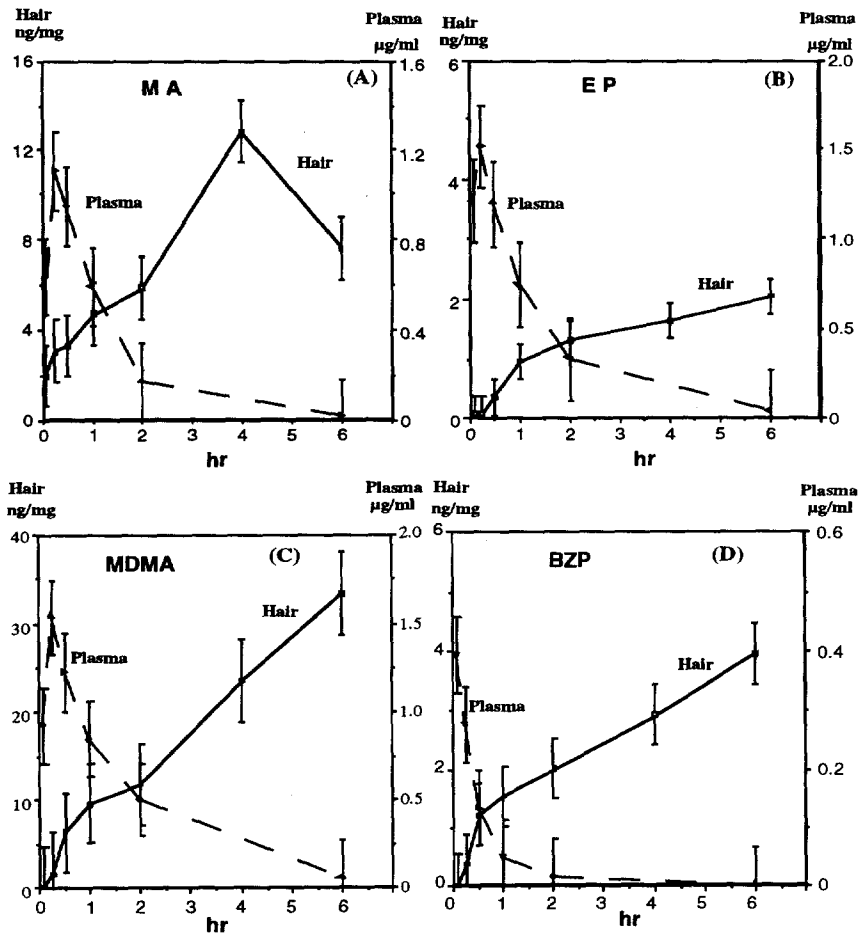

FIG. 4A

The concentration of (A) MA; (B) EP; (C) MDMA; and (D) BZP, in the plasma of the rat, as well as in the hair root, up through 6 h after the drug was administered.

Figures 4A and 4B show the graphs of the concentration of the drug in the plasma, and the respective drug in the hair root, up through 6 h after administration of each of the 7 phenethylamines. The times at which each of the 7 drugs reached its highest concentration in the hair root (Tmax[HR]) were between 2 h and 6 h after drug administration, while Tmax in

plasma(Tmax[PL]) were between 5 min and 30 min after drug administration. In all cases except for AcMA, even after the drug had disappeared from the plasma, the concentration of the drug in the hair root was still increasing forty-eight hours after administration. Although the Tmax[PL]s of MA and EP were both 15 min after intraperitoneal injection, the behaviors of the two compounds in the hair root were very different from each other, as can be seen in Figs. 4A and 4B. The Tmax[HR] of MA and EP occurred at 4 h and 6 h, respectively, after administration. The Tmax[PL]s of BZP and MDMA occurred at 15 and 5 min, respectively, after the drug was administered. The half-life of BZP in the plasma (33 min), is shorter than that of MDMA (72 min); however, the length of time for which it took BZP to reach its peak accumulation in the hair root (24 h), was much longer than the respective length of time for MDMA (6 h). This fact suggests that BZP is more slowly incorporated into hair than MDMA. The Tmax[PL] of NO2MA occurred at 30 min. However, the concentration of NO2MA in the hair root increased up through 32 h after administration.

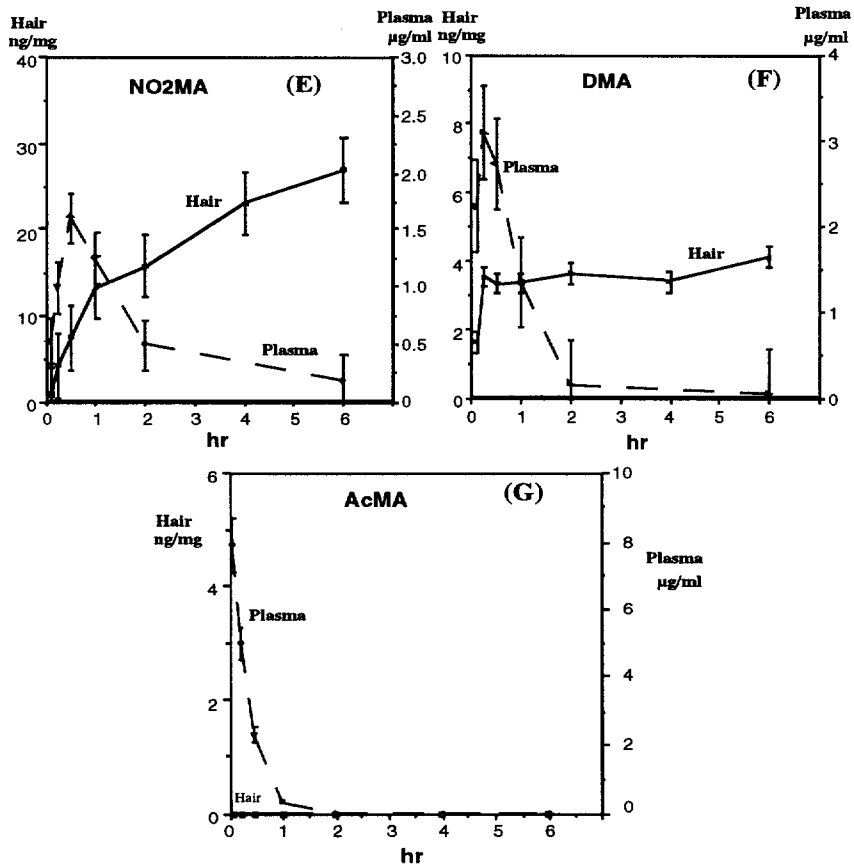

FIG. 4B

The concentration of (E) NO2MA; (F) DMA; and (G) AcMA, in the plasma of the rat, as well as in the hair root, up through 6 h after the drug was administered.

Table 1 shows the pharmacokinetic parameters of the 7 phenethylamines, as measured in the hair root, and in the plasma of the rats. The time at which 4 of the 7 drugs (except for BZP, AcMA and NO2MA), reached its peak accumulation in the hair root, occurred between 2 h and 6 h after administration. As previously reported, the Tmax[HR] of phencyclidine also occurs at 6 h after

administration (4). In contrast, Tmax[HR] of BZP and NO2MA occurred at 24 h and 33 h, respectively, after drug administration. As shown in Table 1, the half-lives of BZP and NO2MA in the plasma are short; the half-life (T1/2) of BZP was 0.72 h, and that of NO2MA was 2.68 h. This time lag would suggest that BZP and NO2MA are very rapidly distributed in the hair bulbs, and that they are continuously incorporated into the hair from the hair bulb. Since the concentration of each drug in the hair root was nearly constant after 48 h, the concentration of drug present in the hair root at 48 h, can be regarded as a steady state. The strength with which a drug is retained in the hair (drug retention), can be compared by calculating the ratio of the concentration of the drug in the hair root at 48 h (C_{48h}[HR]), vs. the value of the maximum concentration (Cmax) in the hair root (Cmax[HR]). The values of C_{48h}[HR] of NO2MA and MDMA, were nearly 90% of the respective value of Cmax[HR], while the C_{48h}[HR] of MA, DMA and EP, had decreased to a value of less than 70% of the respective value of Cmax[HR]. The C_{48h}[HR] of BZP had decreased to a value of 80% of the Cmax[HR] of this drug.

TABLE 1

Pharmacokinetic parameters of the 7 drugs in the hair root and plasma of the rat

	Plasma		Hair Root						
	Cmax (ng/ml)	T1/2 (min)	ACmax*1	Cmax (ng/mg)	C_{48h}	Percentage of C_{48h} to Cmax	AUC[HR]0-48h (μg•min/g)	AUC[PL] (μg•min/ml)	Ratio of AUC[HR]/[PL]
BZP	395	43	24 h	6.00	4.85	80.8 %	13.2 ± 2.55*2	13.7 ± 1.41	0.96
MA	1104	64	4 h	12.85	8.99	70.0 %	22.4 ± 3.89	93.7 ± 16.3	0.24
EP	1512	73	6 h	2.44	1.34	54.9 %	7.0 ± 2.05	142.5 ± 28.8	0.05
MDMA	1535	73	6 h	33.32	30.42	91.3 %	82.4 ± 11.3	173.3 ± 36.7	0.48
DMA	3085	108	2 h	3.70	2.42	65.0 %	9.7 ± 1.63	203.2 ± 65.4	0.05
AcMA	7875	181	-	0	0	-	0.0	187.2 ± 41.3	0.00
NO2MA	1605	161	33 h	41.51	37.98	91.5 %	97.4 ± 36.9	203.6 ± 77.3	0.48

*1 This abbreviation means the maxumum of accumulation in hair root.
*2 Mean data from three experiments ± S.E.M. are presented.

The value of AUC[HR] of NO2MA and MDMA, were overwhelmingly high, compared to that of the other drugs in this study. The AUC[HR] of NO2MA is especially high, in spite of the low AUC[PL] of this drug. By comparing the values of the plasma AUCs with its respective hair AUCs, the characteristics of the distribution of a particular drug to the hair, may be evaluated. The data distinctly shows that NO2MA is distributed to the hair, rather than remaining in the plasma. On the other hand, EP and DMA remain in the plasma, while showing a relatively low distribution to the hair shaft. As mentioned earlier, it was reconfirmed that any AcMA is hardly incorporated into hair. These new findings on LE rats are consistent with our previous findings on the incorporation of drugs into the hair of Dark Agouti rats (5, 6).

Discussion

In hair analysis, it is hard to know if a detected drug had existed inside or outside the hair, even if the hair sample is washed. Especially in the hair root (or bulb), it is very hard to isolate drugs that

exist only within the hair bulb, because plucking hair bulb is bare and drug inside hair is also susceptible to effect of wash. However, it was considered that it would be possible to remove any blood adhered to the hair bulb by several rapid washes. It seems likely that any blood which existed on the hair bulb as a result of surface adhesion of the capillary vein onto the hair bulb, was washed away by a few simple washes with SDS; however, a portion of the drug which exists inside the hair may also have been affected by the washings. Nevertheless, to compare the concentration of various drugs incorporated inside the hair shaft, it presumably would be necessary to wash the hair samples before extraction.

Although six drugs, except for AcMA, were detected at relatively high concentrations in the black hair, the concentration of each of the 7 drugs in the white hair, was below the detectable limit. It is reasonable to consider that our finding that barely any of the 7 drugs were incorporated into the white hair, is due to the absence of melanin in white hair. It has already been demonstrated that melanin in hair, especially eumelanin, plays an important role as a host compound with which the drug interacts, and becomes incorporated into the hair shaft (5, 7-12). In regards to the contribution of melanin to drug incorporation in hair, the evidences showing the binding or affinity between drugs and melanin have been shown in many reports. Potsch, et al.(7), showed in animal experiments using guinea pigs having hair of three colors, that the presence of eumelanin in hair, rather than pheomelanin, is the decisive factor for codeine-melanin binding in hair to occur. Joseph, et al.(8), reported that melanin was considered to be the most likely binding site for cocaine in hair. Gygi, et al. (9), and Slawson, et al. (10), reported that pigmented hair possesses a greater capacity to bind and incorporate drugs, than nonpigmented hair. Gerstenberg, et al. (11), demonstrated that hair pigmentation has a major influence on systemic uptake of nicotine in hair. Finally, Uematsu, et al. (12), found that haloperidol is selectively incorporated into black hair, but not into white hair. We previously demonstrated by in vitro experiments that a good correlation exists between the amount of a particular drug that becomes incorporated into the hair shaft, and the degree of that drug's affinity to melanin (5). In this study, concentration of phenethylamine in the black hair of LE rats reached as high as 41.51 ng/mg, while the concentration of phenethylamine in the white hair was nearly zero. This fact suggests that white hair, which does not contain melanin, lacks the ability to incorporate drugs and/or the ability to retain drugs in the hair.

On the other hand, drugs that are regarded as being a guest compound, seem to need specific properties of basicity and lipophilicity, for that drug to become incorporated into hair (5). Despite the finding that a larger concentration of AcMA exists in the plasma than MA, barely any AcMA was detected in the hair root. This phenomenon occurred because AcMA lost its basicity due to N-acetylation. This indicates that the basicity of a particular drug is an essential factor in determining if that drug becomes incorporated into hair.

Differences were seen in the ways that MA, and its nitro-analog (NO2MA), became incorporated into hair. This suggests that nitro-compounds are incorporated for a longer period of time into hair, and are more strongly retained in the hair shaft, compared to the original compound (MA).

Comparing the rates of drug incorporation into the hair root up through 6 h after drug administration, MDMA, MA and NO2MA were classified as being rapidly incorporated into hair, while BZP and EP were more slowly incorporated into hair. Among the drugs classified as being rapidly-incorporated drugs, MDMA and NO2MA were incorporated into the hair over a long period of time. DMA was rapidly incorporated into hair over a relatively short period of time, while EP was slowly incorporated into hair over a relatively long period of time. Summarizing these phenomena, the way in which a particular drug becomes incorporated into the hair root, was classified into 4 modes. These are rapid and prolonged incorporation; rapid and short incorporation;

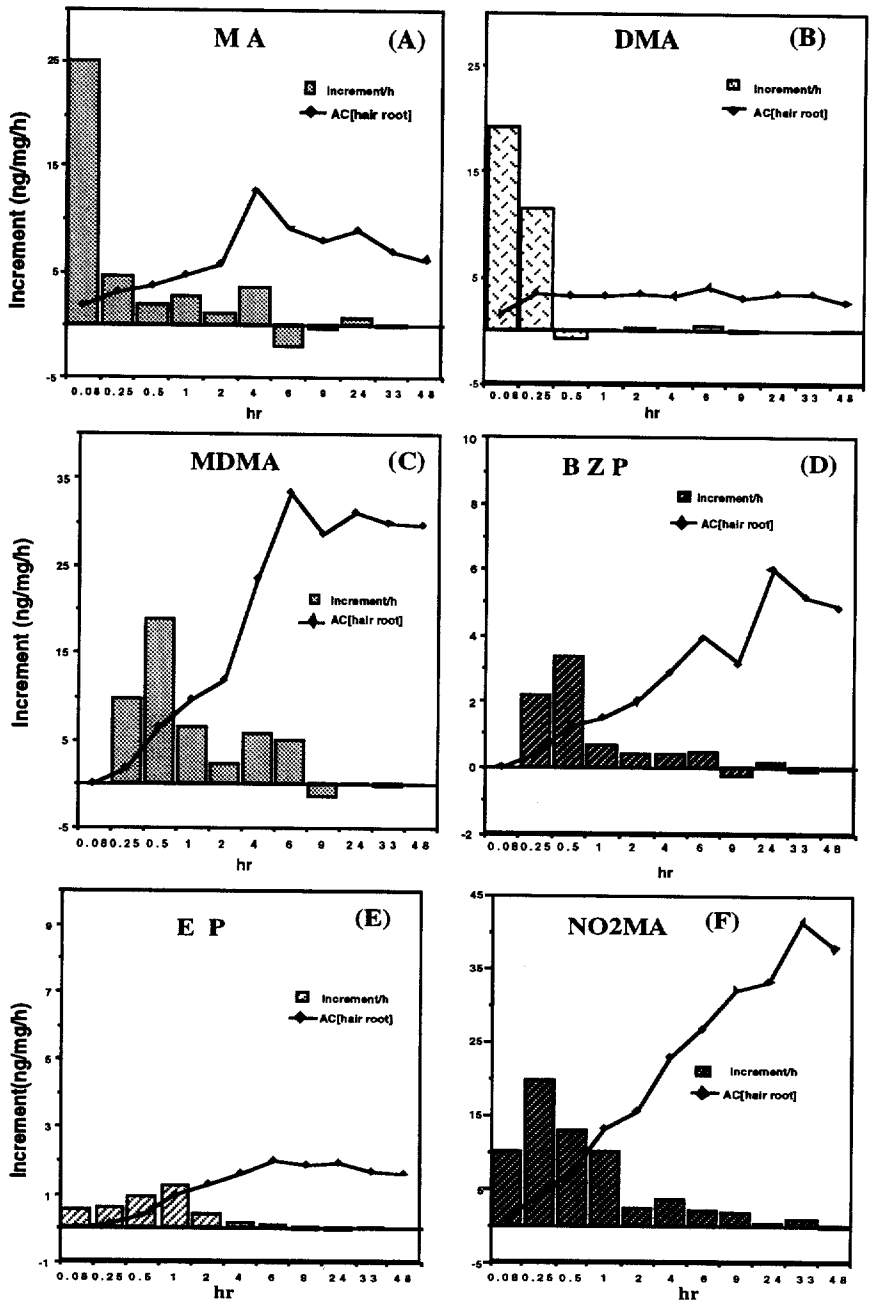

FIG. 5

Graphs of the incremental changes in the concentration of a drug incorporated into rat hair, at each measured time point. The incremental change at each data point was calculated to be the percentage that the drug concentration had changed, compared to the drug concentration measured at the time point immediately previous to that. The line graph represents the concentration of drug incorporated into the hair over the 48-h measurement period. (A) MA; (B) DMA; (C) MDMA; (D) BZP; (E) EP; (F) NO2MA.

slow and prolonged incorporation; and slow and short incorporation, which includes barely any incorporation. These factors would determine the amount that a particular drug becomes incorporated into hair.

The drug concentrations in the hair root obtained in this study, are the values of accumulation after dosing. In order to examine the dynamic behavior of drug incorporation into the hair root, we calculated the incremental change in the concentration of a particular drug in the hair root at each data point, based on the previous measurement point. Figure 5 shows a graph of the incremental changes in drug concentration in the hair root, at every measured time point; a graph was drawn for each of the 7 phenethylamines in this study. MA and DMA showed the largest positive incremental change in drug concentration in the hair root, within the first 5 min after the drug was administered. The largest positive incremental change of NO2MA in the hair root, occurred between 5 and 15 min after drug administration; that of MDMA, and that of BZP, occurred between 15 and 30 min after drug administration. Thereafter, MDMA and BZP showed relatively small, positive incremental changes up through 4 h after drug administration. NO2MA maintained large, positive incremental changes up through 9 h after drug administration. A relatively small amount of EP was incorporated into hair; for EP, the largest, positive incremental change occurred between 30 min and 1 h, which is relatively late in time compared to the other drugs.

The conclusions of this study are as follows. The combination of host (melanin), and guest (basic compounds), is essential for a drug to become incorporated into hair. Our results suggest that a portion of a drug which reached hair bulb is incorporated in the hair shaft, and that the remaining portion is redistributed to the other part from hair bulb. The amount of drug incorporated into hair is determined mainly by two processes - incorporation into hair, and retention in hair. Four modes of drug behavior in the hair root were seen: rapid and prolonged incorporation; rapid and short incorporation; slow and prolonged incorporation; and slow and short incorporation, including barely any incorporation. Drug incorporation into hair is greatly affected by the physicochemical properties of a particular drug, and the functional groups of the drug compound.

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