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FACTORS INFLUENCING THE PLASMA LEVELS OF AMPHETAMINE AND ITS METABOLITES IN CATHETERIZED RATS

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Abstract—Rats fitted with chronically indwelling Silastic tubing catheters in the right jugular vein were given orally a low dose (0.067 mg/kg) of *d*-amphetamine sulfate (A) alone or in combination with other chemicals or environmental conditions. In general, A levels increased slowly over 1 hr, peaked and declined during the 4-hr test period. Levels of metabolites (M) were already appreciable at 15 min, slowly increased to 1 hr, and remained constant over the rest of the testing period. A variety of chemicals and environmental conditions that were meant to mimic some human situations (social interaction, food, stress, alcohol, bicarbonate and ammonium chloride) selectively affected the half-lives, the areas under the time-curve, and the levels of A and M at different times during the experiment. In two cases, individual differences in A and M blood levels were found in these rats, which belong to a genetically homogenous group.

The pharmacokinetics of amphetamine (A) have been studied in rats [1–3]. However, these studies have usually employed i.p. administration, much higher doses than those used in man, and blood collection by decapitation which requires a large number of animals and permits data collection only once in an animal. In addition, this method of blood collection does not allow for the detection of small variations, which can only be observed if the animal is used as its own control, and of individual differences that are known to exist [4, 5]. Furthermore, these data are difficult to extrapolate to the human situation where smaller quantities of the drug are usually taken orally, often in conjunction with other drugs or foods, and where the drug is subjected to individual genetic influences.

For this reason, we modified and improved an existing animal model with an indwelling catheter [6] for successive blood drawings. This model requires a much smaller number of animals and allows for the detection of small and individual differences. We then used this model to study the effects of some environmental and nutritional factors on the pharmacokinetics of A and its metabolites (M) under some conditions which perhaps more closely resemble human situations.

METHODS

Male Wistar rats weighing 275–375 g (Perfection Breeders, Douglasville, PA) were anesthetized with pentobarbital (40 mg/kg, i.p.), and fitted with a Silastic tubing catheter in the right jugular vein as described previously [6].

Modifications of this procedure were as follows: (1) the tubing was extended to a total length of 85 cm to allow more freedom of movement for the animal and for blood drawings without disturbance of the rat, (2) the tubing was shielded by spring steel (3/16 in. i.d.; 80 turns/in.) which was attached to the skin by at least four stitches and cemented (dental acrylic) to the skin as one unit, and (3) the spring steel containing the catheter was supported by a small swivel pulley allowing vertical and circular movements while being counterbalanced by a small weight (about 10 g).

These modifications protected the catheter and the animals did not seem to be affected; with this procedure we have kept animals for up to 3 weeks.

After a post-surgical recovery period of at least 5 days, animals were given by gavage the human equivalent of about 5 mg/70 kg or 0.067 mg/kg *d*-amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) containing 2×10^6 cpm [^{3}H]*d*-amphetamine sulfate (15–30 Ci/mmole, New England Nuclear, Boston, MA) in 0.5 ml water alone or in combination with Nutrament, 43 mg/kg NH_4Cl, 29 mg/kg $NaHCO_3$ [7], or 0.2 ml of 100% ethanol [8]. Amphetamine was also given by intraperitoneal injection at the beginning of a restraint stress [9] period done by taping the animals to a wooden table for the first 60 min of the study and in the presence of a second, non-experimental animal [4]. Each animal was used for these experiments with 2 days rest between experiments. The types of experiments were rotated to avoid possible influences of previous exposure on following tests.

Sampling syringes were 1 ml insulin syringes with 23 gauge × 1 in. needles containing 100 units/ml heparin in saline. Blood sampling was initiated by withdrawing the heparinized saline from the catheter until blood could be seen entering the syringe. The

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syringe was removed and a sample of approximately 0.25 ml blood was drawn into a second syringe. The first syringe was then re-attached and the total fluid withdrawn was replaced with heparinized saline.

The sampling syringe without a needle was used to obtain the plasma. The syringe was cut with strong scissors to remove the flanges and plunger top so that the cut syringe could be placed in an inverted position inside a centrifuge trunion.The plastic case that surrounds new needles was positioned over the cut plunger so that the plunger would not be depressed on centrifugation. The syringe was spun immediately after blood sampling to obtain plasma. Once separated, the plasma was pumped out of the syringe into a test tube. The exact plasma weight (g) was obtained by subtracting the weight of the syringe after plasma removal from the weight of the syringe filled with whole blood. At the end of the experimental period, the red blood cells were given back to the animal.

Amphetamine was isolated by alkaline extraction into an organic solvent [10]. Two hundred μl of plasma were made alkaline with 200 μl of 0.1 N NaOH to which 3 ml of toluene–isoamyl alcohol (97:3) were added. The tubes were vortexed, centrifuged at 1000 rev/min for 3 min, and the aqueous phase was quick frozen by placing the tubes in a $-70°$ freezer for 4 min. The top organic phase was decanted into a scintillation vial. This was repeated with an additional 3 ml of organic solvent. To the vials were added 10 ml of scintillation fluid (16.5 g PPO,* 0.5 g POPOP, and 500 ml Triton X-100 in 2.5 l. of toluene) and the radioactivity was determined. Verification of isolation of A was done by radiochromatography using *n*-butanol–acetic acid–water (25:4:10); only one radioactive spot was found in the organic phase corresponding to the R_f of externally applied amphetamine.

The remaining aqueous phase was thawed and poured into a scintillation vial; the test tube was washed twice with 5 ml Biofluor (New England Nuclear) and then the Biofluor aliquots were added to the vial. Radioactivity was counted and assumed to be present from the metabolites. Chromatography (see above) of the aqueous phase showed no radioactivity in the area where externally added amphetamine was found.

To assure proper functioning of the catheter, seven animals received orally 0.067 mg/kg of A. After 1 hr, blood was obtained from four rats through the catheter and from three rats by decapitation. No significant difference was found between the two groups; A levels were 2.4 ± 0.7 and 2.8 ± 0.6 ng/g and M levels were 11.1 ± 3.1 and 9.3 ± 0.3 ng/g, respectively.

Statistical evaluations were performed by using the paired *t*-test and Student's *t*-test.

RESULTS

During our studies we found that plasma levels of A and M varied among animals after oral intubation, with some animals showing low and other animals showing higher A and M levels. To see whether or not the same animal would show the same pattern on a different day, two rats were selected which exhibited low and high blood levels of A and M. These rats received a second dose of A 2 days later. Figure 1 shows that the blood levels of A and M were similar in each rat on both occasions, indicating individual differences in this genetically quite homogenous population.

Figure 2 shows data on the pharmacokinetics of A and M alone, and when influenced by the co-administration of food and chemicals as well as social interactions and stress.

Generally, rats dosed orally with A showed already appreciable levels of A and M at 15 min. Levels of A increased only slightly over the next hour before a decrease could be seen. Levels of M increased for the first 2 hr and then remained con-

* PPO =2,5-diphenyloxazole; and POPOP = 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene.

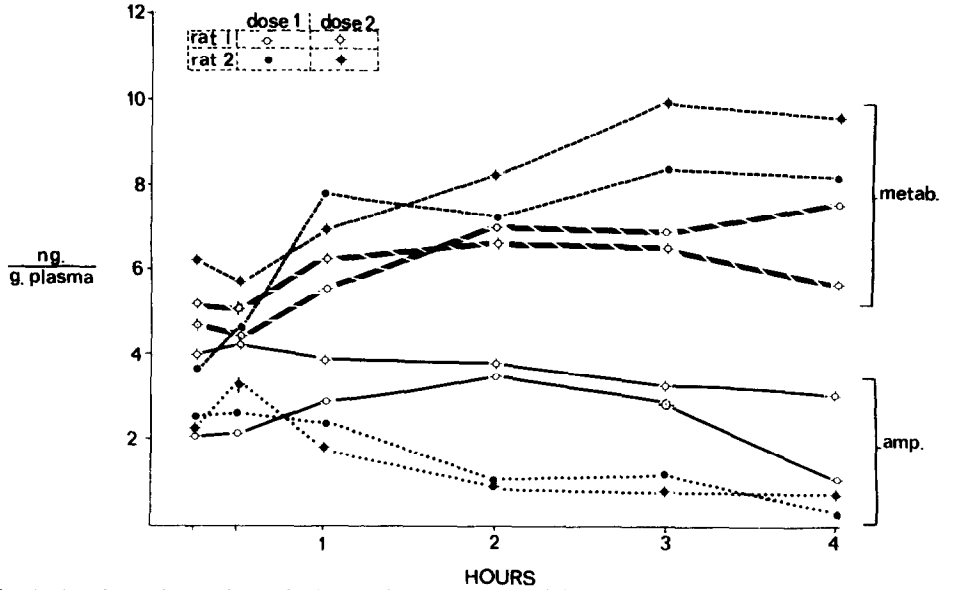

Fig. 1. Amphetamine and metabolite levels after oral administration of two doses of 0.067 mg/kg *D*-amphetamine sulfate which were given 2 days apart.

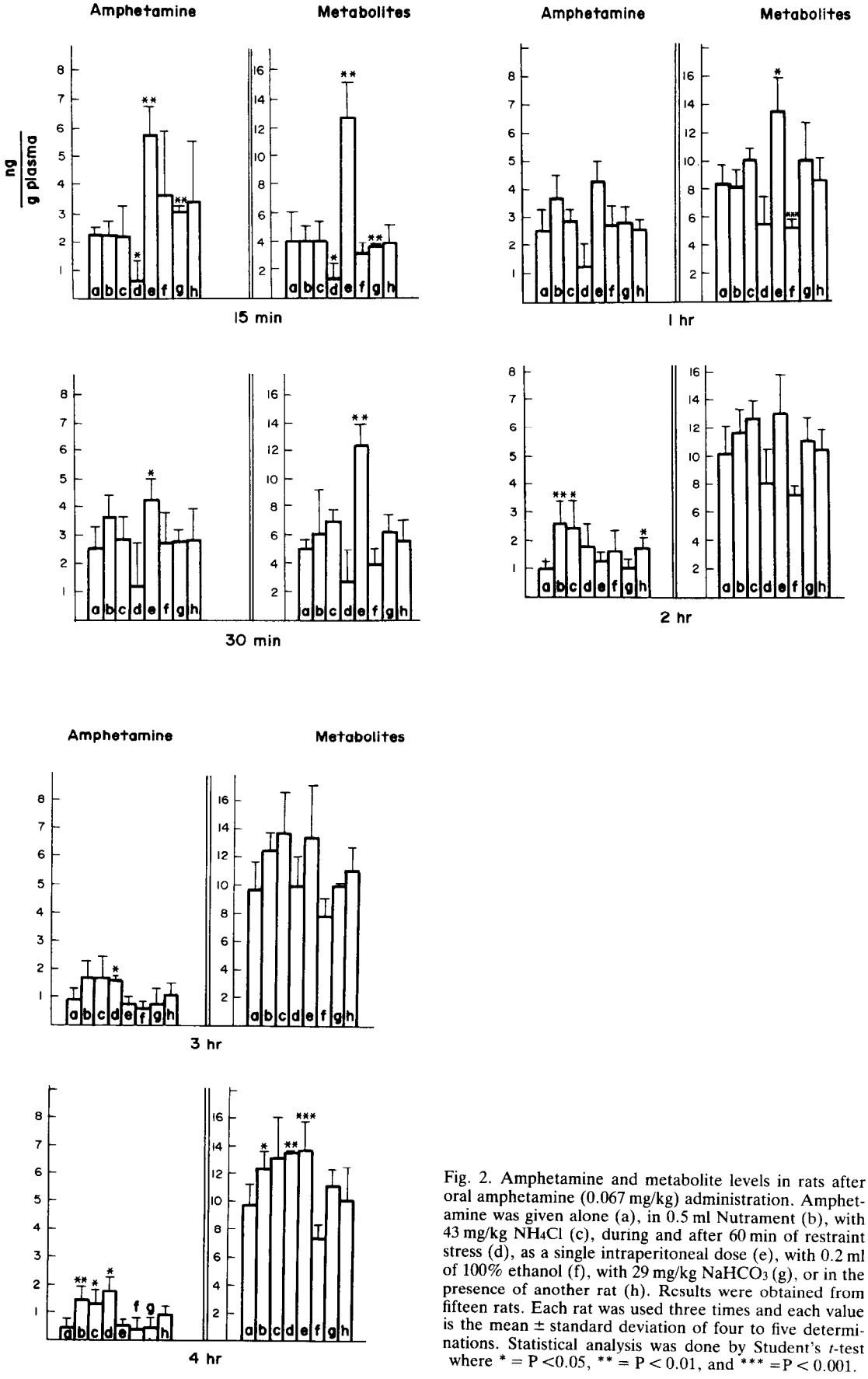

Fig. 2. Amphetamine and metabolite levels in rats after oral amphetamine (0.067 mg/kg) administration. Amphetamine was given alone (a), in 0.5 ml Nutrament (b), with 43 mg/kg NH_4Cl (c), during and after 60 min of restraint stress (d), as a single intraperitoneal dose (e), with 0.2 ml of 100% ethanol (f), with 29 mg/kg $NaHCO_3$ (g), or in the presence of another rat (h). Results were obtained from fifteen rats. Each rat was used three times and each value is the mean ± standard deviation of four to five determinations. Statistical analysis was done by Student's *t*-test where * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

stant for the rest of the test period. Not shown in the graph are the 24-hr values; at this time no A was detectable and M had declined to about 10 per cent of the 4-hr values.

The simultaneous administration of Nutrament was without effect except at 2 and 4 hr when A was increased and at 4 hr when M was increased.

The co-administration of a dose of ammonium chloride comparable to the human dose changed urinary pH from 6.5 to 5.8 in our rats and resulted in an increase in A levels at 2 and 4 hr without significant effects on M levels. This increase is somewhat surprising since NH_4Cl decreases A levels in humans by increasing urinary excretion. In our case, NH_4Cl and A were given simultaneously and elevated levels can perhaps be explained by increased absorption into the blood stream which is greater than the increase in urinary excretion.

Rats restrained during the first hour of the experiment showed lower levels of A at 15 min and higher levels over the last 2 hr. Levels of M were lower at 15 min and higher at 4 hr.

A comparison of oral and intraperitoneal administration shows the expected higher levels of A and M during the first 30 and 60 min respectively. Later on, levels of A and M are similar to controls except for 4 hr when M is again significantly elevated.

The simultaneous administration of ethanol was generally without effect on the A levels, but significantly depressed M levels at 1 hr.

Co-administration of sodium bicarbonate at the human dose, to alkalinize the urine, changed urinary pH from 6.5 to 7.4 in our rats and increased levels of A and decreased levels of M only at 15 min; no effects were seen at other times.

It is interesting that placing another rat in the cage of the experimental rat during the 4-hr experiment resulted in higher A levels at 2 hr with no effects on the levels of M.

The half-life of A in our rats was found to be 63 min which is in agreement with others who gave higher doses and found half-lives of 52 and 75 min respectively [5, 11]. Nutrament, NH_4Cl and social interaction significantly ($P < 0.05$) increased half-lives to 147, 140 and 108 min respectively.

A calculation of the area under the curve (ng/g × 4 hr) for A and M levels resulted in values of 5.1 and 33.1 respectively. The value for A was significantly ($P < 0.05$) increased by NH_4Cl (8.8) and Nutrament (10), and the value for M was significantly changed by ethanol (22.2), i.p. administration (51.6), and NH_4Cl (44.4).

DISCUSSION

The animal model reported in this paper requires only minor surgery, inexpensive equipment, and can be learned readily by the experimenter. The modifications allow the animal to move freely over prolonged periods of time and the experimenter to draw blood without touching or disturbing the rat. Our animals behave normally, as judged by gross observations, and appeared not to be encumbered by the catheter. Drawing of 0.2 ml of blood five times during a 1-hr period did not change the hematocrit, and values for corticosterone and catecholamines were at basal levels (unpublished observation). The animals did not try to bite or remove the catheter after the first 24 hr, and a second animal placed into the cage paid no unusual attention to the catheter. The latter observation allows the study of the effects of social interactions on drug kinetics in individual animals, and our results show that such interactions can indeed influence the half-life of A and, possibly, of other drugs as well.

The use of the catheter reduced the number of animals drastically, since one animal can be used for more than one blood sampling or dose of A. We used a total of 15 rats, and each rat was dosed three times with A alone and in combination with the conditions studied; however, more experiments could have been performed. This schedule gave us four to six determinations for each time point. In contrast, blood sampling by decapitation would have required five determinations × eight experimental conditions (a–h) × six times (15 min to 4 hr) = 240 rats.

More important than the decreased cost is the detection of small and/or individual differences which cannot be observed with the traditional studies using trunk blood. Although blood levels of A and M after a given dose were within a relatively narrow range, we found in one experiment two animals which were consistently low or high in A or M levels. Implantation of the catheter and the repeated use of the same rat is one important feature of this model, since it allows us to detect and study individual differences in a genetically homogenous population. Furthermore, using the same rat as its own control eliminates individual variations and allows the detection of more subtle changes. Such changes became apparent during our studies, as mentioned later.

Although the blood levels of A and its metabolites, as well as their interactions with other agents, have been studied, no information is available in rats about the pharmacokinetics of A after oral administration of the drug at a dose which is comparable to that used in man (about 5 mg/70 kg) and its interaction with other substances or environmental conditions. In general, A is rapidly absorbed after oral administration and peak levels are present after about 15 min. The level of A reaches a plateau, declines after 1 hr and decreases to about one-tenth after 4 hr. Levels of M are already appreciable after 15 min, slowly increase until 1 hr and then remain fairly constant for up to 4 hr.

The effects of various chemicals and conditions on A and M were evaluated by using blood level determinations at different times, and calculations of half-lives and areas under the curve. Since the same animal was used in these comparisons, individual variations were eliminated and small differences became statistically significant. All of the chemicals and conditions produced changes in one or the other evaluation procedure. The half-life of A was affected by Nutrament, NH_4Cl and social interaction. The areas under the curves for A and M were altered by NH_4Cl and Nutrament, and by ethanol, i.p. administration and NH_4Cl respectively. Differences in A and M levels at various times were usually not marked and were seen only at some time(s) during

the period of study. Such small changes became only significant in our model since the animal served as its own control, and suggest that blood levels of A and M (and probably other drugs) do not follow a smooth curve but can be slightly affected at any time during an experiment.

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