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Biliary Excretion of Amphetamine and Methamphetamine in the Rat

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1. ^{14}C-labelled amphetamine and methamphetamine were injected into rats cannulated at the bile duct under thiopentone anaesthesia and the output of their metabolites in urine and bile was determined. 2. With amphetamine, 69% of the ^{14}C was excreted in the urine and 16% in the bile in 24h. The main metabolite in bile was the glucuronide of 4-hydroxyamphetamine. The output of unchanged amphetamine was much greater in cannulated rats than in intact rats. 3. With methamphetamine, 54% of the ^{14}C appeared in the urine and 18% in the bile. The main metabolite in the bile was the glucuronide of 4-hydroxynorephedrine. The output of amphetamine, a metabolite of methamphetamine, was much greater in cannulated rats than in intact rats. 4. Evidence has been obtained for the enterohepatic circulation of certain amphetamine and methamphetamine metabolites in the rat. 5. Thiopentone anaesthesia appeared to inhibit the ring hydroxylation of amphetamine administered as such or formed as a metabolite of methamphetamine.

Dring *et al.* (1970) have shown that orally administered (±)-amphetamine (2-amino-1-phenylpropane) is excreted by the rat mainly in the urine as conjugated 4-hydroxyamphetamine [2-amino-1-(4′-hydroxyphenyl)propane; 60% of dose]. The faecal excretion of metabolites was small and in the region of 4–5% of the dose. For (±)-methamphetamine (2-methylamino-1-phenylpropane), Caldwell *et al.* (1972) showed that the metabolites of this drug were also excreted mainly in the urine (about 80% of the dose) in the rat, the faecal excretion being only 2% of the dose. The main metabolic route of methamphetamine was also aromatic hydroxylation, the major metabolite being conjugated 4-hydroxymethamphetamine [1-(4′-hydroxyphenyl)-2-methylaminopropane] together with significant amounts of 4-hydroxynorephedrine [2-amino-1-(4′-hydroxyphenyl)propan-1-ol] and 4-hydroxyamphetamine.

The biliary excretion of these two drugs has now been examined in rats, and we show that there is a significant biliary excretion of the conjugates of the hydroxylated forms of these drugs. But since there is little faecal excretion of these drugs or their metabolites, the significant biliary excretion suggests that there may be an enterohepatic circulation of the hydroxylated forms of amphetamine and methamphetamine.

Materials and Methods

Compounds

(±)-[^{14}C]Amphetamine sulphute {(±)-2-amino-1-phenyl[1-^{14}C]propane; 16.4 μCi/mg} was the gift from Smith, Kline and French Laboratories, Philadelphia, Pa., U.S.A. (±)-[^{14}C]Methamphetamine hydrochloride {(±)-2-methylamino-1-phenyl[1-^{14}C]propane} was prepared, and other compounds were obtained, as previously described (Caldwell *et al.*, 1972).

Animals

Female Wistar albino rats (200±10g; Allington Farm, Porton, Wilts., U.K.) were bile-duct cannulated under thiopentone anaesthesia as described by Abou-El-Makarem *et al.* (1967). They were put in restraining cages (Bollman, 1948) and kept warm with suitable lamps above the cages. The bile was collected in graduated tubes and the urine in polythene trays fitted underneath the cages. The labelled drugs were administered intraperitoneally dissolved in water 2h after administration of the anaesthetic. If the rats were dosed with these drugs, which are powerful stimulants of the central nervous system, while still under thiopentone anaesthesia, they woke up rapidly in a very agitated state and died. They were therefore allowed to recover from the anaesthetic before the drugs were administered.

Chromatography

The solvents used were A (3-methylbutan-1-ol–2-methylbutan-2-ol–water–formic acid, 5:5:10:2, by vol.) for paper chromatography on Whatman no. 1 paper, and C (methanol–chloroform, 1:1, v/v) for t.l.c. as described by Caldwell *et al.* (1972).

Identification of metabolites

(*a*) *Amphetamine.* Radiochromatograms of the 24h urine collected from bile-duct-cannulated rats were prepared by using solvent A. Two major ^{14}C-

labelled peaks were found with R_F 0.0 and 0.49, and two minor ones of R_F 0.26 and 0.81. Treatment with β-glucuronidase (Ketodase; Warner–Chilcott, Eastleigh, Hants., U.K.) caused the large peak at R_F 0.0 to disappear, and a corresponding increase occurred at R_F 0.26 which corresponds to 4-hydroxyamphetamine. The large peak at the origin was therefore the glucuronide of 4-hydroxyamphetamine. The large peak at R_F 0.49 corresponds to amphetamine and the minor one at R_F 0.81 to hippuric acid. The bile was similarly chromatographed and the same four peaks were found, except that the one at the origin was a major peak and the other three were minor ones. The paper was cut into suitable strips and the radioactivity was determined in a Packard Tri-Carb liquid scintillation counter (Caldwell *et al.*, 1972).

(*b*) *Methamphetamine.* The urine and bile were chromatographed on paper in solvent A and then on t.l.c. plates in solvent C and scanned for ^{14}C as described by Caldwell *et al.* (1972). The bile (1 ml used) gave, in solvent A, a broad peak of R_F 0.0 and three very minor peaks at R_F 0.3, 0.49 and 0.82. On hydrolysis with HCl or β-glucuronidase, the peak of R_F 0.0 disappeared with an equivalent increase at R_F 0.3, whereas those at R_F 0.49 and 0.82 were unaffected. Before hydrolysis the peak at R_F 0.0 gave a naphtharesorcinol test for glucuronic acid and that at R_F 0.3 after hydrolysis gave positive tests for phenols. The material of R_F 0.0 was eluted with water and then hydrolysed with β-glucuronidase. The hydrolysate was subjected to t.l.c. in solvent C, in which three radioactive peaks were found of R_F 0.04 (4-hydroxynorephedrine), 0.40 (4-hydroxyamphetamine) and 0.65 (4-hydroxymethamphetamine), The first peak contained enough material to give a purple colour for a phenol with diazotized *p*-nitroaniline. These peaks were scraped off the chromatogram and their ^{14}C contents determined by scintillation counting for radioactivity.

The peak of R_F 0.30 in solvent A before hydrolysis was eluted with water and chromatographed on thin layers in solvent C. Three peaks, R_F 0.04, 0.40 and 0.65, were found corresponding to the above three phenols.

The peak of R_F 0.49 in solvent A was eluted and similarly chromatographed in solvent C. Two ^{14}C-labelled peaks were obtained, one at R_F 0.45 corresponding to amphetamine and the other at R_F 0.80 corresponding to methamphetamine. The peak at R_F 0.82 in solvent A corresponded to hippuric acid.

Results

The excretion of amphetamine and its metabolites in the bile-duct-cannulated rat over a period of 24h is shown in Table 1. In this period 68–70% of the dose is excreted in the urine and about 15% in the bile. The bile contains small amounts of amphetamine (0.9% of the dose) and hippuric acid (0.1%), but the main metabolite in bile is the glucuronide of 4-hydroxyamphetamine (12.4%), together with a little free 4-hydroxyamphetamine (2.2%).

Table 2 shows the results obtained with methamphetamine. In 24h about 54% of the dose is excreted in the urine and 18% in the bile. The main metabolite in bile in this case is the glucuronide of 4-hydroxynorephedrine, which amounts to nearly 12% of the dose.

Table 1. *Biliary excretion of amphetamine and its metabolites in the rat*

[^{14}C]Amphetamine sulphate (7.5mg/kg; 11.5μCi/kg) was injected intraperitoneally in water into bile-duct-cannulated rats. Urine and bile were collected as described in the text. The values quoted are the averages for three rats, with the ranges in parentheses.

	% of dose excreted in 24h*	
Material determined	Urine	Bile
Total ^{14}C excreted†	69.1 (68.6–70.1)	15.5 (11.5–19.2)
Amphetamine	24.4 (18.2–29.5)	0.9 (0.7–1.0)
4-Hydroxyamphetamine		
Free	4.7 (3.7–5.7)	2.2 (1.2–2.8)
Conjugated	33.8 (28.8–37.6)	12.4 (8.2–17.0)
Total	38.5 (33.6–43.3)	14.6 (10.7–18.2)
Hippuric acid	6.3 (4.3–7.0)	0.1 (0.1–0.1)
Sum of metabolites	69.2 (68.6–70.1)	15.6 (11.5–19.1)

* In the next 24h little further ^{14}C was excreted, and in two of the rats the values were urine, 0.9 and 2.8%, and bile, 0.2 and 0.3%, of the dose.

† Total recovery (urine+bile) in 24h was 84.6 (80.1–87.8)%.

Table 2. *Biliary excretion of methamphetamine and its metabolites in the rat*

[^{14}C]Methamphetamine hydrochloride (7.5mg/kg; 1.25μCi/kg) was injected intraperitoneally in water into bile-duct-cannulated rats. Urine and bile were collected as described in the text. The values quoted are the average for three animals with the ranges in parentheses. N.D., Not detected.

	% of dose excreted in 24h	
Material determined	Urine	Bile
Total ^{14}C excreted*	53.7 (43.5–70.0)	18.1 (13.5–20.8)
Methamphetamine	16.2 (9.2–27.4)	0.5 (0–1.1)
Amphetamine	22.0 (19.9–25.5)	1.8 (0.3–2.6)
4-Hydroxymethamphetamine		
Free	1.1 (0.5–1.8)	Trace
Conjugated	5.3 (5.0–5.5)	1.0 (0–2.5)
Total	6.4 (5.9–7.3)	1.0 (0–2.5)
4-Hydroxyamphetamine		
Free	1.1 (0.3–2.0)	0.1, Trace
Conjugated	3.2 (2.8–3.5)	0.9 (0.7–1.0)
Total	4.3 (3.1–5.4)	1.0
4-Hydroxynorephedrine		
Free	0.1 (0–0.2)	1.8 (1.0–3.8)
Conjugated	1.9 (1.7–2.1)	11.6 (8.4–14.2)
Total	2.0 (1.8–2.1)	13.4 (9.4–16.0)
Hippuric acid	0.0	0.4 (0.3–0.5)
Unknown	2.9 (2.4–3.3)	N.D.
Sum of metabolites	53.8 (43.6–69.9)	18.1 (13.5–20.9)

* About 6% more ^{14}C was excreted in the next 24h in the urine.

Discussion

Amphetamine

The excretion of amphetamine and its metabolites in the urine and bile of bile-duct-cannulated rats and in the urine of intact rats is summarized in Table 3. In the cannulated rats there is an increased urinary excretion of unchanged amphetamine (25% of dose) compared with the intact rats (13%) and there is consequently a decrease in the output of total 4-hydroxyamphetamine. It would appear that the cannulation of the rats under thiopentone anaesthesia has to some extent inhibited the aromatic hydroxylation of amphetamine. It is probable that this is an effect of thiopentone, since a similar inhibition of the hydroxylation of amphetamine has been observed in rats pretreated with desmethylimipramine (Dingell & Bass, 1969), iprindole and chlorpromazine (Lemberger *et al.*, 1970*a*,*b*).

In the intact rat little ^{14}C is excreted in the faeces after administration of [^{14}C]amphetamine, but the output of ^{14}C in the urine and bile of the cannulated rats in 1 day is roughly equal to the output of ^{14}C in the urine of intact rats in 2 days. This suggests that the metabolites of amphetamine in bile (some 16% of the dose) are reabsorbed and subsequently excreted in the urine. The main metabolite in bile appears to be the *O*-glucuronide of 4-hydroxyamphetamine, which could be reabsorbed as such or after hydrolysis to the free phenol by the gut flora and/or secretions (see Smith & Williams, 1966).

Methamphetamine

The summarized results for methamphetamine administration are shown in Table 4. For the metabolites, the main difference between the cannulated and intact rats is in the urinary output of amphetamine, which is 22% of the dose in the cannulated rats and 3% in the intact rats. The output of methamphetamine (17%) is only slightly higher in the cannulated rats than in the intact rats (11%). It seems that, as suggested above, the ring hydroxylation of amphetamine is inhibited by the thiopentone used as an anaesthetic, but that the demethylation of methamphetamine to amphetamine is not. It would appear also that there is an inhibition of the ring hydroxylation of methamphetamine, for the output of total 4-hydroxymethamphetamine is only 7% in the cannulated rats as compared with 19% in the intact rats. The output of total 4-hydroxyamphetamine is low and similar (5 and 6%) in both types of

Table 3. *Metabolites of amphetamine in bile-duct-cannulated and intact rats*

The values quoted are taken from Table 1 and Dring *et al.* (1970) and are given to the nearest whole number

Metabolite	Mol.wt. of metabolite	Cannulated rats (% of dose excreted) Urine	Bile	Total in 1 day	Intact rats* (% of dose excreted) Urine in 2 days
^{14}C		69	16	85	85
Amphetamine	135	24	1	25	13
4-Hydroxyamphetamine					
Free	151	5	2	7	7
Conjugated	327	34	12	46	53
Total	—	39	14	53	60
Hippuric acid	179	6	0	6	3
Sum of metabolites		69	16	85	76

* Results from Dring *et al.* (1970)

Table 4. *Metabolites of methamphetamine in bile-duct-cannulated and intact rats*

The values quoted are taken from Table 2 and Caldwell *et al.* (1972) and are given to the nearest whole number.

Metabolite	Mol.wt. of metabolite	Cannulated rats (% of dose excreted) Urine	Bile	Total in 1 day	Intact rats* (% of dose excreted) Urine Day 1	Day 1+2
^{14}C	—	54	18	72	47	74
Methamphetamine	149	16	1	17	10	11
Amphetamine	135	22	2	24	2	3
4-Hydroxymethamphetamine						
Free	165	1	Trace	1	3	3
Conjugated	341	5	1	6	13	16
Total	—	6	1	7	16	19
4-Hydroxyamphetamine						
Free	151	1	Trace	1	1	1
Conjugated	327	3	1	4	3	5
Total	—	4	1	5	4	6
4-Hydroxynorephedrine						
Free	167	Trace	2	2	2	2
Conjugated	343	2	11	11	9	14
Total	—	2	13	15	11	16
Hippuric acid	179	0	0.4	0.4	2	4
Unknown	—	3	0	3	4	4
Sum of metabolites	—	54	18	72	47	74

* Results from Caldwell *et al.* (1972).

rats. The output of total 4-hydroxynorephedrine is similar (15 and 16%) in cannulated and intact rats. This suggests that thiopentone anaesthesia tends to inhibit the ring hydroxylation of amphetamine and methamphetamine, but not the subsequent reaction of β-hydroxylation to give 4-hydroxynorephedrine or the demethylation of methamphetamine.

In the intact rat after [^{14}C]methamphetamine administration very little ^{14}C is excreted in the faeces (Caldwell *et al.*, 1972), but in cannulated rats some 18% of the dose appears in the bile. A comparison of the results (Table 4) for cannulated and intact rats

suggests that the metabolites excreted in the bile are reabsorbed and excreted in the urine. The main metabolite of methamphetamine in bile is the glucuronide of 4-hydroxynorephedrine (12% of the dose), together with smaller amounts of the conjugates of 4-hydroxymethamphetamine (1%) and 4-hydroxyamphetamine (1%). As mentioned above these could be reabsorbed as such or after deconjugation in the gut.

Main metabolites in bile

In a previous paper from this laboratory (Millburn *et al.*, 1967) it was postulated that, for significant biliary excretion (5–10% of dose or more) in the rat, a compound should be polar and possess a molecular weight not less than 325±50. With amphetamine, the only metabolite that meets these requirements is the *O*-glucuronide of 4-hydroxyamphetamine, which is polar and has a molecular weight of 324. As shown in Table 3, its biliary excretion is 12% of the dose and therefore fulfils the above hypothesis.

With methamphetamine, there are three metabolites (see Table 4) that could meet the above criteria for significant biliary excretion, namely the glucuronides of 4-hydroxyamphetamine, of 4-hydroxymethamphetamine (mol.wt. 341) and of 4-hydroxynorephedrine (mol.wt. 343). However, only the last is excreted in the bile in significant amounts (13% of dose), but it is formed in greater amounts than the other two. It is possible that it is given preference for biliary excretion since it contains a free hydroxyl group. Minor differences in molecular structure are known to have a marked effect on the extent of the biliary excretion of foreign compounds (Hirom *et al.*, 1972). The possession of an extra free hydroxyl group is known to increase biliary excretion, as with protocatechuic acid and salicylic acid (Williams *et al.*, 1965) and 4-hydroxybiphenyl and 4,4′-dihydroxybiphenyl (Millburn *et al.*, 1967).

It is clear that in the rat an enterohepatic circulation of certain metabolites of amphetamine and methamphetamine can occur to a certain extent. However, this is unlikely to occur in man, since the molecular-weight requirement for significant biliary excretion is probably in the region of 500 (Millburn, 1970), which is much higher than that of any of the metabolites of these drugs.

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