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The effect of phenothiazine and dibenzazepine pretreatment on the metabolism of methamphetamine in rats

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There was marked variation between *p*-hydroxylation and *N*-demethylation of methamphetamine in rats. Within 24 h, 10·4, 24·7 and 4·1% of the administered methamphetamine was excreted in urine unchanged, *p*-hydroxymethamphetamine (*p*-OH-MP, free plus conjugated) and also as amphetamine, respectively. Treatment by imipramine, desipramine, chlorpromazine, perphenazine and propericiazine 8 h before the administration with methamphetamine completely inhibited the urinary excretion of *p*-OH-MP whereas the excretion of amphetamine was enhanced by about 700 to 800%. This effect was also observed in rats treated with imipramine 16 and 24 h before methamphetamine. Phenothiazine and dibenzazepine derivatives reverse the degree of *p*-hydroxylation and *N*-demethylation of methamphetamine in-vivo in rats.

In rats methamphetamine is first metabolized by *p*-hydroxylation and *N*-demethylation to give *p*-hydroxymethamphetamine (*p*-OH-MP), a major metabolite, and amphetamine, a minor metabolite (Caldwell et al 1972). Yamada et al (1984) have provided evidence for the involvement of cytochrome P-450 (P-450) and flavin-containing mono-oxygenase (FMO) in the rat liver microsomal *N*-demethylation of methylamphetamine. Yamamoto et al (1984) have shown that, in rats, microsomal *p*-hydroxylation of the drug is catalysed by different forms of P-450 and that the microsomal *N*-demethylation is mediated by both P-450- and FMO-dependent systems.

The present investigation sets out to show that whilst the phenothiazine and dibenzazepine derivatives inhibit *p*-hydroxylation (and certain forms of P-450) they do not inhibit *N*-demethylation.

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Materials and methods

Methamphetamine hydrochloride and amphetamine sulphate were gifts from Dainippon Pharmaceutical Co. Ltd and Takeda Chemical Industries, Ltd; imipramine hydrochloride, desipramine, chlorpromazine hydrochloride, perphenazine malate, propericiazine (10-(3-(4-hydroxy-1-piperidinyl)-propyl)phenothiazine-2-carbonitrile) were from Yoshitomi Pharmaceutical Co. Ltd and β-glucuronidase (H-1) was from Sigma Chemical Co. Ltd; *p*-OH-MP hydrochloride was prepared as described by Buzas & Dufour (1950).

Animal treatment. Male, Wistar albino rats (180–200 g) were housed individually in metabolism cages with free access to food and water. Groups of 6 rats were treated intraperitoneally (i.p.) 8 h before the i.p. administration of methamphetamine (10 mg kg^{-1}) with the following: 0·9% NaCl (saline) 0·2 ml, imipramine (40 mg kg^{-1}), desipramine (40 mg kg^{-1}), chlorpromazine (30 mg kg^{-1}), propericiazine (40 mg kg^{-1}), perphenazine (40 mg kg^{-1}). In one experiment, imipramine (40 mg kg^{-1}) was given 8, 16 and 24 h before methamphetamine (10 mg kg^{-1}). Urine samples were collected for 24 h. Urinary pH was recorded and the samples frozen at −15 °C until assayed.

Assay. The procedure was based on that of Sakai et al (1983). urine samples (5 ml) were made alkaline with 2 M Na_2CO_3 (pH 9·0–9·5) and extracted four times with chloroform–isopropanol (3 : 1) (5 ml). The remaining aqueous layer was adjusted to pH 5·0 with acetic acid and was incubated at 37 °C for 48 h in 0·2 M acetate buffer (pH 5·0) with β-glucuronidase (1000 iu). The incubation mixture was adjusted to pH 9·0–9·5 and was extracted as above. The pooled extracts were evaporated to dryness, and the residue was dissolved in ethyl acetate (0·2 ml) and was trifluoroacetylated with anhydrous trifluoroacetic acid (0·2 ml). The acetylated samples (1 μl) were injected into a Shimadzu GC-7AG gas chromatograph with a hydrogen flame ionization detector. The glass column (3 m × 3 mm i.d.) was packed with 2% OV-17 on chromosorb W, 80/100 mesh. Injection port temperature was 180 °C, column 160 °C and detector 200 °C. Helium flow rate was 50 ml min^{-1}, and air 150 ml min^{-1}. The internal standard, diphenylmethane was added to samples before extraction at a concentration of 0·15 μg μl^{-1}. Under these conditions, diphenylmethane and trifluoroacetyl derivatives of methamphetamine, amphetamine and *p*-OH-MP gave retention times of 5·6, 4·4, 2·5 and 7·0 min, respectively. Suitable controls indicated that the five drugs did not interfere with the determination of urinary methamphetamine or its two metabolites. The amounts of unchanged drug and metabolites (free or conjugated) were measured by taking the ratio of area to that of the internal standard and was expressed as a percentage of methamphetamine administered.

Results

The result shows a marked variation in the *p*-hydroxylation and *N*-demethylation of methamphetamine (see Tables 1, 2). About 10, 4 and 25% of the dose was excreted as unchanged drug, amphetamine and *p*-OH-MP (free plus conjugated) respectively, in control rats, which agrees with the data of Caldwell et al (1972).

The five drugs completely inhibited urinary *p*-OH-MP while, the unchanged drug and amphetamine were markedly enhanced by about 100 to 200% and 700 to 800%, respectively. The marked variation of imipramine could be seen in the pretreatment 16 and 24 h before the administration of methamphetamine.

The pH of urine was in all instances maintained ca 7·0 since Beckett & Rowland (1965) have shown that the amounts of methamphetamine and amphetamine excreted unchanged and metabolized by rats increase with acidity of the urine.

Discussion

The complete inhibition of urinary *p*-OH-MP agrees with the result of Yamada et al (1984) who observed complete inhibition of *p*-hydroxylation of 0·1 mM of methamphetamine by iprindole (0·05 nM) added directly to the system or by the microsomal fraction from the livers of rats given 50 mg kg^{-1} 1 h before death.

Table 1. Effect of phenothiazine and dibenzazepine derivatives on the urinary methamphetamine (MP) and its metabolites amphetamine (AP) and *p*-OH-MP.

	% Dose excreted in 24 h as				
			p-OH-MP		
	MP	AP	Free	Conjugated	Total
Control	10·4 ± 1·7	4·1 ± 0·8	12·9 ± 2·3	11·8 ± 2·1	39·2
Imipramine	23·8 ± 5·1 (129+)	37·0 ± 6·5 (802+)	n.d.	n.d.	60·8 (55+)
Desipramine	32·1 ± 5·6 (208+)	37·6 ± 5·8 (817+)	0·2 (98−)	n.d.	69·9 (78+)
Chlorpromazine	29·2 ± 1·9 (181+)	32·5 ± 2·5 (693+)	0·3 (98−)	n.d.	62·0 (58+)
Perphenazine	21·5 ± 4·8 (107+)	38·4 ± 6·9 (837+)	1·2 ± 0·3 (91−)	0·3 (97−)	61·4 (57+)
Propericiazine	33·0 ± 5·9 (217+)	32·9 ± 4·8 (702+)	0·1 (99−)	n.d.	66·0 (68+)

Values are the mean ± s.e.m. % Effect (− = inhibition; + = enhancement) indicated in parentheses; n.d., not detectable.

Table 2. Effect of time of imipramine-pretreatment on the urinary metabolites of methamphetamine in 24 h.

	% Effect			
			p-OH-MP	
Time of pretreatment	MP	AP	Free	Conjugated
8 h	130+	803+	99−	n.d.
16 h	135+	780+	99−	n.d.
24 h	128+	813+	n.d.	n.d.

Control values listed in Table 1 were used for % effect. − = Inhibition. + = Enhancement; n.d., not detectable.

The total (parent compound plus measured metabolites) 0–24 h urinary recovery of the dose is greater in all the drug-pretreated animals by about 65% (Table 1). This increase is due, in the main, to an increase in the urinary excretion of the unchanged drug (as opposed to metabolites).

Although perhaps not a complete explanation, increase in methamphetamine metabolized by the minor *N*-demethylation pathway may be due, at least in part, to a compensatory effect occurring because the normally major cytochrome P-450-dependent *p*-hydroxylation pathway is blocked.

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