J. Pharm. Pharmac., 1970, **22**, 828–831 *Received May* 14, 1970

The interaction of ethanol and amphetamine metabolism

P. J. CREAVEN*, THERESA BARBEE, AND MARY K. ROACH

Section of Biochemistry, Texas Research Institute of Mental Sciences, 1300 *Moursund Avenue, Houston, Texas, U.S.A.*

Ethanol, 1, 3, and 5 g/kg, depresses the hydroxylation of amphetamine by the rat *in vivo*. At 5 g/kg, ethanol does not affect the hydroxylation of acetanilide or biphenyl *in vivo*. Amphetamine hydroxylation is unaffected by phenobarbitone or benzo[a]pyrene pretreatment but is depressed by pretreatment with 2-diethylaminoethyl-2,2-diphenylvalerate (SKF 525-A), 2,4-dichloro-6-phenylphenoxyethylamine (DPEA), and 2,4-dichloro-6-phenylphenoxy-*NN*-diethylethylamine (Lilly 18947).

We have reported previously that ethanol treatment markedly inhibits the hydroxylation of amphetamine by the rat *in vivo* (Creaven & Barbee, 1969). We have now made further investigations of this and other aspects of amphetamine hydroxylation in an attempt to determine the mechanism of this inhibition by ethanol and to elucidate the differences between amphetamine hydroxylation and that of other drugs.

EXPERIMENTAL

Materials and methods

Male Sprague-Dawley rats, 100 to 150 g, received intraperitoneal injections of (±)-[2-^{14}C]amphetamine sulphate 5 mg/kg (1·6 mCi/mM), acetanilide (250 mg/kg in isotonic saline) or biphenyl (200 mg/kg in arachis oil). The animals were placed in metabolism cages and urine samples collected in flasks at 0 to 2°. Urinary pH was recorded and the urine frozen.

Animals were pretreated with 1, 3, or 5 g/kg ethanol by stomach tube as a 25% solution (v/v) in water 30 min before the administration of amphetamine. Control animals received an equal volume of saline.

Benzo[a]pyrene (2 mg/kg) and phenobarbitone (80 mg/kg) were administered intraperitoneally daily for three days; amphetamine was injected 24 h after the final dose. 2-Diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A), 2,4-dichloro-6-phenylphenoxyethylamine hydrochloride (DPEA), and 2,4-dichloro-6-phenylphenoxy-*NN*-diethylethylamine hydrobromide (Lilly 18947), 35 mg/kg, were injected intraperitoneally 45 min before the administration of amphetamine.

Pyrazole (100 mg/kg in isotonic saline) and disulfiram (100 mg/kg in arachis oil) were injected intraperitoneally 15 and 30 min, respectively, before oral administration of 3 g/kg of ethanol. Amphetamine was injected 30 min after ethanol administration.

Metabolites were identified by two-dimensional paper chromatography of urine to which authentic amphetamine and *p*-hydroxyamphetamine had been added. Chromatograms were developed in 1-butanol–acetic acid–water (4 : 1 : 1 v/v) followed by 2-propanol–ammonia–water (8 : 1 : 1 v/v) (Asatoor, Galman & others, 1965).

* Present address: Section of Oncology, Veterans Administration Hospital, 50 Irving Street, Washington, D.C., U.S.A.

After exposure to Kodak no-screen X-ray film NS 54-T, the films were developed and the chromatograms sprayed with diazotized *p*-nitroaniline. The conjugated *p*-hydroxyamphetamine was identified after incubation overnight at 37° with β-glucuronidase at pH 5·2.

For quantitation of metabolites, aliquots of urine were chromatographed on Whatman 3 MM or Whatman No. 1 paper in 1-butanol–acetic acid–water (4 : 1 : 1 v/v) or formic acid–isoamyl alcohol–t-amyl alcohol–water (2 : 5 : 5 : 10 v/v) (Alleva, 1963; Ellison, Gutzait & Van Loon, 1966).

Strips were dried and cut in ½ inch segments beginning ½ inch behind the origin. Each segment was placed in a liquid scintillation vial in 15 ml of liquid phosphor and counted in a Model 3375 Packard Tri-Carb Liquid Scintillation Spectrometer. Amphetamine metabolites are reported as percentages of excreted radioactivity.

Blood alcohol levels were sampled in amphetamine treated rats 12½ h after 5 g/kg of ethanol and measured by the method of Roach & Creaven (1968). *p*-Aminophenol was determined by the method of Brodie & Axelrod (1948); 4-hydroxybiphenyl by the method of Creaven, Parke & Williams (1965). These metabolites are reported as percentages of the dose.

RESULTS

The excretion of unchanged amphetamine is increased and that of *p*-hydroxyamphetamine is decreased by doses of 1, 3, and 5 g/kg of ethanol (Table 1). At the

Table 1. *Effect of ethanol pretreatment on the percentage of* p-*hydroxyamphetamine excreted in rat urine during various time periods after* (±)-[2-^{14}C]*amphetamine.*

Pretreatment	0–3 h	3–6 h	6–12 h	12–24 h
Saline (4)	44·7 ± 3·0	72·3 ± 3·4	74·4 ± 5·2	79·5 ± 6·2
Ethanol 1 g/kg (3)	23·2 ± 10·7‡	43·1 (1)‡	66·4 ± 9·1	66·7 ± 2·9‡
Ethanol 3 g/kg (5)	14·8 ± 7·1*	27·0 ± 5·8*	47·1 ± 9·2*	62·4 ± 8·2†
Ethanol 5 g/kg (2)	11·2 ± 2·3*		40·0 ± 0·5*	50·8 ± 6·9†
Ethanol 5 g/kg 14 h before amphetamine (4)	38·6 ± 5·5		72·2 ± 5·2	72·8 ± 9·7

Numbers of animals are shown in parentheses. Values are reported as mean ± s.d. * $P < 0·01$. † $P < 0·02$. ‡ $P < 0·05$.

beginning of the 12 to 24 h period, maximum blood ethanol levels after 5 g/kg of ethanol measure 1·2 mg/100 ml. Excretion of *p*-hydroxyamphetamine between 12 and 24 h after dosage remains significantly lower than the controls for all ethanol doses. However, hydroxylation of amphetamine injected 14 h after ethanol (5 g/kg) is not significantly different from controls (Table 1).

Combined pretreatment with ethanol and pyrazole produces a more profound effect on amphetamine metabolism which does not return to normal values by 24 h (Table 2). However, some inhibition of amphetamine hydroxylation occurs with pyrazole alone. Pretreatment with disulfiram does not increase the effect of ethanol on amphetamine metabolism although disulfiram without ethanol has an effect similar in magnitude to that of pyrazole alone (Table 2).

The hydroxylation of amphetamine is markedly inhibited by pretreatment with

Table 2. *Effect of pretreatment with ethanol in combination with pyrazole or disulfiram on the percentage of* p*-hydroxyamphetamine excreted in rat urine during various time periods after* (±)-[2-^{14}C]*amphetamine.*

Pretreatment	0–3 h	3–6 h	6–12 h	12–24 h
Ethanol (5)	14·8 ± 7·1	27·0 ± 5·8	47·1 ± 9·2	62·4 ± 8·2
Pyrazole and ethanol (5)	7·1 ± 0·8	16·0 ± 4·8	12·7 ± 6·5	15·5 ± 6·8
Disulfiram and ethanol (4)	7·1 ± 3·8	29·1 ± 7·7	45·6 ± 3·8	60·4 ± 4·0
Pyrazole (5)	20·3 ± 5·7	38·3 ± 6·4	40·3 ± 3·6	46·3 ± 5·3
Disulfiram (3)	24·8 ± 9·9	36·3 ± 6·5	55·3 ± 5·5	65·4 ± 5·0

Numbers of animals are shown in parentheses. Values are reported as mean ± s.d. Doses were: ethanol, 3 g/kg, pyrazole and disulfiram, 100 mg/kg.

SKF 525-A, DPEA, and Lilly 18947 (Table 3), but is unaffected by pretreatment for three days with phenobarbitone or benzo[a]pyrene (Table 3).

Pretreatment with ethanol (5 g/kg) has no effect on the amount of 4-hydroxyacetanilide or 4-hydroxybiphenyl excreted in 24 h after dosage with acetanilide or biphenyl. The amount of acetanilide hydroxylated is 53·8 ± 3·0% after ethanol; 55·9 ± 5·4% after saline. Biphenyl hydroxylation is 16·1 ± 2·3% after ethanol; 22·6 ± 4·9% after saline.

DISCUSSION

The results show that ethanol in doses of 1, 3, and 5 g/kg produces a marked depression of hydroxylation of the aromatic ring of amphetamine, the major metabolic pathway of this compound in the rat (Axelrod, 1954). The effect is most marked in the period immediately after ethanol treatment but can be seen 12 to 24 h after dosage. When amphetamine is injected 14 h after a dose of 5 g/kg of ethanol, no inhibition in *p*-hydroxylation is seen.

Pyrazole, an inhibitor of alcohol dehydrogenase (Lester, Keokosky & Felzenberg, 1968; Goldberg & Rydberg, 1969) greatly enhances the inhibition of amphetamine hydroxylation by ethanol. Disulfiram, an inhibitor of aldehyde dehydrogenase (Graham, 1951) does not enhance this inhibition. These results indicate that the inhibition of amphetamine metabolism is probably mediated through ethanol itself rather than by the metabolism of ethanol or by acetaldehyde. Pretreatment with pyrazole or disulfiram also depresses amphetamine hydroxylation, but to a lesser extent than when combined with ethanol. The reason for the inhibition of amphetamine metabolism by these agents is not presently known.

Table 3. *Effect of some inhibitors and inducers of liver microsomal oxidation on* p*-hydroxyamphetamine excretion in rat urine.* Urine was collected for 24 h.

Compound	*p*-Hydroxyamphetamine (% ± s.d.)	*P*
Saline (6)	65·7 ± 2·6	
DPEA (4)	11·6 ± 2·2	<0·01
SKF 525-A (4)	6·5 ± 0·8	<0·01
Lilly 18947 (3)	1·6 ± 0·6	<0·01
Benzo[a]pyrene (3)	65·5 ± 2·4	NS
Phenobarbitone (3)	63·4 ± 3·6	NS

Numbers of animals are shown in parentheses. Compounds were given as described under methods.

The failure of ethanol to affect the hydroxylation of acetanilide and biphenyl, both of which are aromatic hydroxylations effected through the microsomal mixed function oxidase system (Mitoma, Posner & others, 1956), suggests that amphetamine hydroxylation may differ from the usual microsomal mixed function oxidation. Microsomal oxidations are generally induced by pretreatment with phenobarbitone and some carcinogenic hydrocarbons (Conney & Burns, 1962). However, Groppetti & Costa (1969) failed to induce amphetamine hydroxylation with 3-methylcholanthrene (20 mg/kg) or with phenobarbitone (1·5 mg/rat twice daily for four days) although Lewander (1969) reported a significant increase in hydroxylation after phenobarbitone (80 mg/kg, daily for five days). In the present work neither phenobarbitone (80 mg/kg, daily for three days) nor benzo[a]pyrene (2 mg/kg, daily for three days) produced significant alteration of amphetamine hydroxylation. However, known inhibitors of microsomal hydroxylation (SKF 525-A, DPEA, and Lilly 18947) (Kato, Vassanelli & Chiesara, 1962) did produce marked inhibition of amphetamine hydroxylation *in vivo*.

Dingell & Bass (1969) were able to demonstrate amphetamine metabolism by liver perfusion, but found no metabolism by the microsomal fraction of rat liver. Preliminary observations in this laboratory have confirmed these results.

Amphetamine hydroxylation thus differs from known microsomal hydroxylations by not being induced by phenobarbitone and carcinogenic hydrocarbons. It resembles microsomal hydroxylations by being inhibited by SKF 525-A and other mixed function oxidase inhibitors. It is further characterized by its dramatic and long-lasting inhibition by ethanol. Investigation of this unique aromatic hydroxylation is continuing.

Acknowledgements

This work was supported by grant M. H. 14434, U.S. Public Health Service. We wish to thank Mrs. Marguerite Knapp for her expert technical assistance. SKF 525-A was kindly supplied by Smith, Kline & French Laboratories; DPEA and Lilly 18947 were kindly supplied by Eli Lilly & Co.

REFERENCES

ALLEVA, J. (1963). *J. mednl. Chem.*, **6**, 621–624.

ASATOOR, A. M., GALMAN, B. R., JOHNSON, J. R. & MILNE, M. D. (1965). *Br. J. Pharmac. Chemother*, **24**, 293–300.

AXELROD, J. (1954). *J. Pharmac. exp. Ther.*, **110**, 318–326.

BRODIE, B. & AXELROD, J. (1948). *Ibid.*, **94**, 22–29.

CONNEY, A. H. & BURNS, J. J. (1962). *Adv. Pharmac.*, **1**, 31–58.

CREAVEN, P. J., PARKE, D. V. & WILLIAMS, R. T. (1965). *Biochem. J.*, **96**, 879–885.

CREAVEN, P. J. & BARBEE, T. (1969). *J. Pharm. Pharmac.*, **21**, 859–860.

DINGELL, J. V. & BASS, A. D. (1969). *Biochem. Pharmac.*, **18**, 1535–1538.

ELLISON, T., GUTZAIT, L. & VAN LOON, E. J. (1966). *J. Pharmac. exp. Ther.*, **152**, 383–387.

GOLDBERG, L. & RYDBERG, U. (1969). *Biochem. Pharmac.*, **18**, 1749–1762.

GRAHAM, W. (1951). *J. Pharm. Pharmac.*, **3**, 160–168.

GROPPETTI, A. & COSTA, E. (1969). *Int. J. Neuropharmac.*, **8**, 209–215.

KATO, R., VASSANELLI, P. & CHIESARA, E. (1962). *Biochem. Pharmac.*, **12**, 353–356.

LESTER, D., KEOKOSKY, W. Z. & FELZENBERG, F. (1968). *Q. Jl. Stud. Alcohol*, **29**, 449–454.

LEWANDER, T. (1969). *Europ. J. Pharmac.*, **6**, 38–44.

MITOMA, C., POSNER, H. S., REITZ, H. C. & UDENFRIEND, S. (1956). *Archs Biochem. Biophys.*, **61**, 431–441.

ROACH, M. K. & CREAVEN, P. J. (1968). *Clin. Chim. Acta*, **21**, 275–278.