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Effects of diethyldithiocarbamate and ethanol on the *in vivo* metabolism and pharmacokinetics of amphetamine in the rat

Recently, Creaven, Barbee & Roach (1970) demonstrated that ethanol and disulfiram diminished the excretion of *p*-hydroxyamphetamine in the urine of rats presumably as a result of blockade of *p*-hydroxylation of amphetamine. We wish to report that pretreatment of rats with the metabolite of disulfiram, diethyldithiocarbamate (DDC), or ethanol causes an increase in the concentrations of subsequently administered amphetamine in brain and plasma.

Male Sprague-Dawley rats, 180–250 g were housed in individual cages at least 24 h before and throughout the experiment. Water and food were freely available. DDC, as the sodium salt, was dissolved in saline and injected in a dose of 400 mg kg^{-1} (s.c.) 1 h before the administration of radioactively labelled amphetamine. Ethanol, 4 g kg^{-1} was given by mouth as a 2·5% solution (v/v) 30 min before the amphetamine injection. (+)-[^{3}H]amphetamine sulphate (generally labelled, New England Nuclear) was adjusted to a specific activity of 1·2 μCi nM^{-1} with unlabelled drug and injected as a saline solution in a dose of 4 mg kg^{-1} (i.p.). (+)-[^{14}C]Amphetamine sulphate (CEA, Gif-sur-Yvette, France) (5 mg kg^{-1}, i.p., 1·3 μCi μM^{-1}) was injected into rats housed individually in metabolic cages. Urine was collected for 24 h. For the determination of brain and plasma concentrations of amphetamine, groups of animals were decapitated at different times (see Fig. 1) after the injection of (+)-[^{3}H]amphetamine. Aliquots of brain extracts (0·4 M perchloric acid, 10 ml/brain) and plasma were adjusted to pH 12 by addition of 1 M sodium hydroxide. (+)-[^{3}H]amphetamine was then extracted into toluene and 1 ml of the organic phase was counted in a scintillation spectrometer provided with an external standard equipment for correction of quenching. Urinary metabolites of [^{14}C]amphetamine were separated by means of paper chromatography and quantified by liquid scintillation counting according to Ellison, Gutzait & van Loon (1966) as described by Lewander (1968).

As shown in Fig. 1a amphetamine disappears from brain and plasma in a polyphasic pattern as previously shown by Maickel, Cox & others (1969) and Lewander (1971). There was a constant concentration ratio between brain and plasma of about 7–8

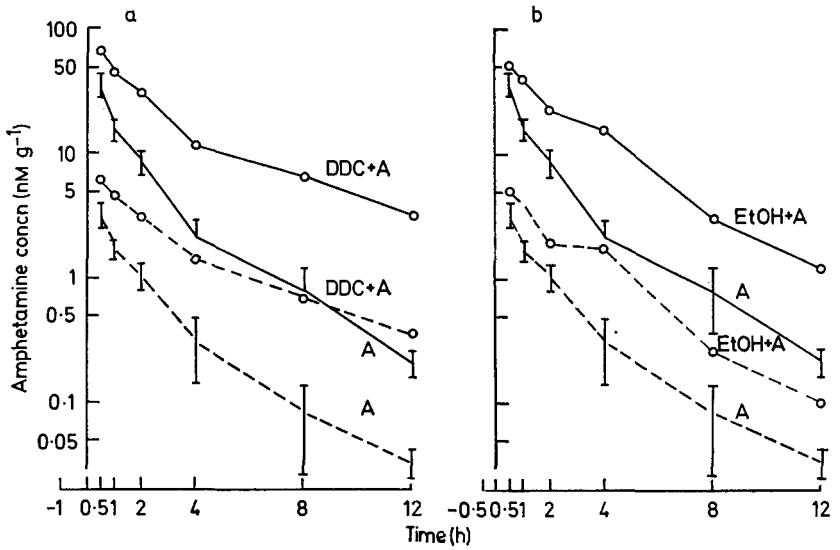

FIG. 1. Disappearance of amphetamine (A) from brain (solid lines) and plasma (broken lines) in diethyldithiocarbamate (DDC, 400 mg kg^{-1}, s.c. at − 1 h), Fig. 1a, or ethanol (EtOH, 5 g kg^{-1}, orally at −0·5 h), Fig. 1b, pretreated rats and rats given amphetamine alone. (+)-Amphetamine [^{3}H] sulphate, 4 mg kg^{-1} was given intraperitoneally at time 0. Each point represents the mean concentration of amphetamine in 4 rats. Vertical bars represent ± s.e. Open circles indicate statistically significant ($P < 0{\cdot}01$) differences from the corresponding controls (Student's *t*-test).

throughout the period studied. In rats pretreated with either DDC or ethanol the concentrations of amphetamine in brain and plasma were significantly higher at all time points compared with the controls. Further, the rates of disappearance of amphetamine through the different phases seemed to be prolonged. Also in the DDC- or ethanol-pretreated rats, the brain and plasma amphetamine concentration curves run parallel with each other and the brain/plasma ratio was about 8–10 at all intervals.

DDC administered 1 h before (+)-7-[^{14}C]amphetamine had a pronounced effect on the pattern of distribution of urinary metabolites of amphetamine (Table 1). There was a reduction of free and conjugated *p*-hydroxyamphetamine by 28% with a corresponding increase in amphetamine by 31%. No change was observed in the percentage of hippuric acid. The urinary pH after DDC was not significantly different from the pH (6·4–7·3) of amphetamine controls.

Table 1. *The effect of diethyldithiocarbamate (DDC) (400 mg kg^{-1}, s.c.), on the distribution of* (+)-[^{14}C]*amphetamine and its metabolites, expressed as percentages of the total urinary radioactivity* (± *s.e.*). Urine was collected from individual rats during 24 h after the i.p. injection of (+)-[^{14}C]amphetamine, 7·2 μCi per rat, together with unlabelled (+)-amphetamine, 5 mg kg^{-1} (n = number of observations; N.S. = not significant).

	Saline+(+)-[^{14}C]Amphetamine	DDC+(+)-[^{14}C]Amphetamine	*P*
Total ^{14}C excreted % of dose administered	63·2 ± 7·6 (n = 4)	58·5 ± 12·4 (n = 3)	N.S.
Amphetamine-^{14}C	18·7 ± 0·7	49·8 ± 6·6	<0·01
p-Hydroxyamphetamine-^{14}C (free + conjugated)	70·9 ± 1·2	43·1 ± 5·5	<0·01
Hippuric acid-^{14}C	4·8 ± 1·2	4·4 ± 0·6	N.S.
Unidentified metabolites-^{14}C	15·2 ± 0·4	3·1 ± 0·2	<0·01

The present results show that DDC, like disulfiram, inhibits the hydroxylation of amphetamine and as a consequence increases the concentrations of amphetamine in brain and plasma. Several reports have appeared showing that amphetamine-induced stereotyped behaviour is prolonged after pretreatment with DDC in mice and rats (Randrup & Scheel-Krüger, 1966; D'Encarnacao, D'Encarnacao & Tapp, 1969; Mayer & Eybl, 1971). This effect of DDC has been interpreted as a result of inhibition of dopamine-β-hydroxylase leading to a decreased synthesis of noradrenaline and increased levels of dopamine in the brain Scheel-Krüger & Randrup, 1967; Mayer & Eybl, 1971). In view of the present findings the increased intensity and the prolongation of the amphetamine-induced stereotyped behaviour in DDC pretreated rats may better be explained by increased tissue levels of amphetamine. However, our data do not rule out the earlier conclusion, that the stereotyped behaviour is dependent on an intact dopaminergic transmission in the central nervous system.

Ethanol also caused an increase in the concentrations of amphetamine in the brain and plasma (Fig. 1b) as might be expected. This result is in agreement with the finding by Creaven & Barbee (1969), that ethanol is an inhibitor of the *p*-hydroxylation of amphetamine in the rat.

Further experiments are needed before the mechanisms of inhibition of amphetamine hydroxylation caused by DDC, ethanol and several other chemical agents (cf. Lewander & Jonsson, 1972) can be established.

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Deacetylation of acetyl sulphapyridine in man

It has been demonstrated that sulphapyridine, like isoniazid, sulphadimidine, and other drugs, is polymorphically acetylated in man (Schröder & Evans, 1972). Sulphapyridine and acetyl sulphapyridine are also subject to ring hydroxylation, the products being recovered in serum and urine as *O*-glucuronides. The further possibility remains, however, that acetyl sulphapyridine like monoacetyl dapsone (Gelber, Peters & others, 1971) also undergoes deacetylation in man.

Deacetylation can, in theory, be effected by the reversible action of the polymorphic N-acetyl transferase enzyme. However, it has been demonstrated that acetyl sulphadimidine is not deacetylated in man (Gelber & others, 1971), nor is acetyl isoniazid deacetylated by the partially purified enzyme (Jenne, 1965). It seems more likely that the deacetylation is effected by specific arylacylamidases (Weber, 1971).