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ACTION OF N,N-DIETHYLACETAMIDE ON HEPATIC MICROSOMAL DRUG-METABOLIZING ENZYMES

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Summary—The action of *N,N*-diethylacetamide on liver microsomal drug-metabolizing enzymes was studied *in vitro* using rat-liver homogenates, and *in vivo* in mice. *In vitro*, *N,N*-diethylacetamide had no effect on a series of mixed-function oxidases or on cytochrome *c* reductase but *in vivo*, these enzymes were inhibited, 45 min after a single, ip dose of 150 mg of the substance/kg body weight and there was also an increase in hexobarbital sleeping time. This discrepancy between the *in vitro* and the *in vivo* results may be due to oxidative *N*-dealkylation of diethylacetamide occurring *in vivo* leading to enzyme inhibition.

Introduction

In pharmacological and toxicological studies and in some other uses, water-insoluble compounds are often administered in water-miscible organic solvents such as dimethylsulphoxide, glycerol, propylene glycol, polyethylene glycol, and *N,N*-diethylacetamide. It has been shown that these vehicle compounds are not as biologically inert as they were originally thought to be (Budden, Kühl & Bahlsen, 1979; Budden, Kühl & Buschmann, 1978a,b,c; Gergely, 1970). Budden *et al.* (1978a,b,c) showed that amongst other effects, they can prolong hexobarbital sleeping time. Thus these substances can interfere with the biotransformation and the pharmacokinetics of compounds to be tested in pharmacological or toxicological studies.

We studied the effect of *N,N*-diethylacetamide on oxidative drug metabolism *in vivo* and *in vitro* because this solvent has structural similarities to the aliphatic amide, 2-hydroxy-2-ethylbutyryl *N,N*-diethylamide (HOE 17,879), a compound which has been shown to prolong drug action times (Beyhl & Lindner, 1976; Lindner, 1960) and to inhibit hepatic microsomal drug-metabolizing mixed-function oxidases, both *in vivo* (Beyhl & Lindner, 1973 & 1976) and *in vitro* (Beyhl, 1980a), but to have no effect on hepatic microsomal NADPH-dependent cytochrome *c* reductase *in vivo* (Beyhl & Lindner, 1976) or *in vitro* (Beyhl, 1980a).

Experimental

Materials. The following chemicals were used: saccharose, inorganic salts, and buffer substances obtained from Merck (Darmstadt) and Riedel-DeHaën (Seelze), biochemicals obtained from Boehringer (Mannheim), *N,N*-diethylacetamide and coumarin obtained from Merck, aminopyrine obtained from Hoechst (Frankfurt), sodium hexobarbital obtained from Bayer (Leverkusen), and 4-methylumbelliferone obtained from EGA-Chemie (Steinheim). 4-Methoxybiphenyl and 7-ethoxycoumarin were the gifts of Dr Sinharay (Hoechst).

In vivo *study*

Treatment of animals. Male mice of the NMRI strain obtained from our breeding station, were divided at random into two groups of 16 animals. The mice of one group were injected ip with an aqueous solution containing 150 mg *N,N*-diethylacetamide/kg body weight and those of the other (control) group were injected with the same amount of water. After 45 min six animals from each group were injected iv with 100 mg sodium hexobarbital/kg body weight for the measurement of hexobarbital sleeping time according to Lindner (1960). The remaining animals were killed by a blow on their heads and bleeding; the livers were removed, weighed, and frozen immediately by immersion into liquid nitrogen. The livers were stored at −20°C until further handling. Activities of drug-metabolizing enzymes were not deteriorated by this procedure (F. E. Beyhl, unpublished data 1980).

Biochemical measurements. After thawing, the livers were homogenized in ice-cold isotonic saccharose solution with a glass/polytetrafluoroethylene homogenizer of the Potter-Elvehjem (1936) system driven by compressed-air motor (Pressluft-Götz, Mannheim). From these crude homogenates, microsomes were prepared by the calcium chloride precipitation method as cited previously (Beyhl & Mayer, 1980). The activities of certain enzymes in these microsomes were assayed as described below. Methoxybiphenyl *O*-demethylase was assayed according to Beyhl (1980b; cf. Bridges, Creaven & Williams, 1965; Creaven, Davies & Williams, 1967; Davies & Creaven, 1964). Aminopyrine *N*-demethylase was determined following Leber, Degkwitz & Staudinger (1969; cf. Volz & Kellner, 1980). Coumarin 7-hydroxylase was assayed by our own method (F. E. Beyhl, unpublished data, 1981; cf. Creaven, Parke & Williams, 1965; Fink & von Kerékjártó, 1966; von Kerékjártó, 1966; von Kerékjártó, Kratz & Staudinger, 1964) with 4-methylumbelliferone as the fluorescence standard, and microsomal NADPH-dependent cytochrome *c* reductase was measured according to Cleveland & Smuckler (1965). All the resulting values were tested for statistical significance using Student's *t* test (Ther, 1965).

In vitro *studies*

For the *in vitro* studies, the livers of untreated Wistar

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rats, of either sex, obtained from our breeding station were homogenized either in isotonic saccharose solution for the preparation of calcium chloride-precipitated microsomes as described above or in isotonic potassium chloride solution for the preparation of 13,000 g supernatants (Beyhl & Mayer, 1980). In the 13,000 g supernatants, the activities of aminopyrine *N*-demethylase and cytochrome *c* reductase were determined as outlined above; in previous studies it had been shown that these microsomal enzymes could be measured without disturbance of the cytosolic components present in these supernatants (F. E. Beyhl, unpublished data, 1980). In the microsomal preparation, the activities of methoxybiphenyl *O*-demethylase and coumarin 7-hydroxylase were measured by the methods cited above, and that of 7-ethoxycoumarin *O*-deethylase was determined by our own method (F. E. Beyhl, unpublished data, 1981; cf. Aitio, 1978; Ullrich & Weber, 1971). All the enzyme activities were assayed in the absence and in the presence of the *N*,*N*-diethylacetamide at concentrations of 0·1, 1·0 and 10 mM, in the assay medium (cf. Beyhl, 1980a). Again, the resulting values were tested for statistical significance using Student's *t* test.

Results

The results are listed in Tables 1 and 2. All the enzyme activities measured in the *in vivo* experiment were significantly decreased and hexobarbital sleeping time was doubled. In the *in vitro* experiment *N*,*N*-diethylacetamide did not have a significant effect on the activity of any of the enzymes.

Discussion

The lack of enzyme inhibition observed *in vitro* shows that *N*,*N*-diethylacetamide does not inhibit either mixed-function oxidases or cytochrome *c* reductase. Other microsomal enzyme inhibitors either inhibit the mixed-function oxidases, as a result of interaction with cytochrome *P*-450, the terminal oxidase of the microsomal redox chain (Beyhl, 1980a,c; Cooper, Axelrod & Brodie, 1954) or inhibit both the mixed-function oxidases and cytochrome *c* reductase (Beyhl, 1981). Thus *N*,*N*-diethylacetamide would not be expected to affect drug-metabolizing enzymes or hexobarbital sleeping time *in vivo*. However our results show that *N*,*N*-diethylacetamide reduces both mixed-function oxidase and cytochrome *c* reductase activities *in vivo* and, as a consequence of the thus impaired drug biotransformation, prolongs hexobarbital sleeping time. The increased hexobarbital sleeping time was also observed by Budden *et al.* (1978a). In view of the *in vitro* results this effect on drug-metabolizing enzymes cannot be explained as resulting from enzyme inhibition by *N*,*N*-diethylacetamide itself. We would like to suggest that *in vivo*, *N*,*N*-diethylacetamide is *N*-dealkylated to form a reactive metabolite which blocks both drug metabolism by mixed-function oxidases and microsomal electron transport by cytochrome *c* reductase. A similar mechanism for inhibition of drug-metabolizing enzymes is being discussed for diethylamino-ethyl 2,2-diphenylvalerate hydrochloride (SKF 525-A; Proadifen), a well-known inhibitor of mixed-function oxidases (Barber & Wilson, 1980; Schenkman, Wilson & Cinti, 1972). Studies on this metabolic activation of *N*,*N*-diethylacetamide are in progress.

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Table 1. *Action of* N,N-*diethylacetamide on hepatic parameters in mice* in vivo

Group	Hexobarbital sleeping time (min)	Aminopyrine *N*-demethylase (U/g liver)	Methoxybiphenyl *O*-demethylase (U/g liver)	Coumarin 7-hydroxylase (mU/g liver)	Cytochrome *c* reductase (U/g liver)
Control	50 ± 11	0·452 ± 0·031	0·179 ± 0·01	5·06 ± 0·93	31·5 ± 3·5
Treated	101 ± 17 (202%)	0·315 ± 0·047 (69·7%)	0·127 ± 0·02 (70·9%)	2·72 ± 1·07 (53·8%)	24·5 ± 3·1 (77·8%)

Values are means ± 1 SD for groups of ten animals (except hexobarbital sleeping time, six animals). Values in parentheses are percentages of the corresponding control value. All values for the treated group differ significantly (Student's *t* test) from those of the corresponding control group ($P < 0·0005$).

Table 2. *Effect of* N,N-*diethylacetamide on rat-liver enzyme activities* in vitro

N,N-Diethylacetamide concn (mM)	Enzyme activities (% of control)				
	Aminopyrine *N*-demethylase	Methoxybiphenyl *O*-demethylase	Ethoxycoumarin *O*-deethylase	Coumarin 7-hydroxylase	Cytochrome *c* reductase
0 (control)	100 ± 3·9	100 ± 1·5	100 ± 8·5	100 ± 3·4	100 ± 2·6
0·1	105·2 ± 11·2	99·2 ± 1·9	100·7 ± 9·2	96·8 ± 3·1	99·8 ± 2·8
1·0	102·1 ± 22·8	103·9 ± 2·7	100·4 ± 6·5	99·2 ± 7·9	111·8 ± 2·7
10	86·4 ± 13·2	106·2 ± 2·4	96·5 ± 5·3	82·8 ± 7·4	111·5 ± 5·0

Values are means ± 1 SD. None of the values differs significantly (Student's *t* test) from that of the corresponding control group.

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