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Induction of the Hepatic Microsomal Mixed Function Oxidase System in Mice by *p*-dioxane

Avinash M. Mungikar and Sitaram S. Pawar

Division of Biochemistry, Department of Chemistry, Marathwada University, Aurangabad (Maharashtra) India

The use of drugs to induce mystical or spiritual experiences is of great antiquity but in recent years the drugs used for mental ill health were regarded as sedatives or stimulants of the central nervous system. Now a days, barbituate abuse has developed extensively. The barbituates have advantages as hypnotics in that tolerance is not marked and they have a generous margin of safety. Differences in the in vivo and in vitro drug metabolism are found in various species of animals at different ages and after treatment of animals with inducers of microsomal drug metabolism e.g. phenobarbital or pentobarbital. Sedative effect of several drugs or sleep duration induced by certain barbituates can be altered by pretreatment of animals with a number of environmental contaminants like pesticides (KOLMODIN-HEDMAN et al 1971, HART and FOUTS 1963), food additives (SPRINCE et al 1966) and industrial organic solvents (MUNGIKAR and PAWAR 1975). In 1963, HART et al. reported shortening of hexobarbital sleeping times in rats housed in quarters which had been sprayed with chlordane. This effect was accompanied by an increase in the activity of drug oxidizing enzyme. In spite of wide use and potential application of several organic solvents, very little efforts have been made to study their effects on in vivo and in vitro drug metabolism in laboratory animals. Our preliminary studies (PAWAR and MUNGIKAR 1976) indicatedthe ability of dioxane to induce mouse liver microsomal mixed function oxidase system. The present work deals with the effect of dioxane of in-vivo and in vitro hepatic microsomal mixed function oxidase system.

MATERIALS and METHODS

Hindustan Antibiotics strain, adult male and female mice (30-50 g) were used in the present studies. They were housed in an air-conditioned room. The animals were provided with water and laboratory diet ad libitum.

One group of male mice was treated daily with p-dioxane by oral administration at 0.5 g/kg for three days. Another group of mice received equivalent amounts of distilled water and served as controls.

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In order to evaluate the effect of dioxane on the rate of biotransformation in the intact organisms, alterations in drug-induced sleeping time studies were carried out. The classic sedative hypnotic used were phenobarbital, pentobarbital, thiopentone and sodium barbital.

Both male and female mice were treated with dioxane (0.5 g/kg and 1 g/kg body weight) 30 minutes prior to the intraperitoneal injections of barbituates. Phenobarbital sodium in physiological saline was given intraperitoneally at a dose of 50 mg/kg. The mice were similarly injected with pentobarbital and sodium barbital at the concentrations of 25 mg/kg and 50 mg/kg respectively. Thiopentone was injected at a dose of 50 mg/kg. Control group of mice were treated with phenobarbital, pentobarbital, thiopentone and sodium barbital.

Twenty four hours after the last injection, the animals were sacrificed by cervical dislocation, livers were removed and microsomes were prepared as reported earlier (MUNGIKAR and PAWAR 1975). The microsomal protein was measured according to the biuret method (GORNALL et al 1949) using crystalline bovine serum albumin as the standard.

Aminopyrine N-demethylation, lipid peroxidation and microsomal electron transport components were determined according to the procedures reported earlier (MUNGIKAR and PAWAR 1975, PATEL and PAWAR 1974).

Microsomes from untreated animals were used to study the effect of dioxane on the in vitro aminopyrine metabolism and the dioxane spectrum.

RESULTS

The duration of the drug induced sleeping time was significantly decreased in both male and female mice pretreated with dioxane (0.5 g/kg and 1 g/kg body wt.) (Table 1). The phenobarbital sleeping time was 90 minutes for male and 52 minutes for female mice, whereas, the values for the mice receiving dioxane (0.5 g/kg) 30 minutes earlier to phenobarbital were 85 minutes in male and 32 minutes in female mice, indicating that a greater magnitude of decrease in sleeping time occured in the case of female mice. Dioxane treatment (0.5 g/kg) caused a significantly higher decrease in pentobarbital sleep time both in male and female mice, with a decrease of 15% in male and 48% in female mice. Contrary to the results for phenobarbital and pentobarbital, dioxane administration caused a higher degree of decrease in thiopentone sleep time in the case of male mice as compared to female mice. The higher dose of dioxane further decreased the magnitude of drug induced sleep. Sodium barbital sleeping time was decreased by 18% in male and 33% in female mice at the lower dose of dioxane administration (0.5 g/kg), and further decreased the higher dose of dioxane (1 g/kg) in both male and female mice.

TABLE 1

Effect of dioxane pretreatment on drug-induced sleep in male and female mice*.

Group and treatment	Sleep Duration (Minutes + SE) Male	Female
Phenobarbital	90±11	52±9
Dioxane (0.5 g/kg) + phenobarbital	85±2	32±2
Dioxane (1 g/kg) + phenobarbital	77±1	42±2
Pentobarbital	72±2	42±6
Dioxane (0.5 g/kg) + pentobarbital	61±9	20±5
Dioxane (1 g/kg) + pentobarbital	56±6	26±10
Thiopentone	80±4	42±5
Dioxane (0.5 g/kg) + thiopentone	54±14	31±3
Dioxane (1 g/kg) + thiopentone	33±1	30±3
Sodium barbital	55±4	45±5
Dioxane (0.5 g/kg) + sodium barbital	45±1	30±5
Dioxane (1 g/kg) + sodium barbital	36±2	25±5

* Values are mean ± SE (6 mice in each group)
a = P<0.05, b = P<0.01, c = P<0.001

Dioxane administration (0.5 g/kg or 1 g/kg body wt.) caused a significant increase in microsomal protein content (Table 2). Increase in microsomal protein content in dioxane treated animals was accompanied by the concurrent increase in the aminopyrine-N-demethylase activity. The magnitude of increase in aminopyrine N-demethylase was significantly high in animals treated with both dioxane and barbituates. A measurable increase in the in vitro rate of aminopyrine metabolism was observed on the addition of dioxane (5mM) to the incubation medium. Dioxane addition to control microsomes exhibited a trough at 397 and peak at 420 mM a typical type II spectrum (Fig. 1).

Dioxane treatment caused a considerable decrease in NADPH linked and ascorbate induced lipid peroxidation both in male and female mice. (Table 3). Dioxane administration prior to inducers resulted into similar decrease in in vitro NADPH linked and ascorbate induced lipid peroxidation.

Dioxane administration for 3 days resulted into significantly higher levles of cytochrome b_5, total heme and cytochrome P-450 and a marginal increase in cytochrome c reductase (Table 4).

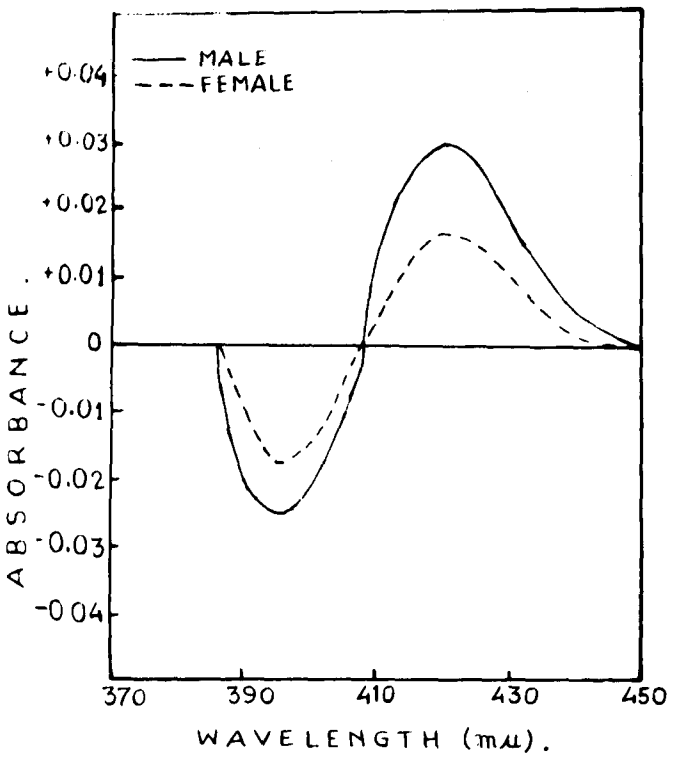

Figure 1. Spectral change induced by dioxane. Microsomes were suspended in 50mM Tris-HCl buffer pH 7.4 to 2 mg protein per ml. The sample contained 20 mM dioxane Difference spectra were recorded with a Hitachi model 124 recording spectrophotometer.

Administration of dioxane 30 minutes earlier to inducing drugs caused the similar effect on microsomal mixed function oxidase system.

DISCUSSION

Increased activity (induction) of the hepatic microsomal oxidative enzymes can be evoked by industrial organic solvents such as dioxane. To study the effect of inducing agents, the drug induced sleeping time procedure is an accepted method (HART and FOUTS, 1963, 1965; HART et al 1963). Several authors have shown a parallel effect between the in vivo tested hexobarbital sleeping time and in vitro tested metabolic reactions of drug metabolism using microsomal enzymes (HART and FOUTS, 1963, 1965; NAIR and CASPER 1969). GHAZAL et al (1964) recorded a shortening of hexobarbital sleeping times in rats after pretreatment of large single doses of α,β or γ-benzene hexachloride. In the present investigation, animals receiving dioxane (0.5 g/kg body wt. and 1 g/kg body wt..) 30 minutes before challenge with different sleep time inducers slept for appreciably short periods than controls. At these relatively low doses of dioxane, no sedative effects were noted in the absence of inducing drugs. Since the time between the administration of dioxane and that of sedative drugs was quite short, it is unlikely that the effects seen were mediated via the adrenal, they may therefore ascribed to the direct action of dioxane upon the drug-oxidizing enzyme

TABLE 2

Changes in liver microsomal protein and aminopyrine N-demethylase due to the treatment of drugs and pretreated with dioxane*

Group and treatment	Sex	Microsomal protein (mg/g liver)	Aminopyrine N-demethylase (nmoles HCHO formed/min/mg protein.
Control	Male	24.18±0.28	8.75±1.25
	Female	48.40±0.40	6.25±1.25
Dioxane (0.5 g/kg)	Male	28.38 ± 1.00^{a}	18.75 ± 1.25^{c}
Dioxane (1 g/kg)	Male	32.60 ± 0.50^{c}	22.50 ± 2.50^{c}
Dioxane (In vitro)	Male	-	11.25 ± 0.75^{b}
Phenobarbital	Male	56.00 ± 4.00^{c}	26.25 ± 1.25^{c}
	Female	66.80 ± 0.40^{c}	17.50 ± 2.50^{c}
Dioxane (0.5 g/kg)	Male	31.58 ± 0.08^{c}	33.00 ± 0.75^{b}
+ phenobarbital	Female	80.00 ± 1.00^{b}	22.50 ± 0.50^{c}
Dioxane (1 g/kg)	Male	37.30 ± 0.30^{c}	18.13 ± 0.62^{b}
+ phenobarbital	Female	52.62 ± 0.02^{b}	18.75 ± 1.25^{a}
Pentobarbital	Male	42.10 ± 0.50^{c}	16.88 ± 1.87^{c}
	Female	35.60 ± 0.60^{b}	10.60 ± 0.60^{c}
Dioxane (0.5 g/kg)	Male	26.40 ± 0.40^{c}	23.13 ± 0.62^{c}
+ pentobarbital	Female	50.45 ± 0.05^{c}	12.25 ± 0.25^{b}
Dioxane (1 g/kg)	Male	33.60 ± 0.60^{b}	13.75 ± 2.50^{c}
+ pentobarbital	Female	63.14 ± 0.04^{c}	16.25 ± 1.25^{c}
Thiopentone	Male	45.75 ± 0.75^{c}	13.75 ± 0.75^{c}
	Female	38.90 ± 0.20^{b}	8.13 ± 0.62^{c}
Dioxane (0.5 g/kg)	Male	31.08 ± 0.08^{b}	18.13 ± 0.62^{c}
+ thiopentone	Female	73.60 ± 0.60^{c}	11.25 ± 1.25^{c}
Dioxane (1 g/kg)	Male	92.60 ± 0.40^{c}	20.00 ± 2.50^{c}
+ thiopentone	Female	75.76 ± 0.05^{c}	11.00 ± 1.00^{c}
Sodium barbital	Male	28.68 ± 0.32^{a}	13.13 ± 0.62^{c}
	Female	58.80 ± 0.40^{b}	8.75 ± 1.25^{b}
Dioxane (0.5 g/kg)	Male	35.80 ± 0.80^{b}	19.38 ± 0.62^{c}
+ sodium barbital	Female	75.30 ± 0.30^{b}	16.25 ± 1.25^{c}
Dioxane (1 g/kg)	Male	42.20 ± 0.20^{c}	14.38 ± 0.62^{a}
+ sodium barbital	Female	76.80 ± 0.80^{b}	8.75±1.25

* Values are mean ± SE (6 mice in each group)
a = $P<0.05$, b = $P<0.01$, c = $P<0.001$

TABLE 3

Hepatic microsomal lipid peroxidation during drug treatment and the effect of dioxane pretreatment in male and female mice.*

Group and treatment	Sex	Lipid peroxidation	
		NADPH linked (nmoles malonaldehyde formed/min/mg protein)	Ascorbate induced (nmoles malonaldehyde formed/min/mg protein)
Control	Male	10.31±0.31	10.40±0.80
	Female	8.50±0.50	8.10±0.50
Dioxane (0.5 g/kg)	Male	7.60 ± 1.60^{b}	2.78 ± 0.06^{c}
Dioxane (1 g/kg)	Male	8.20 ± 0.20^{b}	3.60 ± 0.40^{c}
Dioxane (In vitro)	Male	3.60 ± 1.20^{c}	3.00 ± 0.20^{c}
Phenobarbital	Male	7.35 ± 0.46^{b}	8.69 ± 0.91^{b}
	Female	6.80 ± 0.16^{b}	6.35 ± 0.25^{b}
Dioxane (0.5 g/kg) + phenobarbital	Male	6.20 ± 0.20^{b}	8.60 ± 0.20^{a}
	Female	2.84 ± 0.04^{c}	7.20 ± 0.20^{a}
Dioxane (1 g/kg) + phenobarbital	Male	5.60 ± 0.12^{b}	3.48 ± 0.20^{b}
	Female	2.88 ± 0.08^{c}	3.92 ± 0.08^{b}
Pentobarbital	Male	7.05 ± 0.15^{c}	6.50 ± 0.10^{c}
	Female	3.12 ± 0.16^{c}	3.40 ± 0.12^{c}
Dioxane (0.5 g/kg) + pentobarbital	Male	5.60 ± 0.08^{b}	6.80 ± 0.40^{a}
	Female	3.32 ± 0.36	3.32 ± 0.20^{a}
Dioxane (1 g/kg) + pentobarbital	Male	4.42 ± 0.06^{c}	3.11 ± 0.15^{c}
	Female	4.24 ± 0.08^{c}	4.76 ± 0.28^{b}
Thiopentone	Male	7.18 ± 2.82^{c}	8.20 ± 2.20^{b}
	Female	2.83 ± 0.19^{c}	3.28 ± 0.12^{c}
Dioxane (0.5 g/kg) + thiopentone	Male	5.20 ± 0.40^{b}	7.60 ± 0.40^{b}
	Female	2.88 ± 0.04^{a}	3.08 ± 0.04^{a}
Dioxane (1 g/kg) + thiopentone	Male	3.40 ± 0.20^{c}	6.24 ± 1.04^{b}
	Female	3.88 ± 0.92^{c}	4.44 ± 0.28^{c}
Sodium barbital	Male	6.56 ± 0.31^{c}	9.00 ± 0.20^{a}
	Female	2.84 ± 0.04^{c}	7.10 ± 0.70^{a}
Dioxane (0.5 g/kg) + sodium barbital	Male	4.40 ± 0.40^{a}	7.60 ± 1.20^{b}
	Female	2.96 ± 0.24^{a}	6.60 ± 0.20^{a}
Dioxane (1 g/kg) + sodium barbital	Male	3.64 ± 0.16^{b}	8.96 ± 0.32^{a}
	Female	2.86 ± 0.32^{a}	4.08 ± 0.32^{c}

* Values are mean ± SE (6 mice in each group).
a = $P<0.05$, b = $P<0.01$ & c = $P<0.001$.

TABLE 4

Effect of dioxane and drug treatments on hepatic microsomal electron transport components in male and female mice*.

Group and treatment	Sex	Cytochrome b5 (nmoles/mg protein)	Cytochrome P-450	NADPH cyto.c ** reductase	Total heme (nmoles/mg)
Control	Male	0.15	0.17	14.0	0.38
	Female	0.14	0.16	12.0	0.34
Dioxane (0.5 g/kg)	Male	0.23	0.335	15.0	0.46
Dioxane (1 g/kg)	Male	0.27	0.44	14.4	0.62
Dioxane (In vitro)	Male	0.16	0.22	12.0	0.92
Phenobarbital	Male	0.27	0.49	16.8	0.61
	Female	0.23	0.68	36.0	0.87
Dioxane (0.5 g/kg) + phenobarbital	Male	0.22	0.36	16.8	0.42
	Female?	0.32	0.60	24.0	0.92
Dioxane (1 g/kg) + phenobarbital	Male	0.24	0.385	26.4	0.615
	Female	0.16	0.55	14.4	0.69
Pentobarbital	Male	0.18	0.24	6.0	0.15
	Female	0.16	0.33	24.0	0.48
Dioxane (0.5 g/kg) + pentobarbital	Male	0.19	0.22	15.6	0.31
	Female	0.19	0.38	16.0	0.54
Dioxane (1 g/kg) + pentobarbital	Male	0.22	0.33	19.6	0.54
	Female	0.19	0.19	9.6	0.23
Thiopentone	Male	0.16	0.26	10.8	0.38
	Female	0.19	0.30	18.0	0.31
Dioxane (0.5 g/kg) + thiopentone	Male	0.16	0.21	9.6	0.38
	Female	0.16	0.22	18.0	0.15
Dioxane (1 g/kg) + thiopentone.	Male	0.08	0.32	19.2	0.15
	Female	0.14	0.35	12.0	0.46
Sodium barbital	Male	0.16	0.18	12.0	0.46
	Female	0.22	0.22	12.0	0.46
Dioxane (0.5 g/kg) + sodium barbital	Male	0.14	0.18	12.0	0.42
	Female	0.11	0.49	24.0	0.61
Dioxane (1 g/kg) + sodium barbital	Male	0.20	0.165	12.0	0.16
	Female	0.10	0.27	14.4	0.54

* Values are the average of three determinations of pooled livers of 3 mice.
** nmoles/min/mg protein.

system. Present studies indicate the dioxane decreased the drug induced sleeping time in both male and female mice possibly due to the fact that dioxane shortens sleeping times by antagonizing the barbituate in the central nervous system. The shortened sleeping times in mice treated with dioxane was probably due to the increased metabolism of drugs.

In order to confirm the effect of dioxane on drug induced necrosis, experiments were carried out using sodium barbital only, since it is not siginificantly metabolized, any change in its duration of action in the presence of dioxane would reflect an additive effect on central nervous system rather than an effect on drug-metabolism. Enhanced synthesis of cytochrome P-450 and the higher level of aminopyrine N-demethylase in dioxane treated animals could be due to induced effect of dioxane. The induction of cytochrome P-450 and benzpyrene hydroxylase observed by HOOK et al (1975) in 2,3,7,8-tetra chlorodibenzo-p-dioxin (TCDD) treated male and female rats support our observations.

Decreased lipid peroxidation in dioxane treated animals could be due to the antioxidant property of dioxane metabolites. Secondly, dioxane may attack the endoplasmic reticulum by virtue of a solvent effect on the lipoprotein structure of the organelle.

The results strongly suggest that dioxane acts as an inducing agent of mixed function oxidase system.

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