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Induction of Dimethylnitrosamine-demethylase by Polar Solvents (40721)

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The hepatic carcinogen, dimethylnitrosamine (DMN) is activated by the microsomal *N*-demethylase, DMN-demethylase I (1), which is in many respects an unusual mixed-function oxidase. Compounds, such as 3-methylcholanthrene, phenobarbital, pregnenolone-16α-carbonitrile, polychlorinated biphenyls, and β-naphthoflavone, which induce typical mixed-function oxidases bring about a decrease of DMN-demethylase I activity (1–6). It has been established that with polycyclic hydrocarbons, at least, the decrease of enzyme activity is due to decrease of *de novo* synthesis, the counterpart of true enzyme induction (7). Pregnenolone-16α-carbonitrile, aminoacetonitrile, and 3-methylcholanthrene, which repress hepatic DMN-demethylase activity, inhibit hepatic tumorigenesis induced by DMN (8–11); on the other hand, β-naphthoflavone, which also represses DMN-demethylase I, substantially enhances the hepatocarcinogenicity of DMN (8). It has been suggested that the conflicting effects of β-naphthoflavone may be due to the action of this agent on pathway(s) other than DMN metabolism (8, 12).

The only compounds known to enhance DMN-demethylase activity following *in vivo* pretreatment are acetone and isopropanol (13), and acetone also stimulates the covalent binding of DMN to RNA, DNA, and protein (14); the increases are, however, not accompanied by an increase of the cytochrome *P*-450 level. *In vitro* addition of the two agents to the assay system produces an inhibition rather than enhancement (13), suggesting that the effect is not due to the direct action of these polar compounds on the mixed-function oxidases. The present investigation explored the possibility that the *in vivo* enhancing effect of DMN-demethylase I is not unique to acetone and isopropanol, but is shared by a variety of structurally unrelated polar compounds.

Materials and methods. Male, weanling Sprague–Dawley rats, weighing 45–60 g at sacrifice were used. The rats were fed for 3–4 days an 18% casein semisynthetic diet (15) with riboflavin doubled, and were injected ip with the reagent-grade compounds: acetone (Baker, distilled), dioxane (Eastman, distilled over sodium), tetrahydrofuran (Aldrich, distilled over $LiAlH_4$), dimethylsulfoxide (Sigma), trioctanoin (Eastman), and polyoxyethylene sorbitan monooleate (Tween 80, Sigma), 16 hr prior to sacrifice, at the dose levels given in Footnote *a* to Table I. Control animals were administered identical volumes of 0.9% NaCl used as administration vehicle. The hepatic postmitochondrial fraction was prepared, the microsomal protein content of this fraction was determined, and DMN-demethylase I and arylhydrocarbon hydroxylase activities were measured, as previously described (1, 16). In the experiments where the compounds were added *in vitro* to the DMN-demethylase I assay system, their concentration was 100 m*M*. Although the ip doses given and the concentration used in the *in vitro* experiments were equimolar or lower than those used by Sipes *et al.* (13, 14) for acetone, they may be regarded as relatively high.

Results and discussion. Table I presents the effect of intraperitoneal administration of the polar solvents on DMN-demethylase I and arylhydrocarbon hydroxylase activities. The data confirm the finding of Sipes *et al.* (13, 14) that *in vivo* (oral) administration of acetone brings about an enhancement of DMN-demethylase I activity.

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TABLE I. ENHANCEMENT OF HEPATIC DMN-DEMETHYLASE I ACTIVITY IN THE RAT BY *IN VIVO* ADMINISTRATION OF POLAR COMPOUNDS (POSTMITOCHONDRIAL FRACTION)[a]

Compound	DMN-Demethylase		Arylhydrocarbon hydroxylase	
	nmol HCHO/hr/mg microsomal protein	Percentage increase	nmol 3-OH BP/hr/mg microsomal protein[b]	Percentage increase
Control	90 ± 13		35 ± 4	
Acetone	248 ± 35	176 $0.01 < P < 0.02$	47 ± 7	34 $0.1 < P < 0.2$
Dioxane	294 ± 19	227 $P < 0.001$	51 ± 4	46 $0.02 < P < 0.05$
Tetrahydrofuran	146 ± 4	62 $0.01 < P < 0.02$	28 ± 4	−20 $0.1 < P < 0.2$
Dimethysulfoxide	209 ± 22	132 $0.001 < P < 0.01$	36 ± 4	None $0.8 < P < 0.9$
Trioctanoin	201 ± 17	123 $P < 0.001$	47 ± 4	34 $0.05 < P < 0.10$
Tween 80	111 ± 8	23 $0.1 < P < 0.2$	37 ± 4	None $0.7 < P < 0.8$

[a] Except trioctanoin and Tween 80, all compounds were administered ip, in 0.9% NaCl, 16 hr prior to sacrifice, at the dose of 30 mmol/kg body wt. Trioctanoin (glyceryl tricaprylate) was given at 20 mmol/kg; Tween 80 was administered at the dose of 2.4 ml/kg. Each value represents the mean ± SE obtained with postmitochondrial fraction from six individual animals.

[b] 3-Hydroxybenzo[*a*]pyrene is abbreviated as 3-OH BP.

However, the effect of acetone does not appear to be specific, since the structurally unrelated polar compounds, dioxane, tetrahydrofuran, dimethylsulfoxide, and trioctanoin also enhance the mixed-function oxidase activity. The highest increase was obtained with dioxane, which was also the only compound eliciting a small but significant ($P \leq 0.05$) increase of arylhydrocarbon hydroxylase activity. The moderate inducing effect of dioxane on cytochrome *P*-450 synthesis and on aminopyrine *N*-demethylase has been reported (17).

The data in Table II show that *in vitro* inclusion of these compounds in the assay system does not enhance but rather substantially inhibits DMN-demethylase activity. This parallels the observations of Sipes *et al.* (13) on the *in vitro* versus *in vivo* effect of acetone on the activation of $^{14}CCl_4$ and its covalent binding to protein.

The molecular mechanism whereby certain chemical agents repress and induce microsomal mixed-function oxidases is unknown; however, it has been proposed (18) that these agents modify the conformation of regulatory proteins and thereby bring about gene-level repression and derepression. It is well known that polar solvents, as acetone and dioxane, are potent protein denaturing agents at high concentrations and it has been well documented (19, 20) that such agents have a concentration-dependent ambivalent effect on protein conformation; they stabilize and increase the helix content at low concentrations and unfold (denature) proteins at high concentrations. Consistent with the conformation-modifying effect are recent results from this laboratory (unpublished) that induction and repression by polycyclic hydrocarbons, unlike the carcinogenic effect, does not require their metabolism.

The data in Table II appear to rule out that the *in vivo* enhancement of DMN-demethylase I activity may be due to activating effect of the polar compounds at the level of existing enzyme. However, the data presented here and those of Sipes *et al.* (13, 14) are not inconsistent with the possibility that the effect of these agents—operating at the level of repressor protein(s) of genetic regulation of DMN-demethylase (18)—may play a role in the observed *in vivo* enhancement of enzyme activity.

Summary. The polar compounds acetone, dioxane, tetrahydrofuran, dimethylsulfoxide, trioctanoin, and polyoxyethylene sorbitan monooleate (Tween 80) induce the activity of hepatic dimethylnitrosamine-demethylase I, a

TABLE II. EFFECT OF *IN VITRO* ADDITION OF POLAR COMPOUNDS ON DMN-DEMETHYLASE I ACTIVITY (POSTMITOCHONDRIAL FRACTION)[a]

Compound	DMN-Demethylase	
	nmol HCHO/hr/mg microsomal protein	Percentage change
Control	117 ± 3	
Acetone	48 ± 5	−59 $0.001 < P < 0.01$
Dioxane	0	−100 $P < 0.001$
Tetrahydrofuran	3 ± 2	−97 $0.001 < P < 0.01$
Dimethylsulfoxide	32 ± 4	−73 $0.001 < P < 0.01$
Trioctanoin	220 ± 2	+88 $P < 0.001$

[a] Polar compounds were at 100 mM in the assay system. Each value represents the mean ±SE obtained with postmitochondrial fraction from three individual animals.

mixed-function oxidase repressed by typical inducer agents such as 3-methylcholanthrene and phenobarbital. The highest activity was reached with dioxane, which is also a weak inducer for arylhydrocarbon hydroxylase, an inducible mixed-function oxidase. On the other hand *in vitro* addition of the polar compounds (with the exception of trioctanoin) to the assay system strongly inhibits dimethylnitrosamine-demethylase I activity; *in vitro* addition of trioctanoin increases this enzyme activity.

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