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EFFECT OF ACETONE ADMINISTERED *IN VIVO* UPON HEPATIC MICROSOMAL DRUG METABOLIZING ACTIVITY IN THE RAT

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Abstract—The effects of acetone administered to female rats *in vivo*, upon the metabolism of drugs by the hepatic microsomal subcellular fraction have been studied. There is a rapid increase, maximal at 0·5 hr in aniline *p*-hydroxylation of 69 per cent, and an inhibition of aminopyrine *N*-demethylation of 61 per cent. Thereafter levels return to control values. There is a slower increase in aniline *p*-hydroxylation of 168 per cent, maximal at 48 hr, whilst aminopyrine *N*-demethylation is unaltered. Cycloheximide has no effect upon the changes in activity after 0·5 hr but blocks the increase in aniline *p*-hydroxylation 48 hr after the administration of acetone. It is suggested that acetone may contribute to the changes in drug metabolizing activity found in diabetic animals.

ACETONE added to the hepatic microsomal subcellular fraction has been shown to increase the rate of aniline *p*-hydroxylation.[1,2] We report the effects upon the drug metabolizing activity of microsomes prepared from rats pretreated with acetone *in vivo*.

METHODS

Acetone 60 m-mole/kg, as a 50 per cent solution in saline, was administered by intraperitoneal injection to 200 g female Wistar rats. The rats were allowed free access to food and water. Hepatic microsomes were prepared in 0·25 M sucrose, 0·05 M Tris, pH 7·4, at various times after the administration of acetone, by the method of Ernster *et al.*[3] Drug metabolizing activity was determined over 30 min using a supporting system utilizing glucose-6-phosphate dehydrogenase, as described by Mazel.[4] The formation of formaldehyde from aminopyrine was measured by the method of Nash,[5] and *p*-aminophenol from aniline by the method of Schenkman *et al.*[6] The cytochrome b_5 and cytochrome P-450 content of the microsomes was determined by the method of Dallner[7] and NADPH-cytochrome *c* reductase activity by the method of Mazel.[4] The protein content of the microsomes was determined as described by Lowry *et al.*[8] Microsomes were washed by resuspending 30 mg microsomal protein in 12·5 ml 0·25 M sucrose, 0·05 M Tris, pH 7·4 and centrifuging at 105,000 *g* for 60 min.

RESULTS

The maximum increase in aniline *p*-hydroxylation by hepatic microsomes 1 hr after pretreatment was given by a dose of 60 m-moles of acetone/kg. This dose produced anaesthesia lasting for around 5 hr. Higher doses proved fatal. The drug metabolizing activity of hepatic microsomes prepared at various times after the

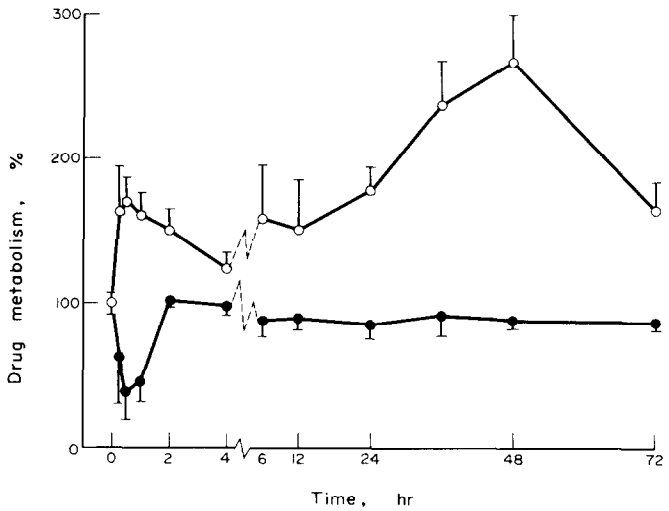

FIG. 1. Effect of acetone 60 m-moles/kg. upon hepatic drug metabolism. (○) Aniline *p*-hydroxylation; (●) aminopyrine *N*-demethylation. Results expressed as a percentage of control and represent the mean of 10 observations. Bars are S.E.M.

administration of acetone is shown in Fig. 1. For convenience the results are expressed as a percentage of the saline injected control, which were; aniline *p*-hydroxylation 29·1 nmoles/mg microsomal protein/30 min, and aminopyrine *N*-demethylation 135·3 nmoles/mg microsomal protein/30 min. Preliminary experiments revealed that the incubation conditions were optimal and that the effects of acetone could not be explained by a change in the requirements for cofactors or buffer. Preparing the microsomes in 0·15 M KCl, the use of various buffers or the replacement of the supporting system by NADPH[4] had no effect upon the changes in drug metabolizing activity caused by the administration of acetone. The *p*-hydroxylation of aniline showed a biphasic response. There was a rapid increase in activity ± S.E.M., of 69 ± 17·4 per cent ($n = 10$, $P < 0{\cdot}002$) by 0·5 hr, after which the levels declined towards control values. After 12 hr the activity again began to increase reaching a maximum of 168 ± 30·9 per cent ($n = 10$, $P < 0{\cdot}001$) by 48 hr. The *N*-demethylation of aminopyrine was inhibited 61 ± 19·6 per cent ($n = 10$, $P < 0{\cdot}01$) by 0·5 hr but had

TABLE 1. EFFECT OF ACETONE PRETREATMENT UPON THE HEPATIC MIXED FUNCTION OXIDASE

	Cyt. P-450 (nmoles/mg microsomal protein)	Cyt. b_5 (nmoles/mg microsomal protein)	NADPH cyt. *c* reductase (nmoles/mg microsomal protein/min)
Control	0·47 ± 0·02	0·27 ± 0·02	89·6 ± 4·2
Acetone pretreatment			
0·5 hr	0·50 ± 0·05	0·26 ± 0·02	139·0 ± 7·0*
48 hr	0·54 ± 0·09	0·27 ± 0·03	213·1 ± 6·2*

Acetone 60 m-moles/kg was administered to female rats by intraperitoneal injection and microsomes prepared after 0·5 and 48 hr. Values represent mean ± S.E.M. if five determinations.

* $P = <0{\cdot}01$.

TABLE 2. EFFECT OF CYCLOHEXIMIDE AND ACETONE PRETREATMENT UPON DRUG METABOLISM BY HEPATIC MICROSOMES

Aniline	nmoles/mg microsomal protein/30 min	
	Aniline *p*-hydroxylation	Aminopyrine *N*-demethylation
0·5 hr Pretreatment		
Saline control	28·7 ± 1·4	131·9 ± 5·7
Cycloheximide	27·2 ± 1·9	148·7 ± 8·6
Acetone	48·2 ± 5·1†	55·4 ± 3·2†
Cycloheximide and acetone	48·3 ± 2·9†	56·7 ± 5·3†
48 hr Pretreatment		
Saline control	28·9 ± 1·9	135·6 ± 8·4
Cycloheximide	31·0 ± 5·7	108·6 ± 4·6*
Acetone	76·2 ± 8·9†	134·9 ± 3·7
Cycloheximide and acetone	22·6 ± 1·1*	100·5 ± 4·3†

Cycloheximide 1 mg/kg was administered simultaneously with acetone 60 m-moles/kg by intraperitoneal injection to female rats. Microsomes were prepared after 0·5 and 48 hr. Values represent mean ± S.E.M. of five determinations.

* P = <0·05.

† P = <0·01 when compared to the saline control values.

returned to control levels by 2 hr. The levels of cytochromes b_5 and P-450 were unchanged after 0·5 hr and 48 hr but NADPH-cytochrome *c* reductase showed an increase in activity corresponding to the increase in aniline *p*-hydroxylation at both 0·5 and 48 hr (Table 1).

Cycloheximide 1 mg/kg, an inhibitor of drug induced protein synthesis[9] had no effect upon the changes in drug metabolizing activity 0·5 hr after the administration

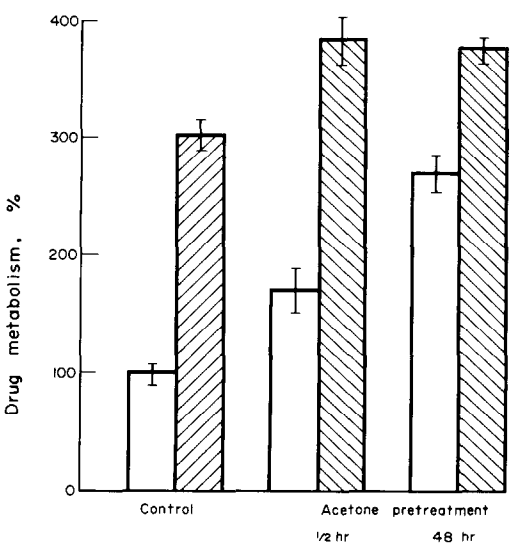

FIG. 2. Effects of acetone 0·5 M, upon aniline *p*-hydroxylation when added directly to the microsomes prepared from control and acetone pretreated rats. Shaded areas represent acetone added directly to the microsomes. Results are expressed as a percentage of the untreated control and represent the mean ± S.E.M. of five observations.

of acetone (Table 2). Cycloheximide completely blocked the increase in aniline *p*-hydroxylation 48 hr after acetone pretreatment and even produced a 22 per cent inhibition in activity. Cycloheximide by itself had no effect upon aniline *p*-hydroxylation but gave rise to a 20 per cent inhibition in aminopyrine *N*-demethylation.

Washing the microsomes prepared after the administration of acetone *in vivo* had no effect upon the changes in drug metabolizing activity. On the other hand the increase in aniline *p*-hydroxylation caused by the direct addition of acetone to the microsomes could be completely reversed by washing the microsomes (results not shown). The inhibition of aminopyrine *N*-demethylation by acetone added *in vitro* was not reversed by washing.

The effect upon aniline *p*-hydroxylation of 0·5 M acetone added directly to the microsomes was additive with the increased activity 0·5 hr after the administration of acetone *in vivo* (Fig. 2). There was no further increase in the direct activation caused by acetone in microsomes prepared 48 hr after the administration of acetone *in vivo*.

DISCUSSION

Acetone administered to rats *in vivo* has a biphasic effect upon drug metabolism by hepatic microsomes. There is a rapid increase in aniline *p*-hydroxylation and an inhibition of aminopyrine *N*-demethylation. This is similar to the effects seen when acetone is added directly to the microsomes. The *in vivo* effects of acetone are however, different from the effects *in vitro*, in two important respects. Washing the microsomes fails to reverse the effects of acetone pretreatment upon drug metabolism whilst the increase in aniline *p*-hydroxylation observed with acetone *in vitro* is completely reversible. The short term effects of acetone *in vivo* upon aniline *p*-hydroxylation are additive with the effects of acetone *in vitro* suggesting a different mechanism of action.

The increase in aniline *p*-hydroxylation 48 hr after the administration of acetone involves the synthesis of new protein and is blocked by cycloheximide, an inhibitor of drug induced protein synthesis.[9] The effects of acetone differ from those of classical inducers of drug metabolism, such as phenobarbital or 3-methylcholanthrene, both of which are associated with an increase in cytochrome P-450.[10] Acetone has no effect upon the levels of cytochrome P-450. Sipes *et al.*[11] have also reported that acetone leads to an increase in the *N*-demethylation of dimethylnitrosamine, but has no effect upon the levels of cytochrome P-450.

Acetone is found in the highest concentration of all the ketone bodies in uncontrolled diabetes and plasma levels as high as 13 mM have been reported.[12] Thus acetone might contribute at least in part, to the changes in drug metabolizing activity found in alloxan induced diabetic rats.[13,14] Neither of the ketone bodies acetoacetate or β-hydroxybutyrate had any effect upon hepatic microsomal drug metabolizing activity when administered *in vivo* in doses up to 20 m-moles/kg, or present *in vitro* in concentrations up to 1 M (results not shown).

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