The effect of a single dose of ethanol on the hepatic microsomal metabolism of foreign compounds in the rat

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The ability of ethanol administered chronically, to increase the metabolism of foreign compounds by the hepatic microsomal fraction is well documented. Relatively little is known however, of the effects of a single dose of ethanol upon the activity of the hepatic microsomal mixed function oxidase.

Ethanol 170 mmol/kg body weight, was administered as a 25% (v/v) solution in water to 250 g male Wistar rats by stomach tube. The hepatic microsomal fraction was prepared by the method of Ernster, Siekevitz & Palade (1962) in 0.25 M sucrose containing 0.05 M Tris buffer pH 7.4. The metabolism of foreign compounds was measured over 30 min at 37°C using the incubation conditions described by Mazel (1971). The formation of p-aminophenol from aniline was measured by the method of Schenkman, Remmer & Estabrook (1967) and formaldehyde formed from aminopyrine by the method of Nash (1953). Cytochrome b_5 and cytochrome P-450 were determined as described by Dallner (1963), NADPH-cytochrome c reductase by the method of Mazel (1971), NADPH-cytochrome P-450 reductase by the method of Gigon, Gram & Gillette (1969) and spectral changes produced by the interaction of foreign compounds with the microsomal fraction determined as described by Schenkman *et al.* (1967). Microsomal protein was measured by the method of Lowry, Rosebrough, Farr & Randall (1951).

A single dose of ethanol resulted in an increase in aniline hydroxylation, from 11.9 ± 0.6 nmol mg^{-1} $30\ min^{-1}$ ($n = 8$) to a maximum of 19.8 ± 2.9 nmol mg^{-1} $30\ min^{-1}$ ($n = 8$, $P < 0.05$) at 24 hour. Aminopyrine demethylation was simultaneously reduced from 124.2 ± 11.9 nmol mg^{-1} $30\ min^{-1}$ ($n = 8$) to 48.4 ± 10.1 nmol mg^{-1} $30\ min^{-1}$ ($n = 8$, $P < 0.01$). There was no change in the liver weight or in the yield of microsomal protein/g liver. The levels of microsomal cytochrome b_5, cytochrome P-450, NADPH-cytochrome c reductase and NADPH-cytochrome P-450 reductase were unchanged. There was however, a $29.0 \pm 6.5\%$ ($n = 4$, $P < 0.01$) decrease in the type I spectral change produced by 5 nM aminopyrine, whilst the type II spectral change produced by 5 mM aniline was reduced by only $10.0 \pm 3.2\%$ ($n = 4$, $P > 0.05$).

Cycloheximide 1 mg/kg, an inhibitor of drug induced protein synthesis (Miller & Jondorf, 1973) completely blocked the increase in aniline hydroxylation but could not prevent a decrease in aminopyrine demethylation. The increase in aniline hydroxylation was due to an increase in the Vmax whilst the Km was unchanged. The effects of ethanol on aniline hydroxylation were not additive with the effects of either of the classical inducers of the microsomal mixed function oxidase, phenobarbitone or 3-methylcholanthrene.

It is concluded that the decrease in the metabolism of aminopyrine produced by the administration of a single dose of ethanol may be associated with a decrease in the binding to the type I site on cytochrome P-450. It is not possible at present to propose a mechanism for the increase in the metabolism of aniline.

References

DALLNER, G. (1963). Studies on the structural and enzymic organization of the membranous elements of liver microsomes. *Acta path. microbiol. scand. suppl.*, **166**, 1-80.

ERNSTER, L., SIEKEVITZ, P. & PALADE, G.E. (1962). Enzyme-structure relationships in the endoplasmic reticulum of rat liver. *J. Cell. Biol.*, **15**, 541-562.

GIGON, P.L., GRAM, T.E. & GILLETTE, J.R. (1969). Studies on the rate of reduction of hepatic microsomal cytochrome P-450 by reduced nicotinamide adenine dinucleotide phoasphate. *Mol. Pharmacol.*, **5**, 109-122.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193**, 265-275.

MAZEL, P. (1971). Experiments illustrating drug metabolism *in vitro*, in *Fundamentals of Drug Metabolism and Drug Disposition*, ed. LáDu, B.N., Mandel, H.G. & Way, E.L., p. 546-582. Baltimore: Williams Wilkins Co.

MILLER, H.H. & JONDORF, W.R. (1973). Glutarimide antibiotics and drug metabolism in the rat. *J. Pharm. Pharmac.*, **25**, 337-338.

NASH, T. (1953). The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.*, **55**, 416-421.

SCHENKMAN, J.B., REMMER, H. & ESTABROOK, R.W. (1967). Spectral studies of drug interaction with hepatic microsomal cytochrome. *Molec. Pharmac.*, **3**, 113-123.