Biochem. J. (1982) **206**, 153–156
Printed in Great Britain

Role of alcohol dehydrogenase activity and of acetaldehyde in ethanol-induced ethane and pentane production by isolated perfused rat liver

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(*Received 22 February 1982/Accepted 10 May 1982*)

The volatile hydrocarbons ethane and n-pentane are produced at increased rates by isolated perfused rat liver during the metabolism of acutely added ethanol. The effect is half-maximal at 0.5 mM-ethanol, and it is not observed when inhibitors of alcohol dehydrogenase such as 4-methyl- or 4-propyl-pyrazole are also present. Propanol, another substrate for the dehydrogenase, is also active. Increased alkane production can be initiated by adding acetaldehyde in the presence of 4-methyl- or 4-propyl-pyrazole. An antioxidant, cyanidanol, suppresses the ethanol-induced alkane production. The data obtained with the isolated organ demonstrate that products known to arise from the peroxidation of polyunsaturated fatty acids are formed in the presence of ethanol and that the activity of alcohol dehydrogenase is required for the generation of the active radical species. The mere presence of ethanol, e.g. at binding sites of special form(s) of cytochrome *P*-450, is not sufficient to elicit an increased production of volatile hydrocarbons by rat liver.

The concept of the involvement of lipid peroxidation in the hepatotoxicity of ethanol (Di Luzio, 1966, 1973; Di Luzio & Stege, 1977) has recently found support with experiments with intact animals where volatile hydrocarbons were detected in the breath after an acute application of ethanol (Köster *et al.*, 1977; Litov *et al.*, 1978, 1981; Burk & Lane, 1979), using the method described by Riely *et al.* (1974). However, Frank *et al.* (1980) have proposed that increased exhalation of hydrocarbons upon acute doses of ethanol cannot be taken as proof for lipid peroxidation, but rather for its inhibitory effect on a specific form of cytochrome *P*-450 capable of metabolizing hydrocarbons that may arise from endogenous sources. Frommer *et al.* (1970), working with isolated rat liver microsomes, have demonstrated, in fact, that n-pentanol is formed from n-pentane at considerable rates, but no information on ethane is available.

In the present work we address the question of whether the increased production of volatile hydrocarbons is due to an action of the alcohol itself, e.g. the binding of a specific form of cytochrome *P*-450 as proposed by Frank *et al.* (1980), or rather the metabolism of ethanol, catalysed by alcohol dehydrogenase. For this purpose, 4-propyl- or 4-methyl-pyrazole were employed, and further the effect of acetaldehyde on hydrocarbon production was studied.

Because breath analysis from the intact animal does not necessarily reflect hepatic metabolism, we have adapted the hydrocarbon sampling to the isolated perfused rat liver (Müller *et al.*, 1981), and it has already been shown that acute application of ethanol leads to a rapid and reversible increase in ethane release.

Materials and methods

Livers from male Wistar rats (150–220 g body wt.), fed on stock diet (Altromin, Lage, Germany) were perfused at 37°C without recirculation of the perfusate (Sies, 1978), using the bicarbonate-buffered salt solution equilibrated with O_2/CO_2 (19:1, v/v) (Krebs & Henseleit, 1932). Perfusate flow (4–5 ml/min per g wet wt.) was maintained constant throughout the individual experiment, O_2 concentration and pH in the effluent perfusate was monitored by appropriate electrodes, and care was taken to maintain effluent O_2 concentration >0.2 mM to avoid pericentral hypoxia.

The collection chamber for hydrocarbons was the one described by Müller *et al.* (1981). Gas chromatography of samples obtained from the collection chamber with Hamilton gas-tight syringes was performed on a Carlo Erba (Frankfurt, Germany) model 51 AC Fractovap gas chromatograph, equipped with a Porasil C column (Linde, München, Germany). The system was calibrated

0306-3283/82/070153-04$01.50/1

with calibration gas (ethane, 0.69 p.p.m.; propane, 0.82 p.p.m.; n-butane, 0.62 p.p.m.; n-pentane, 0.66 p.p.m.) provided by Messer-Griesheim (Duisburg, Germany). Some details of the gas-chromatographic analysis are discussed by Wendel & Dumelin (1981).

Chemicals and biochemicals were obtained from Boehringer (Mannheim, Germany), Merck (Darmstadt, Germany) or Sigma Chemical Co. (München, Germany), except for n-propylpyrazole (a gift from Professor T. Yonetani, Johnson Research Foundation, Philadelphia, PA, U.S.A.) and (+)-cyanidanol (a gift from Dr. G. Hennings, Zyma, München, Germany).

Results

Perfused liver rapidly responds to the infusion of ethanol with an increase in the rate of ethane release, half-maximal at about 0.5 mM-ethanol (Fig. 1). Upon cessation of the infusion of ethanol, there is a rapid reversal of the effect (Müller *et al.*, 1981). To maintain stable rates of increased ethane release, the further experiments were usually carried out with concentrations of around 30 mM-ethanol. The hydrocarbon release elicited by ethanol is sensitive to the addition of inhibitors for alcohol dehydrogenase. As shown in Fig. 2(*a*), n-propylpyrazole decreases ethane release towards controls levels, and conversely, as shown for methylpyrazole in Fig. 2(*b*), prior addition of the inhibitor makes the subsequent addition of ethanol ineffective with respect to ethane release. Control experiments in which no addition was made throughout the experimental period also showed stable conditions of ethane release as in Fig. 2(*b*) (results not shown).

As a parameter for following general cellular damage, lactate dehydrogenase activity released across the plasma membrane into the effluent perfusate was routinely measured. The time course shown in Fig. 2(*b*) was similar to control experiments in which no additions were made, indicating that the organ remained intact for approx. 1 h. In the experiment shown in Fig. 2(*a*), there was a slight increase in lactate dehydrogenase efflux on addition of n-propylpyrazole, but this had no influence on the control level of ethane production, which was re-established at 50–60 min in this experiment.

These experiments indicate that the increase in ethane release due to the addition of ethanol is dependent on flux through alcohol dehydrogenase. If this is so, then the product of the reaction, acetaldehyde, should be able to cause the effect by itself. As shown in Fig. 3, acetaldehyde elicits ethane release; to suppress the back-reaction to ethanol, this experiment was carried out in the presence of methylpyrazole. Reduction of cytosolic NAD^+ to NADH in itself is not responsible for the effects observed here. L-Lactate or D-sorbitol (added at

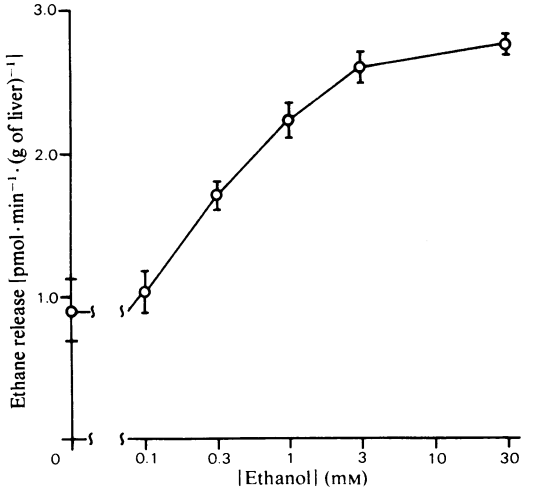

Fig. 1. *Dependence of ethane production by the perfused rat liver on ethanol concentration*
Results are means ± S.D. (represented by the bars) from three different perfusion experiments

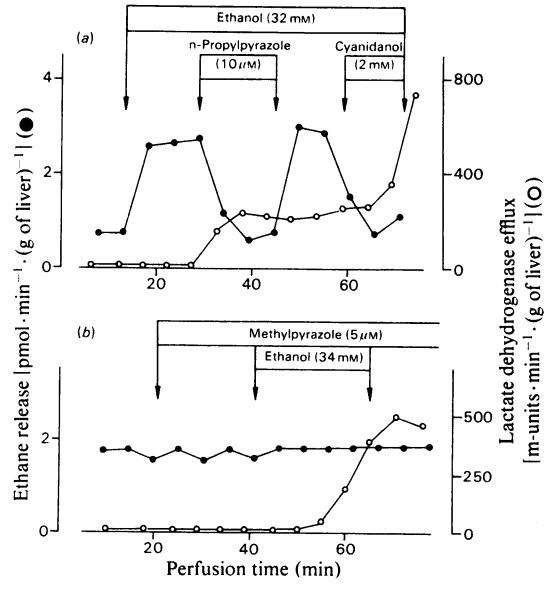

Fig. 2. *Increase of ethane release from perfused rat liver during addition of ethanol (time as indicated), and decrease by n-propylpyrazole and (+)-cyanidanol (a) and suppression of extra ethane release on addition of ethanol in presence of methylpyrazole (b)*

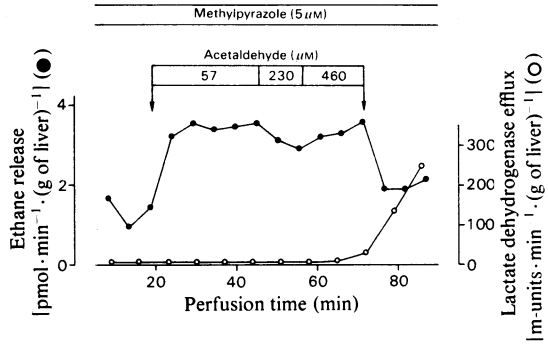

Fig. 3. *Increase of ethane release during addition of acetaldehyde in the presence of methylpyrazole*

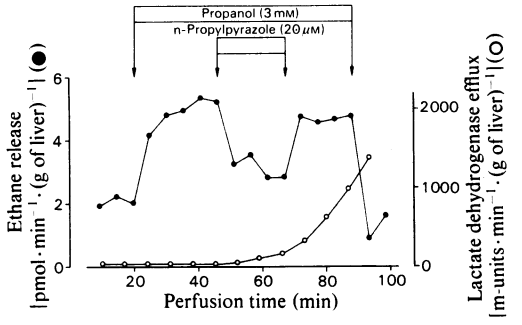

Fig. 4. *Inhibitory effect of n-propylpyrazole on propanol-induced ethane release*

10 mM concentration) were ineffective regarding ethane release (results not shown).

To further substantiate that alcohol dehydrogenase activity is responsible for the enhanced hydrocarbon production, another substrate, n-propanol, was used (Fig. 4). The effects are similar to those observed with ethanol. t-Butanol, on the other hand, which is not a substrate for alcohol dehydrogenase, had only a slight effect, which was not sensitive to n-propylpyrazole (results not shown). That t-butanol causes a slight rise in ethane production at all may be attributable to the capability of the endoplasmic reticulum to metabolize this tertiary alcohol to formaldehyde (Cederbaum & Cohen, 1980) and formaldehyde also caused a slight increase in ethane release (results not shown).

n-Pentane is known to be another product of lipid peroxidation. As shown in Fig. 5, this hydrocarbon is also released on addition of ethanol. However in

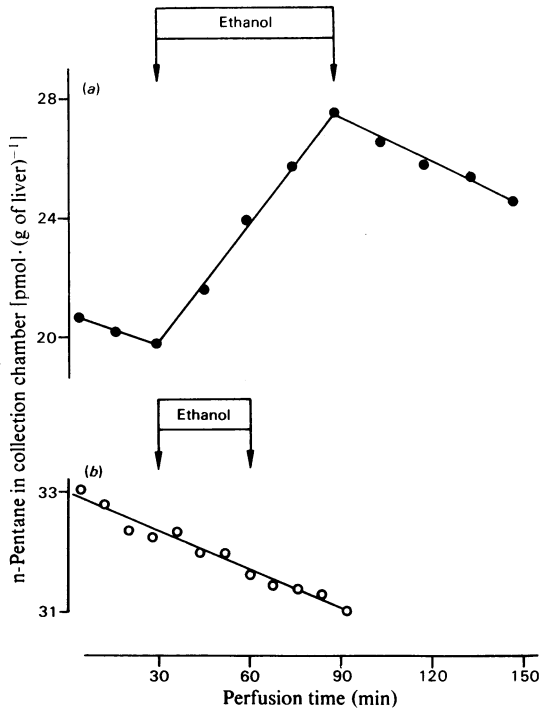

Fig. 5. *Metabolism of added n-pentane to the collection chamber in the presence* (○) *or absence* (●) *of n-propylpyrazole* (*10 μM*) *and the effect of infusion of ethanol* (*31 mM*)
Results are from two separate infusion experiments.

this case the experimental situation is complicated somewhat by the fact that n-pentane is metabolized whereas ethane is not, as we have shown by the addition of these hydrocarbons to the collection chamber (Müller *et al.*, 1981). Therefore, in the experiment of Fig. 5(*a*), n-pentane was added to the collection chamber at an initial amount of about 21 pmol/g wet wt. of liver, so that the metabolism of n-pentane by the liver can be assessed. If one assumes that the rate of n-pentane metabolism by the liver did not change significantly during the time in which ethanol was present, a net production of n-pentane of about 2.5 pmol/min per g wet wt. is calculated for this experiment (see also Table 1). The response is also sensitive to n-propylpyrazole (see Fig. 5*b*).

Discussion

The evidence presented here on the production of ethane and n-pentane by isolated perfused rat liver is in support of the concept that not ethanol by itself, but reaction product(s) arising from the action of

Table 1. *Ethane and n-pentane release from perfused rat liver*

Results are means ± S.E.M. for numbers of different perfusion experiments shown in parentheses. n.d., not determined. n-Pentane production was calculated from the accumulation of n-pentane in the collection chamber (see Fig. 5) assuming a partitioning between gas phase and perfusate similar to that of ethane (Müller *et al.*, 1981) and, further, assuming a constant rate of metabolism throughout the experimental period, as substantiated by the n-propylpyrazole experiment (Fig. 5*b*). n-Pentane uptake was 0.6 ± 0.1 ($n = 8$) pmol/min per g wet wt. of liver, with added n-pentane in the collection chamber ranging from 15 to 35 pmol/g wet wt. of liver.

Additions	Production [pmol·min^{-1}·(g of wet wt. of liver)$^{-1}$] of: Ethane	n-Pentane
None	1.1 ± 0.1 (23)	
Ethanol (31 mM)	3.2 ± 0.3 (13)	1.5 ± 0.4 (4)
Plus n-propylpyrazole (10 μM)	1.1 ± 0.3 (4)	n.d.
Plus cyanidanol (2 mM)	1.2 ± 0.3 (3)	n.d.
Acetaldehyde (1 mM)		
Plus n-propylpyrazole (10 μM)	2.5 ± 0.3 (4)	1.0 ± 0.2 (3)
Acetate (10 mM)	1.2	n.d.

alcohol dehydrogenase, lead to hydrocarbon production. Thus the binding of the alcohol to a specific form of cytochrome *P*-450, which, in turn, would lead to an inhibition of hydrocarbons arising from endogenous sources (Frank *et al.*, 1980), is considered immaterial in the present context.

In the present experiments, ethane in the collection chamber was about 0.3 p.p.m., so that the slight decrease observed within 4 h with the intact animal exposed to 5000 p.p.m. of ethane (Frank *et al.*, 1980) may not apply; further, we are not aware of any data on ethane metabolism in isolated microsomes, whereas the study of Frommer *et al.* (1970) showed such activity for n-butane and longer-chain aliphatic hydrocarbons, including n-pentane.

The observation of increased ethane production after the exogenous addition of acetaldehyde in the presence of methylpyrazole (Fig. 3) points to a role for acetaldehyde. At present, the identification of the oxidative species responsible for alkane production is not known. However, it appears that radical species are involved, as shown by the inhibitory effect of cyanidanol (Fig. 2*a*, Table 1), a flavonoid known to exhibit antioxidant activity (Köster-Albrecht *et al.*, 1979).

Excellent technical assistance was provided by Gunda Böttger. This study was supported by Deutsche Forschungsgemeinschaft, Schwerpunktsprogramm 'Mechanismen toxischer Wirkungen von Fremdstoffen'.

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