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Inhibition of Ethanol- and Aldehyde-Induced Release of Ethane from Isolated Perfused Rat Liver by Pargyline and Disulfiram

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MÜLLER, A. AND H. SIES. *Inhibition of ethanol- and aldehyde-induced release of ethane from isolated perfused rat liver by pargyline and disulfiram.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 429–432, 1983.—Acute addition of ethanol or acetaldehyde to the isolated, perfused rat liver leads to an increase in ethane and n-pentane release. These volatile hydrocarbons are known to originate from the peroxidation of polyunsaturated fatty acids. The effects are half-maximal at 0.5 mM ethanol or 20 μM acetaldehyde in the entering perfusate. Propionaldehyde and benzaldehyde are also able to elicit ethane release. Pargyline and disulfiram, inhibitors of aldehyde oxidation, inhibited the extra ethane release in all cases. The inhibitory effect of pargyline is suppressed during addition of metyrapone. The study indicates that the oxidation of acetaldehyde and not of ethanol itself is the step responsible for increased ethane formation by the perfused rat liver during ethanol infusion.

Acute ethanol and acetaldehyde administration Ethane release Pargyline

VOLATILE hydrocarbons such as ethane and n-pentane are known to originate from the peroxidation of polyunsaturated fatty acids [13]—ethane comes from the ω-3 family and n-pentane from the ω-6 family; additional metabolic sources for these volatile hydrocarbons have not yet been characterized. These alkanes were found in increased amounts upon acute ethanol administration in the exhaled breath of animals [1, 7, 9, 10] and in the gas space around the isolated perfused rat liver [11,12]. That lipid peroxidation occurs during ethanol metabolism has recently been supported by experiments with cyanidanol [12,18], a radical scavenger [15]. Likewise, cyanidanol inhibited lipid peroxidation induced with $CBrCl_3$ [8].

It was recently shown with the perfused rat liver that not ethanol by itself but its metabolism via alcohol dehydrogenase is required for an increase in alkane release [12]. It was concluded that acetaldehyde, the reaction product, plays a crucial role regarding enhanced alkane release.

In the present work we studied the effect of inhibitors of aldehyde oxidation, pargyline [4,5] and disulfiram [3], on ethane formation by the perfused rat liver during infusion of ethanol or various aldehydes such as acetaldehyde, propionaldehyde and benzaldehyde.

METHOD

Chemicals

Ethanol and acetaldehyde were from Merck (Darmstadt, Germany), propionaldehyde and benzaldehyde from Fluka (Buchs, Switzerland), 4-methylpyrazole from Calbiochem-Behring (La Jolla, CA), pargyline and disulfiram from Sigma (Munich, Germany) and other chemicals from Merck (Darmstadt, Germany) or Boehringer (Mannheim, Germany). The reagents used were of the highest purity available.

Animals

Male Wistar rats (150–220 g body wt) fed on stock diet (Altromin, Lage, Germany) were used.

Hemoglobin-Free Liver Perfusion

Perfusion of the livers was carried out at 37°C without recirculation of the perfusate as described [14]. Perfusate flow (4–5 ml/min per g liver wet wt) was maintained constant throughout individual experiments. O_2 concentration in the effluent was monitored with a Clark-type electrode, and care was taken to maintain 0.2 mM O_2 in the effluent to avoid pericentral hypoxia.

Alkane Assay

Gas chromatography was performed with a Carlo Erba Model 2151 AC Fractovap chromatograph (Frankfurt, Germany), equipped with a Porasil C column (Linde, Munich, Germany). The system was calibrated with calibration gas

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TABLE 1

ETHANE AND n-PENTANE RELEASE FROM PERFUSED RAT LIVER

Additions	Production (pmol/min per g wet wt of liver) 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.

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 assuming a partitioning between gas phase and perfusate similar to that of ethane [11] and, further, assuming a constant rate of metabolism throughout the experimental period. 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.

(ethane, 0.69 ppm; propane, 0.82 ppm; n-butane, 0.62 ppm; n-pentane, 0.66 ppm) obtained from Messer-Griesheim (Duisburg, Germany).

The collection chamber for hydrocarbons was the one described in [11]. Gas samples (5 ml) obtained from the collection chamber were taken with Hamilton gas-tight syringes, and exactly 3 ml were introduced by a sampling loop into the chromatographic column. Some details of the gas chromatographic analysis are discussed in [20].

Enzyme Assay

The estimation of leakage of a cytoplasmic enzyme, lactate dehydrogenase, into the effluent perfusate was employed as a parameter of cellular damage.

RESULTS

Relationship of Acetaldehyde to Ethane Release by Perfused Rat Liver and Effect of Pargyline

Some properties of ethane and pentane release from perfused liver are collected in Table 1. The increase in ethane release upon addition of ethanol was half-maximal at about 0.5 mM ethanol [12]. Figure 1 shows the dependence of ethane production on acetaldehyde concentration which was half-maximal at about 20 μM acetaldehyde. The experiments with acetaldehyde were carried out in the presence of 4-methyl pyrazole to suppress the back reaction to ethanol.

To prove whether this effect is an action of acetaldehyde itself or a result of its oxidation, we employed pargyline, an inhibitor of aldehyde oxidation [4,5]. Pargyline is metabolized by the cytochrome P-450 system producing propiolaldehyde, which is thought to be the actual inhibitor [4]. In isolated hepatocytes, acetaldehyde uptake was inhibited up to 60% by pargyline [2].

The increase in ethane formation by both acetaldehyde

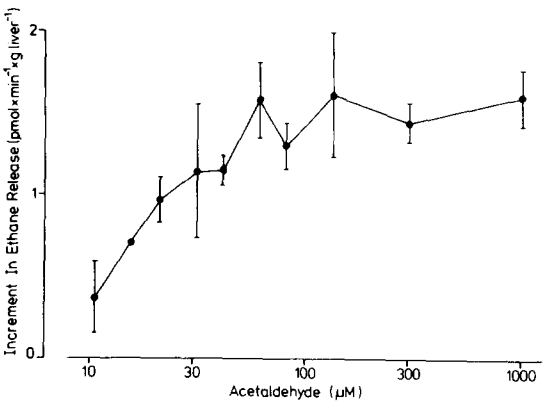

FIG. 1. Dependence of ethane production on acetaldehyde concentration in the presence of 4-methyl pyrazole (5 μM). Results are means±S.E.M. from 2–8 different perfusion experiments.

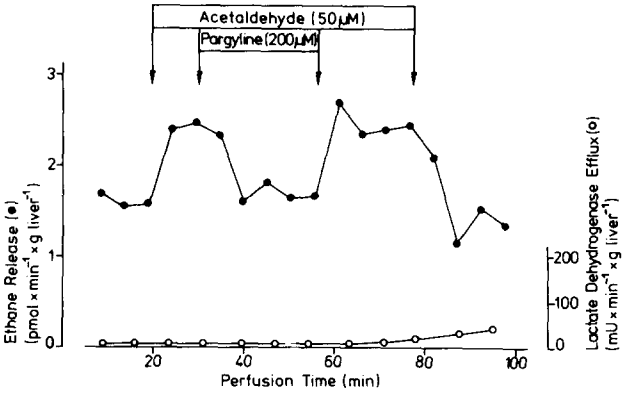

FIG. 2. Inhibitory effect of pargyline on acetaldehyde-induced ethane release. The experiment was carried out in the presence of 4-methyl pyrazole (5 μM).

(Fig. 2) and ethanol (Fig. 3) was diminished by pargyline. Figure 4 shows that the extra ethane release upon addition of ethanol is abolished when pargyline is already present. These observations indicate that in the reaction sequence from ethanol to acetaldehyde and from acetaldehyde to acetic acid the latter step is responsible for enhanced ethane production. This is in agreement with the abolition of the extra ethane release during infusion of ethanol in the presence of 4-methyl pyrazole [12].

Metyrapone, an inhibitor of monooxygenations catalysed by cytochrome P-450, suppresses the inhibitory effect of pargyline (Fig. 5), in agreement with similar findings with SKF 525 A in intact animals [4]. Other aldehydes, such as propionaldehyde and benzaldehyde, are also capable of eliciting an increase in ethane formation, and these effects are also sensitive to pargyline (Figs. 6 and 7).

Effect of Disulfiram on Ethane Release

Disulfiram is another inhibitor of aldehyde oxidation [3] and it is also effective in diminishing ethane release induced by ethanol (not shown) or acetaldehyde (Fig. 8). It is yet unclear which aldehyde metabolizing enzyme activities are

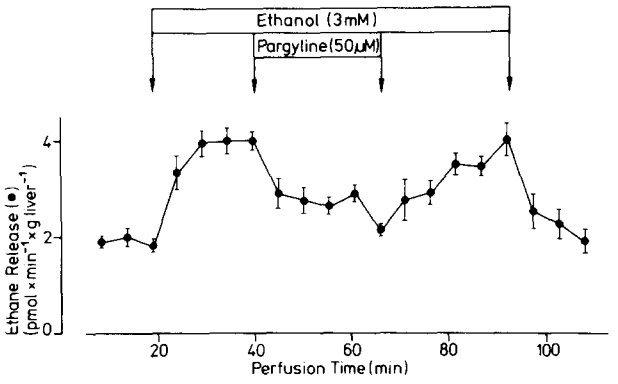

FIG. 3. Inhibitory effect of pargyline on ethanol-induced ethane release. Results are means±S.E.M. from four different perfusion experiments.

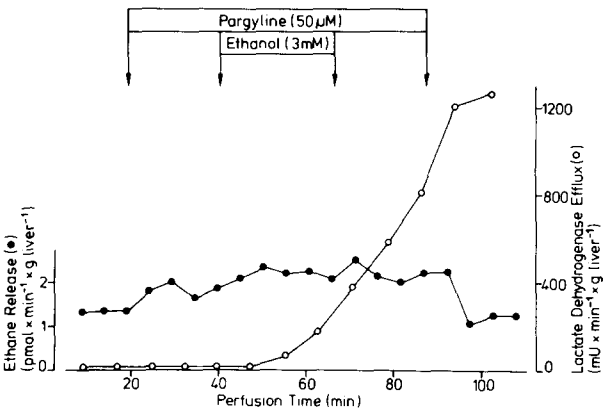

FIG. 4. Suppression of extra ethane release on addition of ethanol in presence of pargyline.

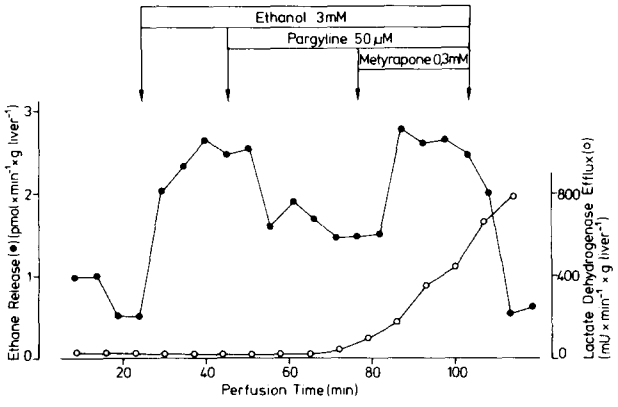

FIG. 5. Suppression by metyrapone of the inhibitory effect of pargyline on ethane release elicited by ethanol.

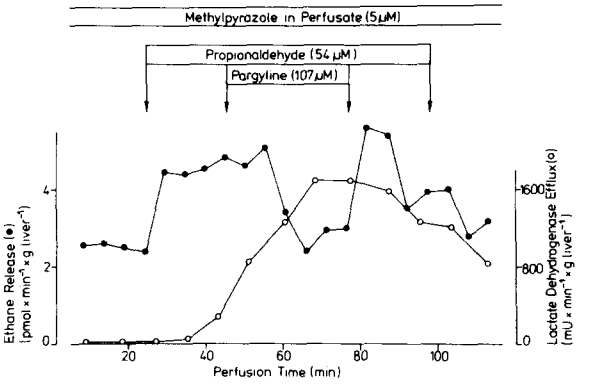

FIG. 6. Inhibitory effect of pargyline on propionaldehyde-induced ethane release.

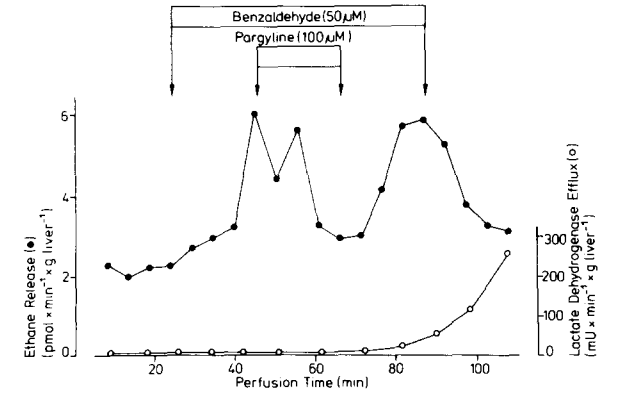

FIG. 7. Inhibitory effect of pargyline on benzaldehyde-induced ethane release.

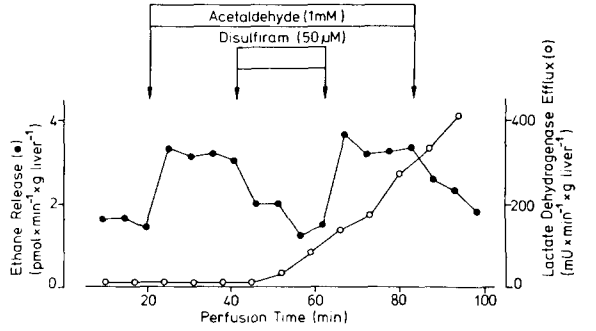

FIG. 8. Inhibition of acetaldehyde-induced ethane release by disulfiram in the presence of 4-methyl pyrazole (5 μM).

inhibited by disulfiram, because the inhibitor is relatively unspecific and reacts with several other enzyme activities as well [16].

DISCUSSION

This study indicates that the oxidation of acetaldehyde and not ethanol itself is the step responsible for the increase in ethane release by the isolated perfused rat liver during ethanol infusion, in agreement with our previous conclusions [12]. This is in support of the concept that acetaldehyde may play an important role in the hepatotoxicity of ethanol. However, whether the observations obtained here with the model of hemoglobin-free perfused rat liver applies to conditions *in vivo* must be further established.

Interestingly, acute application of ethanol has been found to elicit a decrease in reduced glutathione (GSH) levels in isolated hepatocytes [19] and in livers from intact animals [6,17]. This may be related on the one hand to the conjugation of GSH with acetaldehyde [19] and on the other hand also to the ongoing "oxidative stress" leading to hydroperoxide formation and subsequent reduction of such hydroperoxides by GSH peroxidases at the expense of GSH.

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