ETHANE PRODUCTION BY ISOLATED PERFUSED RAT LIVER

A system to study metabolic effects related to lipid peroxidation

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1. Introduction

Alkanes produced from polyunsaturated fatty acids have been detected in exhaled air in experimental animals, and the measurement of alkane exhalation has been proposed as an index for lipid peroxidation [1]. Correlations between lipid peroxidation and in vivo ethane or pentane production have been made with intact animals [1–6], and this parameter is generally considered useful for non-invasive monitoring in toxicological studies (cf. review [7]). The increased formation of malondialdehyde [1,6] and diene conjugation [8] together with decreased levels of glutathione [6] in the liver are in support of the assumption that the liver is the main site for alkane production. However, direct evidence for hepatic alkane production is lacking. Therefore, we have modified the system of non-recirculating hemoglobin-free perfusion of rat liver [9] to permit measurement of hepatic alkane production.

In this work, we have studied alkane release under a variety of conditions including the metabolism of *t*-butyl hydroperoxide, paraquat and ethanol. Parallel determination of glutathione disulfide release showed that ethane formation provides useful information also for the isolated organ. In particular, this experimental system may be suited for assessment of the influence of metabolic manipulations on the process of lipid peroxidation in the liver.

2. Experimental

Livers from male Wistar rats (130–200 g body wt) fed on stock diet (Altromin), were perfused at 37°C as in [9] without recirculation of the perfusate (open system), using the bicarbonate-buffered salt solution [10] equilibrated with O_2/CO_2 (19/1, v/v). Where indicated, the perfusion medium also contained, as sodium salts, L-lactate (2.1 mM) and pyruvate (0.3 mM). Perfusate flow (4–5 ml . min^{-1} . g liver wet wt^{-1}) was maintained constant throughout the individual experiment. $[O_2]$ in the effluent was monitored with a Clark-type electrode, and care was taken to maintain $\geq$0.2 mM O_2 to avoid pericentral hypoxia.

The perfusion table was fitted with a 135 ml cylindrical plexiglass chamber, serving as a collection chamber for evolved alkanes. Sampling was performed through a sampling port using Hamilton gastight syringes. Further, the effluent perfusate was collected into a 1.5 litre and a 1.0 litre vessel (heated to 60°C) connected in series. The gas phases above 0.5 litre of stirred perfusate in these vessels were also analysed for alkanes. Thus, by addition of the amount of alkane released from the liver surface into the plexiglass chamber and of the amount of alkane found in the gas phases above the 2 vessels, the total amount of alkane produced by the organ was determined.

Gas chromatography was performed with a Carlo Erba model 2151AC Fractovap chromatograph, equipped with a Porasil C column, essentially as in [7,11]. The system was calibrated with calibration gas (ethane, 0.69 ppm; propane, 0.82 ppm; *n*-butane, 0.62 ppm; *n*-pentane, 0.66 ppm) provided by Messer-Griesheim (Duisburg). In preliminary experiments using the perfusion apparatus as described, but without a liver, it was ascertained that unspecific absorption of alkanes to the surfaces or leakage of alkanes from the apparatus was negligible, even in the pmol

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range of interest with the liver perfusion. Glutathione efflux from the liver was detected as in [12].

t-Butyl hydroperoxide was a gift from Peroxid-Chemie (Höllriegelskreuth), paraquat was from Fluka (Buchs), and diethylmaleate was from Merck (Darmstadt). Other chemical and biochemicals were from Boehringer (Mannheim) or Merck (Darmstadt).

3. Results

3.1. *Alkane evolution from perfused liver into collection chamber and the release with effluent perfusate*

When massive lipid peroxidation was initiated by infusion of $FeCl_2$ (0.26 mM), alkanes were released from the surface of the perfused liver into the air space of the collection chamber, amounting to 0.7 pmol . min^{-1} . g liver wet wt^{-1} for ethane and pentane (not shown). In other types of initiation of lipid peroxidation, much less alkane is produced (see below), and in those circumstances the metabolism of the gases generated by the liver may become significant. Therefore, we have examined the rate of disappearance of the calibration gas mixture when added into the collection chamber. As shown in fig.1, propane, *n*-butane and *n*-pentane disappear from the chamber when a perfused liver is also present, whereas without the organ there are no changes in alkane content. Ethane, however, showed a net accumulation due to significant production by the liver as is discussed further below.

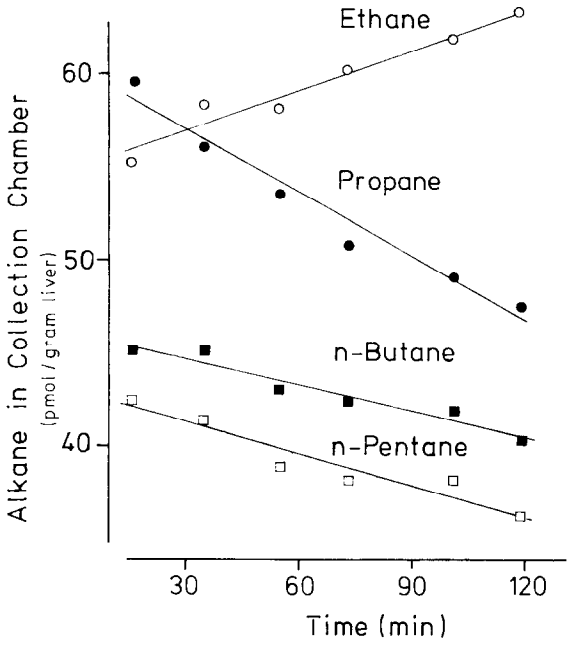

Fig.1. Changes in alkane content in collection chamber equilibrated with the calibration gas mixture (see section 2) in the presence of a perfused liver from a fed rat. Without a liver in the chamber, the lines were practically horizontal.

The partitioning between the evolution from the liver surface and the release into the effluent perfusate was determined in 7 different perfusion experiments. The amount of ethane observed in the collection chamber was remarkably invariant, amounting to 7.1 ± 1.0% of the total alkane release. Further, this percentage did not depend on the rate of ethane production within the range studied here. Therefore, it was considered acceptable to express the results from the accumulated chamber concentration in terms of rates of ethane production per gram liver wet weight per minute by using this factor (table 1, fig.2–4). It is noted that this implies the assumption of a homogeneous response of the organ with respect to ethane production.

3.2. *Ethane release during metabolism of ethanol and of drug substrates*

The release of ethane from the liver amounted to 1.4 pmol . min . g liver wet wt^{-1}, and a slightly lower value was consistently found with livers from rats pretreated with phenobarbital, 1 g/litre drinking water for at least 7 days (table 1). These rates of ethane production could account for those observed with intact rats in previous studies. For example, ethane production in rats fed a control diet was ~400 pmol . h^{-1} . 100 g body wt^{-1} [5], corresponding to 1.7 pmol . min^{-1} . g $liver^{-1}$ with an assumed liver weight of 4 g/100 g body wt. Similar data from other groups were 0.3 [2] and 0.8 [3] pmol ethane . min^{-1} . g $liver^{-1}$.

Table 1
Ethane release from perfused rat liver

Condition	Additions	Ethane production (pmol . min^{-1} . g wet wt^{-1})
Control, fed	None	1.42 ± 0.33 (6)
	Ethanol (44 mM)	7.93 ± 1.54 (4)
Phenobarbital-treated, fed	None	0.85 ± 0.09 (13)
	Paraquat (3 mM)	4.20 ± 1.41 (3)

Data are given as means ± SEM (*n* = number of different perfusion experiments)

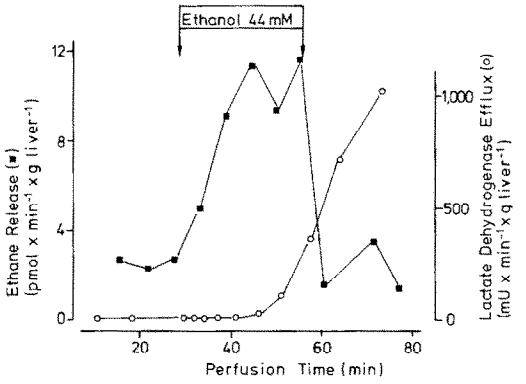

Fig.2. Reversible increase in ethane release from perfused liver during infusion of ethanol.

The increase in ethane release upon addition of an acute dose of ethanol is shown in fig.2 and table 1. The time course shows that the release of ethane is an early and reversible event, occurring a few minutes after the onset of the ethanol infusion, and well before the increased release of lactate dehydrogenase.

The addition of some drugs to perfused livers of phenobarbital-pretreated rats was also included. Paraquat, added to 3 mM, led to a rise in ethane formation of 4.2 pmol . min^{-1} . g wet wt^{-1} within a few minutes after onset of the infusion (table 1) in a time-course similar to that shown for ethanol. Since in whole animal studies on ethane exhalation during paraquat intoxication no significant increase in ethane production was observed [13,14] except for the state of selenium deficiency [13], it was of interest to attempt modification of the production rates in

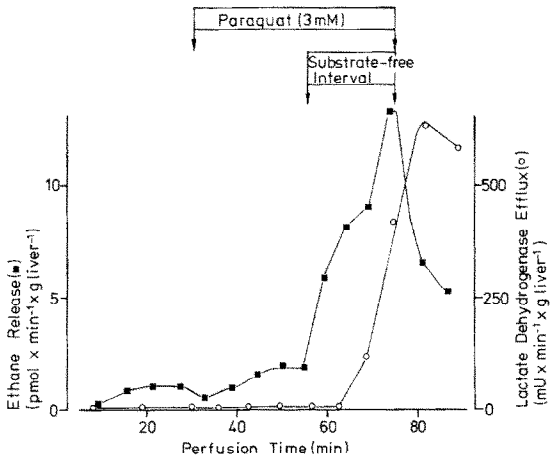

Fig.3. Ethane release from perfused liver from phenobarbital-pretreated fed rat in the presence of paraquat. The perfusion medium contained lactate (2.1 mM) and pyruvate (0.3 mM) except for the period marked as substrate-free interval. Similar experiments in which lactate and pyruvate were not added are reported in table 1.

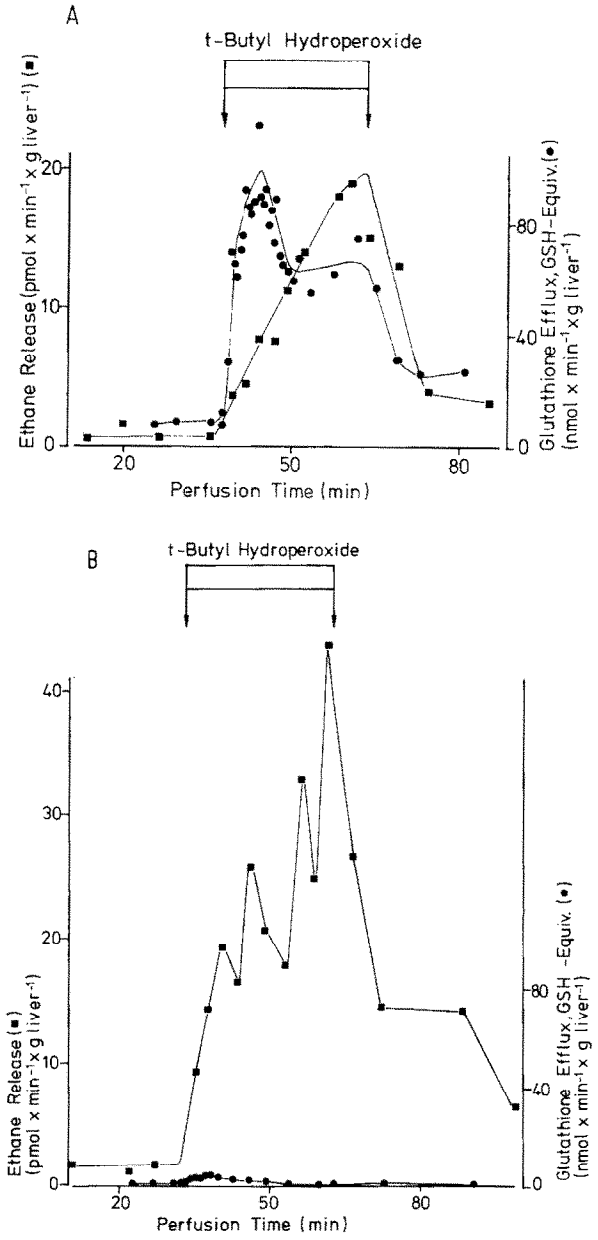

Fig.4. Ethane release and glutathione efflux from perfused livers of fed rats in the presence of *t*-butyl hydroperoxide (0.3 mM) in controls (A) and after pretreatment with diethylmaleate to deplete glutathione (B).

the isolated organ system. In fact, the addition of lactate and pyruvate to the perfusion medium substantially restricted paraquat-induced ethane formation by the liver (fig.3); however, when lactate and pyruvate were omitted, as indicated by the substrate-free interval from 56–76 min in fig.3, there was a swift increase in ethane formation, again well before a rise of lactate dehydrogenase activity in the effluent perfusate. Such observation illustrates the potential use of this experimental system in assessing the role of metabolic manipulations in the control of the rate of lipid peroxidation.

Under conditions of limitation in the capacity of the glutathione 'defense system' the addition of *t*-butyl hydroperoxide elicits the process of lipid peroxidation in intact cells and subcellular fractions [12]. The experiments of fig.4 provide further information on this point; ethane released and glutathione efflux are compared for a control rat (A) and a rat in which liver glutathione was decreased to 25% of the control by prior treatment of the animal with diethyl maleate, 6 mmol/100 g body wt, 1 h prior to the experiment (B). Compared to (A), there is little glutathione disulfide release due to the lack of intracellular glutathione in (B) and concomitantly the rate of ethane production is substantially increased in (B). The ethane release in diethylmaleate-pretreated rats was not significantly above the control level, amounting to 2.0 ± 0.3 pmol . min^{-1} . g liver wet wt^{-1}.

4. Conclusions

These experiments demonstrate directly that isolated perfused liver produces alkanes, the rate of spontaneous ethane formation amounting to 1.4 pmol . min^{-1} . g wet wt^{-1}. The bulk of ethane exhaled from the intact animal can, therefore, be accounted for by hepatic production; this may be different, however, if extrahepatic tissues heavily peroxidize lipids in special cases. Using the paraquat-induced ethane release as an example, the system is shown to qualify for further studies on the metabolic influences in the control of lipid peroxidation occurring with the intact organ. Further, topical information may be gained from simultaneously following other indicators such as malondialdehyde, or also chemiluminescence [15]. The time-correlation between ethane formation and GSSG release as well as the reversibility of both processes after cessation of infusion of oxidants demonstrate that an immediate metabolic response of the organ is measured by this technique.

Acknowledgement

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References

[1] Riely, C., Cohen, G. and Lieberman, M. (1974) Science 183, 208–210.
[2] Köster, U., Albrecht, D. and Kappus, H. (1977) Tox. Appl. Pharmacol. 41, 639–648.
[3] Dillard, C. J., Dumelin, E. E. and Tappel, A. L. (1977) Lipids 12, 109–114.
[4] Hafeman, D. G. and Hoekstra, W. G. (1977) J. Nutr. 107, 666–672.
[5] Burk, R. F. and Lane, J. M. (1979) Toxicol. Appl. Pharmacol. 50, 467–478.
[6] Wendel, A., Feuerstein, S. and Konz, K.-H. (1979) Biochem. Pharmacol. 28, 2051–2055.
[7] Wendel, A. and Dumelin, E. E. (1981) Methods Enzymol. in press.
[8] Lindstrom, T. and Anders, M. (1978) Biochem. Pharmacol. 27, 563–567.
[9] Sies, H. (1978) Methods Enzymol. 52, 48–59.
[10] Krebs, H. A. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33–66.
[11] Wendel, A. and Heidinger, S. (1980) Res. Commun. Chem. Pathol. 28, 473–482.
[12] Sies, H. and Summer, K. H. (1975) Eur. J. Biochem. 57, 503–512.
[13] Burk, R. F., Lawrence, R. A. and Lane, S. M. (1980) J. Clin. Invest. 65, 1024–1031.
[14] Steffen, C., Muliawan, H. and Kappus, H. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 310, 241–243.
[15] Boveris, A., Cadenas, E., Reiter, R., Filipkowski, M., Nakase, Y. and Chance, B. (1980) Proc. Natl. Acad. Sci. USA 77, 347–351.