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THE CRUCIAL ROLE OF HYPOXIA IN HALOTHANE-INDUCED LIPID PEROXIDATION

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Halothane-induced lipid peroxidation in NADPH-reduced liver microsomes from phenobarbital-pretreated male rats was studied under defined steady state oxygen partial pressures (Po_2). Under anaerobic conditions, as well as at a Po_2 above 10 mm Hg no halothane-induced formation of malondialdehyde was detected. At a Po_2 below 10 mm Hg, however, with a maximum near 1 mm Hg oxygen, significant halothane-induced malondialdehyde formation was found. This evidence supports the hypothesis that halothane can induce lipid peroxidation. The Po_2 (i) must be low enough to permit the reductive formation of $^{\cdot}CF_3CHCl$-radicals but (ii), it must be high enough to promote formation of lipid peroxides.

Lipid peroxidation, mediated by reductively-formed $^{\cdot}CF_3CHCl$-radicals, might explain the hepatotoxicity of halothane ($CF_3CHBrCl$), a commonly used anaesthetic (1). Thus far, however, experiments along this line gave contradictory or negative results. Although in in vivo experiments an increase in conjugated dienes of microsomal lipids was observed following anaesthesia with halothane (2,3), in in vitro experiments with isolated, NADPH-reduced rat liver microsomes the formation of malondialdehyde, another indicator of lipid peroxidation, remained unaffected or even decreased during incubation with halothane (2,4). Moreover, Wood et al. (4) could demonstrate that the halothane-induced increase in conjugated dienes is maximal under anaerobic conditions, a situation which virtually excludes lipid peroxidation.

In this paper, the problem of halothane-induced lipid peroxidation is studied with special emphasis on the oxygen partial pressure (Po_2) present. This because of our working

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hypothesis (5) that halothane-induced lipid peroxidation should proceed only under a Po_2 which fulfills both requirements: (i) to be low enough to permit the reductive formation of $\cdot CF_3CHCl$-radicals, a process which is inhibited by oxygen (6-9), but (ii) to be high enough to promote formation of lipid peroxides.

METHODS

The experiments were performed with microsomes from phenobarbital-pretreated (0.1 % Na-phenobarbital, w/v, dissolved in drinking water, for 4 days) male rats. The incubation system consisted of microsomes (about 3.0 mg microsomal protein/ml), NADPH (about 0.7 mM, regenerating system), halothane (0.5 mM), and $MgCl_2$/KCl/Tris-HCl buffer (6 mM/104 mM/50 mM, pH 7.4). A given Po_2 was maintained constant throughout the experimental period by the addition of O_2-saturated buffer, using a feedback control system. This oxy-stat system provided constant Po_2 from 7 - 15 mm Hg with a signal-to-noise ratio of about 50 and below 7 mm Hg with a signal-to-noise ratio of about 5. Lipid peroxidation (the amounts of thiobarbituric acid-reactive substances produced) was estimated by the 2-thiobarbituric acid method (10) and expressed as equivalents of malondialdehyde. Protein was determined with the method of Lowry et al. (11). Further experimental details are as in (12,13).

RESULTS

During the incubation of NADPH-reduced liver microsomes from phenobarbital-pretreated male rats under different steady state Po_2 significant amounts of malondialdehyde were only formed at a Po_2 above 1 mm Hg (Fig. 1). As seen in Fig. 2, this NADPH-induced lipid peroxidation continuously increased with increasing Po_2. On the whole, however, only small amounts of malondialdehyde were formed under these conditions.

Addition of halothane to the NADPH-reduced liver microsomes was without effect on the formation of malondialdehyde at Po_2-values above 10 mm Hg (Figs. 1,2). Below 10 mm Hg, however, with a maximum near a Po_2 of 1 mm Hg, a marked rise in the formation of malondialdehyde was observed. This NADPH/halothane-induced lipid peroxidation started following a short lag phase, was almost linear for about 10 min and slowed down at about 20 min, possibly due to an inactivation of cytochrome P-450 (14). Under anaerobic conditions no lipid peroxidation

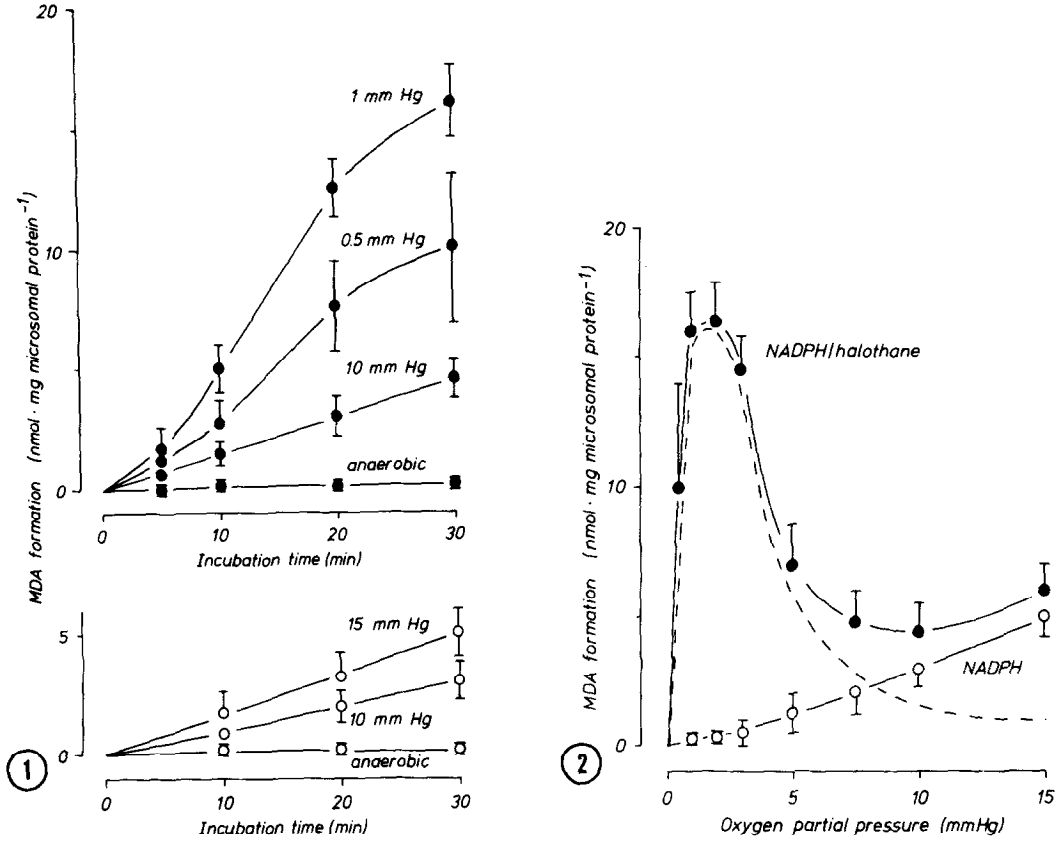

Fig. 1 NADPH- and NADPH/halothane-induced lipid peroxidation in rat liver microsomes from phenobarbital-pretreated male rats. NADPH-reduced microsomes were incubated in absence and presence of halothane (0.5 mM) under anaerobic conditions and at Po_2 of 0.5, 1, 10 and 15 mm Hg. Lipid peroxidation is expressed as malondialdehyde (MDA) formation. Each value represents a mean ± S.E.M. of at least 3 incubations at 37°.

Fig. 2 Effects of increasing Po_2 on NADPH- and NADPH/halothane-induced lipid peroxidation. NADPH-reduced liver microsomes from phenobarbital-pretreated male rats were incubated in absence and presence of halothane under varying steady state Po_2 for 30 min. Experimental details are as in Fig. 1. The dashed line represents the difference between NADPH- and NADPH/halothane-induced formation of malondialdehyde.

was detected, as would be expected (Fig. 1). Similarly, in presence of halothane but without NADPH no formation of malondialdehyde was measured under all Po_2 studied (data not shown). Subtraction of NADPH- from NADPH/halothane-induced lipid peroxidation (Fig. 2) reveals the specific effect of halothane on lipid peroxidation within the Po_2-range from 0.5 to 10 mm Hg.

DISCUSSION

The critical role of hypoxia in the generation of cell damage by lipid peroxidation (15) is exemplified also by the results shown here. Halothane induces lipid peroxidation exclusively under hypoxic conditions with a maximum near a Po_2 of 1 mm Hg. Above a Po_2 of 10 mm Hg, halothane loses its capability of inducing lipid peroxidation, a result which can explain the negative reports thus far (2,4). Probably, in those experiments Po_2 was not controlled rigorously enough. In agreement with our working hypothesis (5), a Po_2 near 1 mm Hg is obviously low enough to permit the reductive formation of $^{\cdot}CF_3CHCl$-radicals at sufficient rate, a process which is inhibited by oxygen and catalyzed by cytochrome P-450, especially those isoenzymes induced by phenobarbital (10-13). On the other hand, a Po_2 around 1 mm Hg appears to be high enough to allow the formation of fatty acid peroxides from fatty acid radicals formed by interaction of $^{\cdot}CF_3CHCl$- or $^{\cdot}CF_3CHClO_2$-radicals with unsaturated fatty acids of membrane lipids (16,17).

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