Cytochrome P-450 and Oxygen Toxicity. Oxygen-Dependent Induction of Ethanol-Inducible Cytochrome P-450 (IIE1) in Rat Liver and Lung†

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ABSTRACT: The ethanol-inducible form of cytochrome P-450 (P-450IIE1) has previously been shown to exhibit an unusually high rate of oxidase activity with the subsequent formation of reactive oxygen species, e.g., hydrogen peroxide, and to be the main contributor of microsomal oxidase activity in liver microsomes from acetone-treated rats [Ekström & Ingelman-Sundberg (1989) *Biochem. Pharmacol.* (in press)]. The results here presented indicate that oxygen exposure of rats causes an about 4-fold induction of P-450IIE1 in rat liver and lung microsomes. The induction in liver was not accompanied by any measurable increase in the P-450IIE1 mRNA levels, but the enhanced amount of P-450IIE1 accounted for 60% of the net 50% increase in the level of hepatic P-450 as determined spectrophotometrically. The induction of P-450IIE1 was maximal after 60 h of O_2 exposure, and concomitant increases in the rates of liver microsomal CCl_4-dependent lipid peroxidation, O_2 consumption, NADPH oxidation, O_2^- formation, H_2O_2 production, and NADPH-dependent microsomal lipid peroxidation were seen. Liver microsomes from oxygen-treated rats had very similar properties to those of microsomes isolated from acetone-treated rats with respect to the P-450IIE1 content and catalytic properties, but different from those of thyroxine-treated animals. Treatment of rats with the P-450IIE1 inducer acetone in combination with oxygen exposure caused a potentiation of the NADPH-dependent liver and lung microsomal lipid peroxidation and decreased the survival time of the rats. The results reached indicate a role for cytochrome P-450 and, in particular, for cytochrome P-450IIE1 in oxygen-mediated tissue toxicity.

The toxicity of oxygen appears to be connected with pulmonary injury caused by oxygen radical dependent damage to membranes (Fridovich, 1978). In the initial steps, various types of oxygen radicals formed cause cell damage by overwhelming antioxidant defenses, which are to a great extent exerted by the action of superoxide dismutase (Freeman et al., 1982). Thus, tolerance to prolonged exposure of 100% oxygen, developed by exposure to 85% oxygen, is probably in part dependent on the induction of superoxide dismutase (Crapo et al., 1980). Entrapment of superoxide dismutase or catalase into liposomes, injected intravenously into rats (Turrens et al., 1984) or added to cell cultures (Lesko et al., 1984), significantly increases survival time of rats or cells during hyperoxia.

During oxygen exposure, increased amounts of lipid peroxides in the microsomal fraction are observed (Iwata et al., 1986), and alterations in microsomal phospholipid composition take place, a reaction that is inhibited by the antioxidant α-tocopherol (Taniguchi et al., 1986). Lipid peroxides formed during hyperoxia might act as chemotactic factors attracting phagocytic cells (Perez et al., 1980). This implies that microsomal lipid peroxidation might be of importance for the oxygen-dependent toxicity.

In addition to working as a monoxygenase, cytochrome P-450 exhibits an oxidase activity, thereby reducing dioxygen to hydrogen peroxide or water according to two- or four-electron reduction pathways, respectively (Kuthan & Ullrich, 1982; Gorsky et al., 1984):

$$NADPH + H^+ + O_2 \rightarrow NADP^+ + H_2O_2$$

$$2NADPH + 2H^+ + O_2 \rightarrow 2NADP^+ + 2H_2O$$

Data are currently accumulating indicating that most of the microsomal production of H_2O_2 originates via autoxidation of the oxycytochrome P-450 complex, which liberates O_2^-, which in turn spontaneously dismutates to give hydrogen peroxide (Kuthan et al., 1978; Kuthan & Ullrich, 1982; Ingelman-Sundberg & Johansson, 1980, 1984; Terelius & Ingelman-Sundberg, 1988). It has recently been shown that the oxidase activity of rabbit liver microsomal cytochrome P-450 LM_2 can bring about lipid peroxidation in reconstituted membrane vesicles (Ekström & Ingelman-Sundberg, 1984, 1986). Experiments with various scavengers of active oxygen species indicated that the generation of superoxide anions, in the presence of non-heme iron, was crucial for the initiation process. The ethanol-inducible form of cytochrome P-450 (P-450IIE1) has an especially high oxidase activity (Johansson & Ingelman-Sundberg, 1984; Gorsky et al., 1984), and this isozyme could therefore be a likely candidate to contribute to microsomal NADPH-dependent lipid peroxidation (Ekström & Ingelman-Sundberg, 1986, 1988). Reinke et al. (1987) have recently described the formation of carbon-centered radicals in vivo in the endoplasmic reticulum in liver of ethanol-treated but not of control rats. P-450IIE1 is present in high amounts in livers from ethanol-induced animals and is almost exclusively localized in the centrilobular region (Ingelman-Sundberg et al., 1988), an area known to be affected by oxidative stress upon ethanol treatment (Di Luzio, 1967; Lieber, 1984). Among the most efficient substrates for this isozyme are acetone (Johansson et al., 1986), carbon tetrachloride (Johansson & Ingelman-Sundberg, 1985), butanol (Morgan et al., 1982), and *N*-nitrosodimethylamine (Levin et al., 1986; Patten et al., 1986).

Results by Gonder et al. (1985) have indicated a role of cytochrome P-450 in the toxic action of oxygen. Mice nonresponsive to cytochrome P-450 induction were found to survive for significantly longer time periods in the presence of 100% oxygen than were mice who responded to oxygen treatment by cytochrome P-450 induction. The results

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presented above indicate the importance of cytochrome P-450 for oxidase activity and lipid peroxidation, reactions that are of importance for oxygen-mediated membrane damage. In the present investigation we have examined relationships between oxygen-dependent cytochrome P-450 induction and these reactions and evaluated whether oxygen might prove an inducer of the ethanol-inducible form of cytochrome P-450.

Experimental Procedures

Animal Treatment. Male Sprague-Dawley rats (140–150 g) were used and had access to food and water ad libitum. During oxygen exposure [95% O_2, 5% N_2 (breathing oxygen, AGA, Stockholm, Sweden)], two rats were kept in a Plexiglas chamber (26 × 36 × 10 cm), through which oxygen was passed at a rate of 300 mL/min. At this flow rate, complete gas exchange occurred about each 30 min. Control rats were exposed to an air atmosphere in similar cages. In some experiments, rats were treated with acetone [5 mL/kg, given intragastrically as 33% (v/v) acetone in 0.9% (w/v) NaCl] 40 h prior to oxygen exposure, just before exposure, and every 20 h throughout the exposure period. Other rats were starved and treated with acetone (5 mL/kg for 2 days) as previously described (Eliasson et al., 1988). Another group of rats was treated intraperitoneally with L-thyroxine (200 μg/kg, Sigma Chemical Co.) for 7 consecutive days.

Preparation of Microsomes. Immediately after oxygen exposure, the animals were killed and the liver and lungs quickly removed and homogenized in 2 volumes of ice-cold 10 mM sodium/potassium phosphate buffer, pH 7.4, containing 1.14% (w/v) KCl. Microsomes were subsequently prepared by differential centrifugation (Johansson et al., 1988) and stored under nitrogen at −70 °C.

Assay Methods. The rate of NADPH oxidation was determined at 340 nm by using an absorption coefficient for NADPH of 6.2 mM^{-1} cm^{-1}. Lipid peroxidation was determined as the formation of thiobarbituric acid (TBA) reactive products according to the method of Bernheim et al. (1948). Microsomes corresponding to 1 mg of protein were suspended in 1 mL of 50 mM potassium phosphate buffer, pH 7.4. After a preincubation period of 3 min at 37 °C, the incubations were started by the addition of 0.25 mg of NADPH and terminated 10 min later by the addition of 250 μL of 40% TCA and 125 μL of 5 M HCl. Lipid peroxidation induced by NADPH-dependent metabolism of carbon tetrachloride was studied by using microsomes corresponding to 0.5 mg of protein in 1 mL of 50 mM potassium phosphate buffer, pH 7.4, containing 10 μM EDTA and 2.15 mM CCl_4 according to the method of Johansson and Ingelman-Sundberg (1985). Oxygen consumption was measured by using a Clarke electrode kindly provided by Drs. Hjördis Thor and Gregory Moore at the Department of Toxicology, Karolinska Institute. The incubation mixture, in a total volume of 2 mL, contained 50 mM potassium phosphate buffer, pH 7.4, 0.1 mM EDTA, and microsomes corresponding to 4 mg of protein. After a preincubation period of 5 min at 37 °C, 1.6 mg of NADPH was added and the rate of oxygen consumption was registered continuously. Superoxide anion production was measured by using succinylated cytochrome *c* essentially as described previously (Johansson & Ingelman-Sundberg, 1984). The system was calibrated by using 10 mM dihydroxyfumarate and corrected for by the quenching of the microsomes themselves (Kuthan & Ullrich, 1982). Hydrogen peroxide was determined according to the method of Hildebrandt and Roos (1975). The incubations were terminated by the addition of 0.1 mL of 1 M $MgCl_2$ and 3 mL of dichloromethane. The reaction vessels were extracted twice with dichloromethane, and the organic phase was evaporated to dryness under N_2 and subjected to thin-layer chromatography and autoradiography.

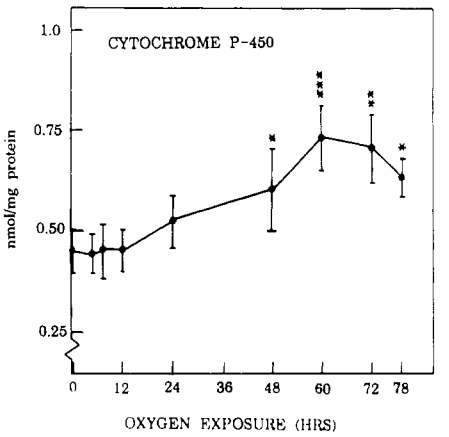

FIGURE 1: Effect of the time of oxygen exposure on the specific content of spectrophotometrically determined cytochrome P-450 in rat liver microsomes. Four different animals were used in each group. Significances are (*) $p < 0.05$, (**) $p < 0.005$, and (***) $p < 0.001$, compared to control (0 h of oxygen exposure).

Western Blot and Northern Blot Analyses. SDS–polyacrylamide gel electrophoresis was performed by using the discontinuous system described by Laemmli (1970) and a Bio-Rad mini Protean II apparatus. Slab gels of an acrylamide concentration of 8.5% were used, and 3 μg of protein in each well was applied. The amount of P-450IIE1 in microsomes was determined by Western blot analysis using a polyclonal antiserum (Johansson et al., 1988; Eliasson et al., 1988). The specificity of the antiserum is characterized by the following: (i) the recognition of only one band in Western blot analysis of liver microsomes from control rats and rats treated with benzene, phenobarbital, β-naphthoflavone, imidazole, ethanol, acetone, Me_2SO, or isoniazid; (ii) the absence of cross reactivity in Western blot analysis, according to the induction response and electrophoretic mobility, with any of rat liver microsomal cytochromes P-450 IIA1, IIB1, IA1, IA2, IIB2, IIC3, IIC7, IIC11, or IIIA (Johansson et al., 1988; Eliasson et al., 1988; Johansson and Ingelman-Sundberg, unpublished data); and (iii) the failure to recognize any type of cytochrome P-450 present in the periportal region of the liver lobule (Ingelman-Sundberg et al., 1988). Total RNA was isolated from homogenized livers by using sucrose gradient centrifugation and phenol extraction [cf. Taylor and Schimke (1983)]. P-450IIE1 mRNA was determined by Northern blot analysis using a ^{32}P-labeled oligonucleotide complimentary to bp 771–820 of the rat P-450IIE1 sequence (Song et al., 1986) as previously described (Johansson et al., 1988).

Results

Induction of Cytochrome P-450 by Oxygen Exposure. A continuous exposure of rats to a 95% oxygen atmosphere caused at maximum (after 60 h) a 50% increase in the hepatic microsomal content of cytochrome P-450 (Figure 1). After 48 h, a significant increase was evident. The relative increase of cytochrome P-450 was much higher in lung microsomes, and the specific cytochrome P-450 content in rat lung microsomes increased from 0.07 to 0.23 nmol/mg after 60 h of oxygen exposure (mean of three experiments performed with pooled fractions from four animals in each group). In control rats, housed in the same type of chambers, but exposed to air, and allowed exactly the same amount of food as the oxygen group had taken, the increase of cytochrome P-450 in liver was from 0.47 ± 0.10 to 0.53 ± 0.09 nmol/mg ($n = 4$, not shown in figure).

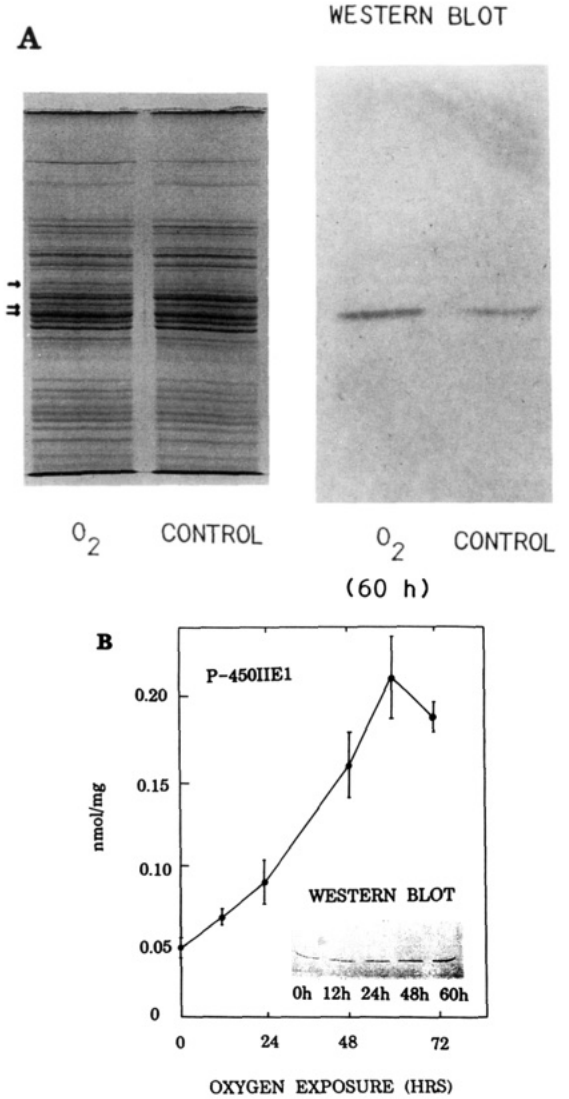

FIGURE 2: SDS gel electrophoretic and Western blot analysis of liver microsomes from control and oxygen-exposed rats (A) and effect of time on the level of P-450IIE1 in liver microsomes of oxygen-treated rats (B). Arrows in (A) indicate protein bands with increased intensity after oxygen exposure. The position of the anti-P-450IIE1 IgG immunoreactive band in the Western blot analysis corresponded exactly to the protein band with higher intensity in microsomes from oxygen-treated rats indicated with the arrow in the lowest position. Insert in (B) shows Western blot analysis with anti-P-450IIE1 IgG of liver microsomes isolated from rats exposed to oxygen for 0, 12, 24, 48, and 60 h. Microsomes corresponding to 3 μg were used in each lane.

Evaluation of Cytochrome P-450IIE1 Induction by Oxygen. Liver microsomes from control and oxygen-exposed rats were subjected to SDS–polyacrylamide gel electrophoresis (Figure 2A). According to Coomassie blue staining, one major protein and two minor bands were increased in liver by the oxygen treatment. Western blot analysis using anti-P-450IIE1 IgG revealed that the major band corresponded to P-450IIE1 (Figure 2A). Immunoquantification using Western blot analysis of this P-450 isozyme in liver microsomes after various exposure times indicated that the amount of P-450IIE1 increased at maximum 4-fold (Figure 2B). This corresponds to an elevation of P-450IIE1 content from 50 to 200 pmol/mg of microsomal protein; i.e., this increase accounts for about 60% of the net elevation in cytochrome P-450 as determined spectrophotometrically (cf. Figure 1).

The level of cytochrome P-450IIE1 mRNA was quantified by using a synthetic oligonucleotide in Northern blot analysis, 24 and 60 h after oxygen exposure [cf. Johansson et al. (1988)]. However, no increase at the mRNA level was observed at either time point [P-450IIE1 mRNA levels of 100 ± 26% in control rat livers and 105 ± 37% in O_2-exposed rat livers at 24 h (n = 4)]. This indicates that the induction mechanism does not involve a transcriptional activation nor any stabilization of P-450IIE1 mRNA.

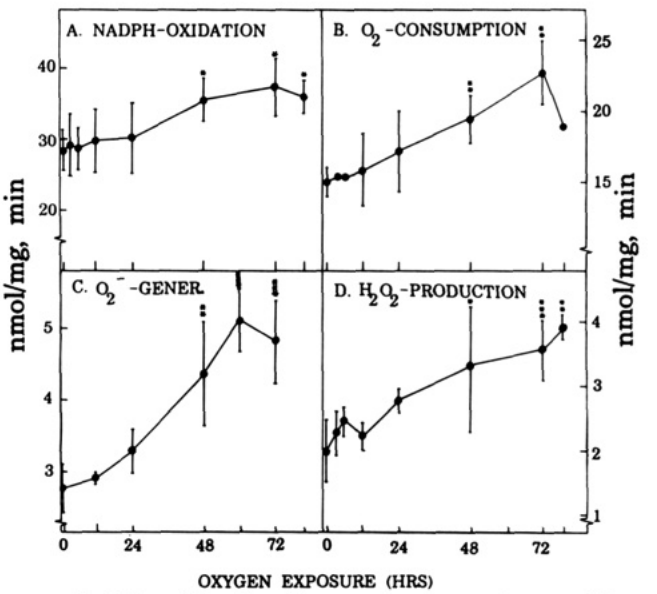

FIGURE 3: Effect of the time of oxygen exposure on the rate of liver microsomal NADPH consumption (A), O_2 consumption (B), superoxide anion generation (C), and hydrogen peroxide production (D). Significances indicated are (*) $p < 0.05$, (**) $p < 0.005$, and (***) $p < 0.001$, compared to control (0 h of oxygen exposure).

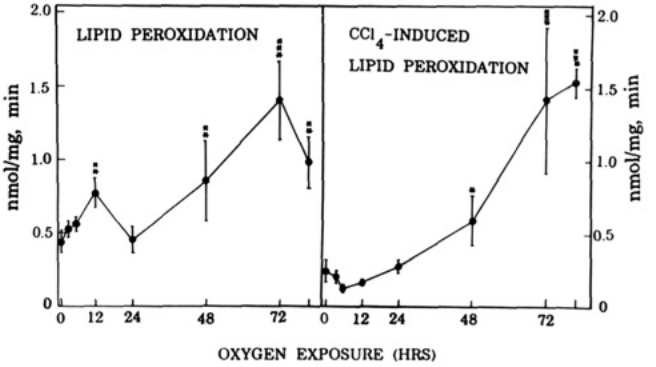

FIGURE 4: Effect of the time of oxygen exposure on the rate of liver microsomal NADPH-dependent lipid peroxidation in the absence of exogenous substrate (left) or in the presence of carbon tetrachloride (right). Products of lipid peroxidation were analyzed as TBA reactive substances (cf. Experimental Procedures).

Effect of Oxygen on NADPH-Dependent Microsomal Oxidase Activities. The rate of cytochrome P-450 dependent oxidase activity in the liver microsomes was monitored by measuring NADPH and O_2 consumption as well as O_2^- and H_2O_2 formation. As shown in Figure 3, concomitant increases in these variables were seen with the cytochrome P-450 content (cf. Figure 1), and significant changes were evident after 48 h of oxygen exposure. At maximum, the rates of O_2 consumption, O_2^- generation, and H_2O_2 production increased by 60–100% after 60 h of oxygen exposure. The corresponding increase of the rate of NADPH consumption was about 30% (Figure 2).

Effect of Oxygen Exposure on Microsomal Lipid Peroxidation. Oxygen exposure of rats significantly increased the rate of NADPH-dependent microsomal lipid peroxidation in the absence of exogenous substrates by about 3-fold, whereas carbon tetrachloride and NADPH dependent lipid peroxidation

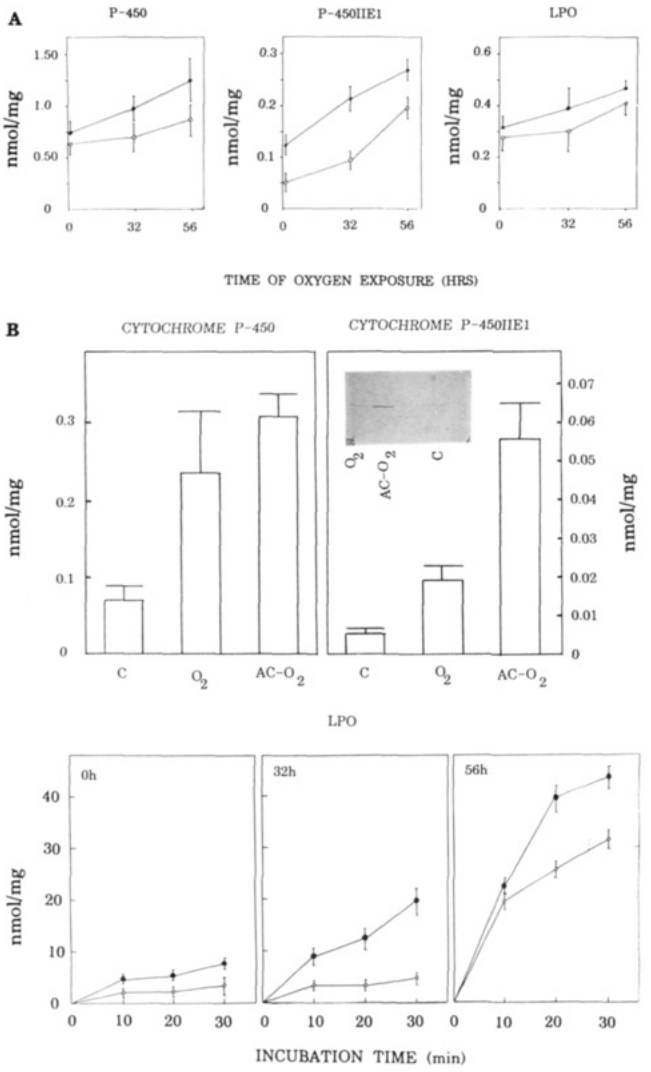

FIGURE 5: Effect of acetone treatment in combination with oxygen exposure on the content of cytochrome P-450, the level of P-450IIE1, and the NADPH-dependent lipid peroxidation (LPO) in microsomes from rat liver (A) and rat lung (B). Where appropriate, solid circles denote animals treated with acetone and oxygen (cf. Experimental Procedures), whereas open circles denote results from animals only exposed to oxygen. In (B) P-450 levels were analyzed in lung microsomes from control (C), oxygen-exposed (O_2), and acetone- and oxygen-treated rats (AC–O_2). Insert in (B) shows Western blot analysis with anti-P-450IIE1 IgG of lung microsomes from control (C) oxygen-treated (O_2), or acetone- and oxygen-treated rats (AC–O_2).

increased about 10-fold (Figure 4). The latter incubations were carried out in the presence of EDTA to inhibit the rate of NADPH-dependent lipid peroxidation not dependent on carbon tetrachloride [cf. Johansson and Ingelman-Sundberg (1985)]. The extent of increase, in relation to the time of oxygen exposure, followed that of cytochrome P-450 content and cytochrome P-450 dependent oxidase activities (cf. Figures 1 and 3).

Effect of Pretreatment with Acetone on Oxygen Toxicity. The contribution of cytochrome P-450 to the oxygen toxicity was further analyzed by comparing the effect of oxygen exposure between control rats and rats treated with acetone before and during the oxygen exposure period. As shown in Figure 5A, acetone treatment caused a further increase of spectrally determined cytochrome P-450, of P-450IIE1, and of the rate of lipid peroxidation in liver microsomes. In rat lung (Figure 5B), oxygen treatment resulted in a 4-fold increased level of P-450IIE1, and acetone treatment in combination with oxygen further elevated the level of P-450IIE1 about 3-fold. The acetone treatment also caused an increased rate of NADPH-dependent lipid peroxidation in lung microsomes, compared to oxygen controls. The largest difference between the two groups was evident after 32 h of oxygen exposure.

In separate experiments the survival time was investigated. After 70 h of oxygen exposure, all animals also treated with acetone ($n = 6$) had died, whereas all animals in the oxygen-treated group ($n = 6$) were still alive.

Comparative Analysis of Oxygen, Acetone, and Thyroxine Treatment. Acetone treatment in combination with fasting is known to increase the amount of P-450IIE1 in rat liver microsomes by about 9-fold (Johansson et al., 1988), and thyroxine treatment of rats is known to increase the oxygen consumption of the hepatocytes and has previously been described to be associated with an enhanced microsomal oxidase activity (Fernandez et al., 1985). It was therefore of interest to compare the rate of oxidase activities and lipid peroxidation in microsomes from variously treated rats. As shown in Table I, oxygen exposure or acetone treatment both caused pronounced elevations, compared to control, in the rate of microsomal NADPH-dependent oxidase activities, oxygen consumption, and O_2^-- or CCl_4-dependent lipid peroxidation, whereas this was not the case in microsomes from thyroxine-treated rats. No increase in the amount of NADPH–cytochrome P-450 reductase activity was seen in the oxygen-exposed group.

Discussion

The results presented indicate that oxygen exposure of rats causes the induction of cytochrome P-450 in liver and lung and that the ethanol-inducible form of cytochrome P-450 accounts for about 60% of the increase of P-450 seen in liver microsomes. The identification of P-450IIE1 in liver microsomes from oxygen-treated rats is based on the following: (i) immunoblotting experiments using an anti-P-450IIE1 IgG preparation in Western blot analysis; (ii) the induction by oxygen treatment of known P-450IIE1-dependent microsomal activities such as NADPH-oxidase and CCl_4 dependent lipid peroxidation and NADPH-dependent lipid peroxidation; and (iii) characteristics of the microsomes with respect to several other variables that are in common with those of microsomes isolated from acetone-treated, but not from thyroxine-treated, rats (cf. Table I). A previous study describing an enhanced rate of microsomal production of superoxide anions following treatment of rats with thyroid hormones (Fernandez et al., 1985) might be explained by the capability of the electron acceptor for O_2^- used, epinephrine, to redox cycle in the microsomal electron transport chain at the level of NADPH–cytochrome P-450 reductase [data not shown; cf. Prough and Masters (1973) and Nakamura and Yamazaki (1969)].

Compared to many other forms of cytochromes P-450, P-450IIE1 is unique since it is constitutively in its high-spin form (Ryan et al., 1985; Patten et al., 1986; Eliasson, et al., 1988). Upon binding of substrate, the ethanol-inducible form of rabbit or rat liver microsomal cytochrome P-450 is converted into its low-spin form (Morgan et al., 1982; Ingelman-Sundberg & Johansson, 1984; Eliasson et al., 1988). This physical property of the enzyme can form a molecular basis for an extensive reduction of the protein by NADPH–cytochrome P-450 reductase in the absence of substrate. The homologous rabbit form exhibits a much higher rate of oxygen consumption (Gorsky et al., 1984) and NADPH-oxidase activity (Ingelman-Sundberg & Johansson, 1984) than other rabbit cytochromes P-450. The high oxidase activity is manifested in a pronounced production of H_2O_2 and O_2^-, which in the presence

Table I: NADPH-Dependent Oxidase Activities and Cytochrome P-450 Content in Liver Microsomes from Variously Treated Rats[a]

	type of treatment			
	control, $n = 5$	O_2 exposed,[b] $n = 4$	acetone,[b] $n = 4$	thyroxine,[b] $n = 7$
cytochrome P-450 (nmol mg^{-1})	0.48 ± 0.05	0.72 ± 0.1**	1.42 ± 0.14***	0.36 ± 0.08*
cytochrome P-450IIE1 (pmol mg^{-1})	50 ± 12	227 ± 30***	454 ± 72***	64 ± 33
P-450 reductase (units mg^{-1})	66 ± 9	76 ± 21	123 ± 5***	99 ± 18*
NADPH oxidation (nmol mg^{-1} min^{-1})	26 ± 1	37 ± 5**	42 ± 3***	22 ± 4
H_2O_2 formation (nmol mg^{-1} min^{-1})	1.3 ± 0.4	3.4 ± 0.2***	4.5 ± 0.4***	1.1 ± 0.09
TBA reactive subst (nmol mg^{-1} min^{-1})	0.44 ± 0.07	1.4 ± 0.3***	1.2 ± 0.15***	0.44 ± 0.07
CCl_4-dependent lip. per.[c] (nmol mg^{-1} min^{-1})	0.19 ± 0.03	1.54 ± 0.07***	3.5 ± 0.5***	0.23 ± 0.06
O_2 consumption (nmol mg^{-1} min^{-1})	15 ± 1	23 ± 3***	25 ± 1***	21 ± 1***

[a] The acetone-treated group was starved for 24 h and subsequently given 5 mL/kg of acetone on 2 consecutive days. Oxygen treatment was for 60 h, and the thyroxine-treated rats received 200 μg/kg of L-thyroxine for 7 days. [b] Significances were, compared to control, (***) $p < 0.001$, (**) $p < 0.005$, and (*) $p < 0.05$. [c] These incubations were performed in the presence of EDTA to suppress the rate of NADPH-dependent lipid peroxidation not dependent on carbon tetrachloride.

of chelated iron can yield reactive hydroxyl radicals (Ingelman-Sundberg & Johansson, 1984). The accentuated oxidase activity of P-450IIE1 thus forms a basis by which superoxide anion induced lipid peroxidation can be brought about. We have recently shown that anti-P-450IIE1 IgG inhibits hydrogen peroxide production in liver microsomes from acetone-treated rats by about 65% and inhibits O_2^--dependent lipid peroxidation extensively in the same type of liver microsomes, whereas preimmune IgG fractions are without effect. Furthermore, a pronounced correlation was evident between the amount of P-450IIE1 in the microsomal membranes and the microsomal rate of NADPH oxidation, O_2^- formation, or H_2O_2 production (Ekström & Ingelman-Sundberg, 1988).

In the present study we observed an accentuated coregulation by oxygen of the total P-450 level, the amount of hepatic P-450IIE1, the rate of microsomal O_2^- and H_2O_2 production, the rate of microsomal O_2 and NADPH consumption, and the rate of microsomal NADPH-dependent lipid peroxidation. These findings are in agreement with the assumption that induction of cytochrome P-450 is a critical event for the exertion of oxygen toxicity. Agents like poly I:poly C and Tilorone, which are interferon inducers, have been shown to prevent the toxic effects of oxygen on rats, thereby reducing the mortality of rats exposed to oxygen and preventing the appearance of oxygen-induced formation of malondialdehyde in lung (Kikkawa et al., 1984). The results were explained by the capability of the interferon inducers to reduce the hemoprotein content in microsomes by about 50% (Kikkawa et al., 1984). Most of the capacity of hydrogen peroxide formation in vitro in subcellular lung fractions resides in the microsomal fraction and is here apparently 7-fold higher compared to the mitochondrial fraction (Turrens et al., 1982). These data thus emphasize the importance of the microsomal electron transport chain and, in particular, of cytochrome P-450 for the toxic effects of oxygen.

In recent studies, Mansour and collaborators failed to observe any increase in oxygen toxicity after treatment of rats or mice with either phenobarbital, 3-methylcholanthrene, or β-naphthoflavone (Mansour et al., 1988a,b). No increase in the rate of microsomal oxidase activity as measured by O_2^- or H_2O_2 formation was observed in these studies. Mansour et al. (1988a,b) could not see any increase in P-450 after oxygen treatment and, in addition, only very small effects on the P-450 levels caused by the inducers. In contrast, Gonder et al. (1985) found a pronounced increase in the total P-450 level in both liver and lung after oxygen exposure and a shorter survival time in the oxygen atmosphere of these animals than of those uncapable of inducing cytochrome P-450. These results further support the concept that an increased P-450 level and enhanced microsomal oxidase activity potentiate the toxic effects of oxygen. In addition, it seems likely that the toxicity is connected with induction of specific forms of cytochrome P-450, e.g., the ethanol-inducible one, as exemplified in the present paper.

The mechanism of oxygen-dependent increase of P-450IIE1 here registered is unknown. The increase at the apoprotein level in liver microsomes was not accompanied by any enhanced amount of P-450IIE1 mRNA, indicating the absence of a pronounced transcriptional activation mechanism or stabilization of mRNA. Possible regulatory sites of action include translational and posttranslational events. P-450IIE1 is known to be mainly regulated at posttranscriptional levels by its common inducers such as ethanol, acetone, imidazole, and pyrazole [cf. Song et al. (1986), Johansson et al. (1988), and Eliasson et al. (1988)]. The absence of induction of P-450IIE1 in rats housed in the same chambers, breathing with a flow of air, and allowed exactly the same amount of food as taken by the oxygen-treated group indicates little influence or exogenous stress factors on the induction process. Similarily, oxygen- and endotoxin-dependent induction of superoxide dismutase in rats is not associated with any increase of the corresponding mRNA, and an elevation of polyribosome concentration in the lung has been suggested (Hass et al., 1987). Thus, the evaluation of the O_2-dependent mechanism of induction of P-450IIE1 awaits experiments aimed to study posttranscriptional events in detail.

Extrapolation of the rate of P-450IIE1 oxygen consumption (Gorsky et al., 1984) or NADPH consumption (Ingelman-Sundberg & Johansson, 1984) reached under in vitro conditions to the in vivo situation with the knowledge of the centrilobular localization of P-450IIE1 (Ingelman-Sundberg et al., 1988) and concentration reveals that, theoretically, more than 3 μmol of oxygen can be consumed by the enzyme per minute per gram of liver in this region, which is a quantitatively important contribution. Thus, one might speculate, on a teleological basis, that induction of P-450IIE1 under hyperoxic conditions might contribute to oxygen removal in vivo and that the induction process may be of functional importance and

thus constitute an adaptive response. In addition, since chronic ethanol treatment of rats is known to cause an accentuation of the oxygen gradient between the perivenous and the centrilobular liver zones (Ji et al., 1982), it might be possible that the ethanol-dependent induction of P-450IIE1 contributes to this heteroacinar concentration of oxygen. Further studies in vivo and in perfused liver systems will be designed to evaluate this possibility.

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Registry No. Ethanol, 64-17-5; cytochromne P-450, 9035-51-2; oxidase, 9035-73-8; acetone, 67-64-1; thyroxine, 51-48-9.

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