Pulmonary Biotransformation of Methoxyflurane:

An In-vitro *Study in the Rabbit*

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The biotransformation of methoxyflurane by rabbit pulmonary microsomal preparations was investigated. The smooth endoplasmic reticulum of rabbit lung metabolized methoxyflurane to organic and inorganic fluoride metabolites in a manner both quantitatively and qualitatively similar to hepatic biotransformation. Pooled pulmonary and hepatic microsomes from 16 rabbits with protein concentrations of 6, 12, and 24 mg/ml incubated with methoxyflurane yielded 420 ± 40 (SD), 621 ± 51, and 903 ± 36 pmol/min/ml of free fluoride, respectively, in the lung, and 363 ± 62, 538 ± 70, and 858 ± 89 pmol/min/ml of free fluoride in the liver. Values of total fluoride obtained in the same preparations were 941 ± 67 (SD), 1,420 ± 77, and 1,685 ± 81 pmol/min/ml in the lung and 888 ± 83, 1,093 ± 109, and 1,838 ± 111 pmol/min/ml in the liver. NADPH cytochrome c reductase was measured in both hepatic and pulmonary microsomes. Untreated, polychlorobiphenyl-induced and phenobarbital-induced rabbits (n = 8) yielded 37 ± 15 (SD), 43 ± 16, and 36 ± 6 nmol/min/mg of NADPH cytochrome c reductase, respectively, in pulmonary microsomes. Hepatic microsomes in the same animals yielded 34 ± 8 (SD), 130 ± 40, and 64 ± 9 nmol/mg/min of NADPH cytochrome c reductase, respectively. Cytochrome P-450 measured in pulmonary and hepatic microsomes in control, polychlorobiphenyl-induced and phenobarbital-induced rabbits (n = 8) yielded 0.16 ± .02 (SD), 0.17 ± .03, and 0.16 ± .02 nmol/mg protein of cytochrome P-450 in the lung and 0.65 ± .17 (SD), 1.9 ± 0.5, and 1.3 ± 0.2 nmol/mg protein of cytochrome P-450, respectively, in the liver. Thus, NADPH cytochrome c reductase and cytochrome P-450 were not inducible in pulmonary microsomes by known hepatic microsomal enzyme-inducing agents. Pulmonary microsomal biotransformation of a volatile anesthetic agent has been demonstrated, and may be an important factor in the disposition of this class of drugs. (Key words: Anesthetics, volatile: methoxyflurane. Biotransformation: microsomes; enzyme induction; fluorometabolites. Lung: microsomes.)

THE LUNG is the primary route through which halogenated anesthetics enter and leave the body. It has often been assumed that the lung plays a passive role handling drugs, and that biological properties of drugs are unaffected by passage through the airways. It has recently been shown that the lung actively metabolizes certain xenobiotics[1,2] and, for some substrates, mixed-function oxidase systems in rabbit lung are as metabolically active as corresponding systems in the liver.[1,2] Similar to the situation in the liver, mixed-function oxidation is of primary importance in pulmonary biotransformation of lipid-soluble compounds. Pulmonary mixed-function oxidase systems appear to be concentrated within the microsomal fraction of homogenized lung. Because of the importance of this nonrespiratory function of the lung, and because biotransformation of halogenated anesthetic agents by the lung had not been studied previously, we investigated the biotransformation of methoxyflurane by the lung.

Methods

Healthy adult New Zealand rabbits of either sex (2–4 kg) were treated with microsomal enzyme-inducing drugs, using the following regimens: A single injection of polychlorobiphenyls (PCB—Arochlor 1254), 500 mg/kg, was administered intraperitoneally to one group, and phenobarbital, 70 mg/kg, intraperitoneally, for four days, was given to a second group. A third group received two hours of administration of halothane, 1 per cent, and a control group received no pharmacologic treatment. All animals were housed in an environment free of known hepatic microsomal enzyme-inducing agents. Four days after PCB treatment, at the conclusion of phenobarbital treatment, and 40 hours after halothane anesthesia, treated and control animals were sacrificed and the lungs removed intact. The parenchyma was excised, weighed, and homogenized. The homogenate was then mixed in 0.05 M Tris and 1.15 per cent KCl, and centrifuged at 9,000 × g for 20 min. The supernatant was then removed and centrifuged in an IEC B/60 ultracentrifuge at 125,000 × g for an hour. The resultant microsomal pellet was resuspended in 0.05 M K_2PO_4 and buffered to pH 7.4. Hepatic microsomes were prepared in a similar manner. The following studies were performed on hepatic and/or pulmonary microsomal preparations:

1) Cytochrome P-450 and NADPH cytochrome c reductase concentrations (lung and liver) were determined in control and treated groups utilizing a spectrophotometric method previously described.[3-5]

2) Malonaldehyde formation as an index of lipo-

peroxidation was measured following pulmonary microsomal incubation with 3 mM methoxyflurane or carbon tetrachloride using a method previously described.[6]

3) Microsomal pellets from control animals were resuspended to concentrations of 6, 12, and 24 mg of microsomal protein per ml. A saturated solution of methoxyflurane was then added to an incubation mixture containing microsomes, EDTA, and an NADPH-generating system, as described by Adler and Brown, and the incubation flasks tightly sealed.[6] All incubations were performed in a shaking water bath at 37 C for 30 min. Free fluoride, total fluoride, and organic fluoride were measured. Free fluoride production was measured at room temperature using an ion-specific electrode. Total fluoride was measured by hydrolyzing the incubation mixture overnight with concentrated sulfuric acid, adjusting the pH to 5.5 with sodium hydroxide, and again assaying for fluoride with the same ion-specific electrode. Organic fluoride metabolites were estimated by subtracting free fluoride from total fluoride. Incubations and fluoride analyses using various concentrations of methoxyflurane ranging from 0.05 mM to a saturated solution were performed (lung only) in a similar manner to determine the kinetics of the reaction.

FIG. 2. NADPH cytochrome c reductase contents of pulmonary and hepatic microsomes in nmol/min/mg protein. Control animals are compared with rats treated with PCB, 500 mg/kg, intraperitoneally (single dose), or phenobarbital, 70 mg/kg, intraperitoneally, for four days. NADPH cytochrome c reductase was inducible by both drugs in hepatic microsomes but was not inducible in pulmonary microsomes. All determinations ±SD. N = 8 for all determinations. * = $P < 0.01$.

The Student t test was used for statistical comparison of data. A minimum of $P < 0.05$ was selected to represent significant differences between means.

Results

Pulmonary microsomes of control, PCB-treated and phenobarbital-treated animals (eight in each group) yielded 0.16 ± .02 (SD), 0.17 ± .03, and 0.16 ± .02 nmol/mg protein, respectively, of cytochrome P-450 (fig. 1). Hepatic microsomal P-450 values in the same animals were 0.65 ± .17 (SD), 1.9 ± 0.5, and 1.3 ± 0.2 nmol/mg protein, respectively. The concentration of cytochrome P-450 was three to four times greater in the liver than in the lung on a per-milligram-microsomal-protein basis. Attempts to induce cytochrome P-450 with PCB or phenobarbital in the lungs were not successful, but cytochrome P-450 was easily inducible in the livers of the same animals. Exposure of rabbits to halothane had no effect on pulmonary cytochrome P-450 levels. Halothane-treated animals yielded 0.14 ± 0.5 nmol/mg protein of cytochrome P-450 and control animals 0.15 ± 0.07 nmol/mg protein of cytochrome P-450. NADPH cytochrome c re-
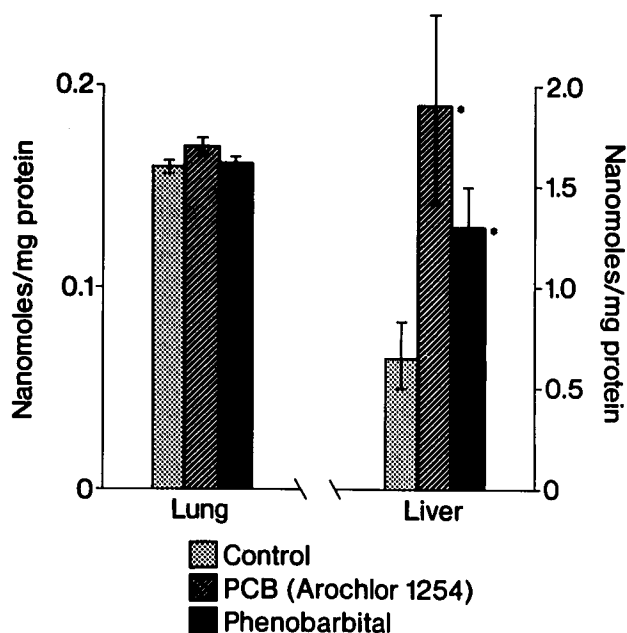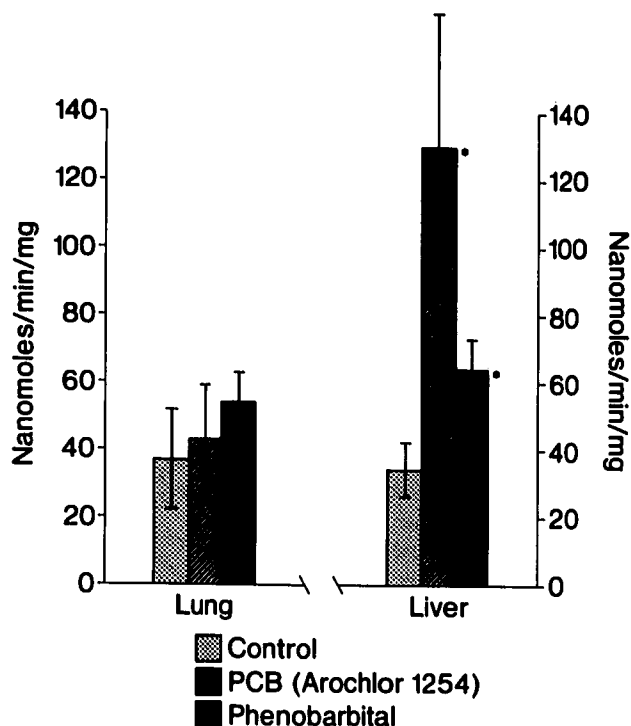

FIG. 1. Cytochrome P-450 contents of pulmonary and hepatic microsomes in nmol/mg protein. Untreated animals are compared with animals receiving PCB, 500 mg/kg, intraperitoneally (single injection), or phenobarbital, 70 mg/kg, intraperitoneally, for four days. The hepatic microsomal cytochrome P-450 was induced with PCB or phenobarbital treatment, whereas pulmonary cytochrome P-450 was not inducible. All determinations are ±SD. N = 8 for all determinations. * = $P < 0.01$.
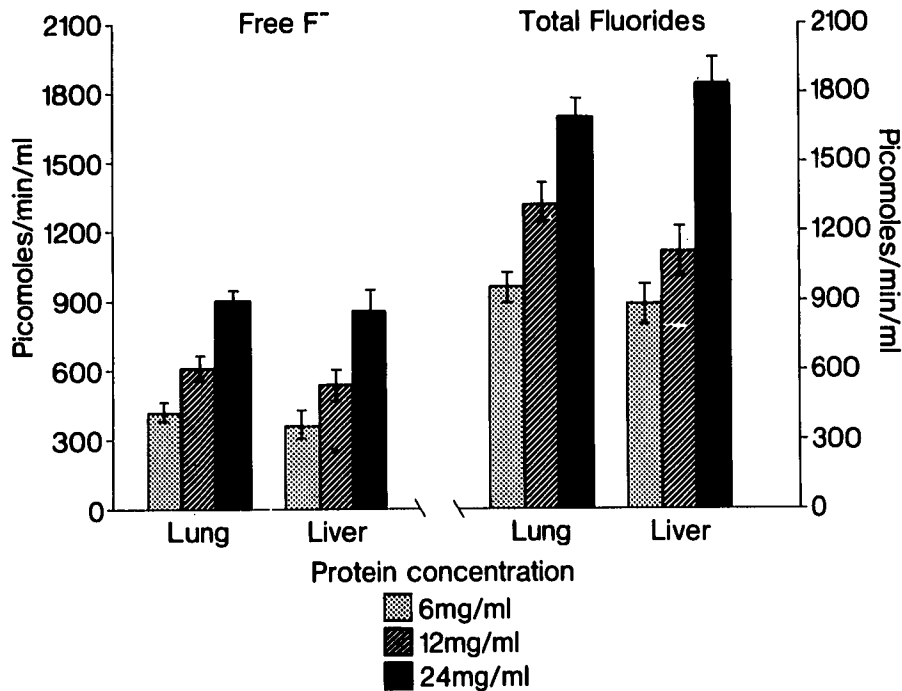
FIG. 3. Free and total fluoride production from methoxyflurane incubations in pmol/min/ml. Protein concentrations are 6, 12 and 24 mg/ml. Free fluoride and total fluoride production was linear with respect to protein concentration in the lung and liver, with the two organs producing comparable amounts of fluoride metabolites on a per-mg-protein basis. All determinations ±SD. N = 8 for all determinations.

ductase was measured in both pulmonary and hepatic microsomes (fig. 2). Pulmonary microsomes in control, PCB-induced, and phenobarbital-induced animals yielded 37 ± 15 (SD), 43 ± 16, and 36 ± 6 nmol/min/mg, respectively, of NADPH cytochrome c reductase. Hepatic microsomes in the same three groups yielded 34 ± 8 (SD), 130 ± 40, and 64 ± 9 nmol/min/mg of NADPH cytochrome c reductase. Thus, NADPH cytochrome c reductase was also not inducible in the lung, whereas induction was accomplished in the liver. No malonaldehyde formation by carbon tetrachloride or methoxyflurane was found in any pulmonary preparation, although carbon tetrachloride produced 50 nmol/min malonaldehyde in the non-induced hepatic microsomal preparation.

Metabolism of methoxyflurane to fluoride was observed at all protein concentrations (fig. 3) and was linear throughout the incubation. Protein concentrations of 6, 12, and 24 mg/ml yielded 420 ± 40 (SD), 621 ± 51, 903 ± 36 pmol/min/ml of free fluoride in the pulmonary microsomes and 363 ± 62 (SD), 538 ± 70, and 858 ± 89 pmol/min/ml of free

y-intercept = 21.4 x 10^{-2}

x intercept = -11.07

$$Km = \frac{1}{11.1} = .09 \text{ mM}$$

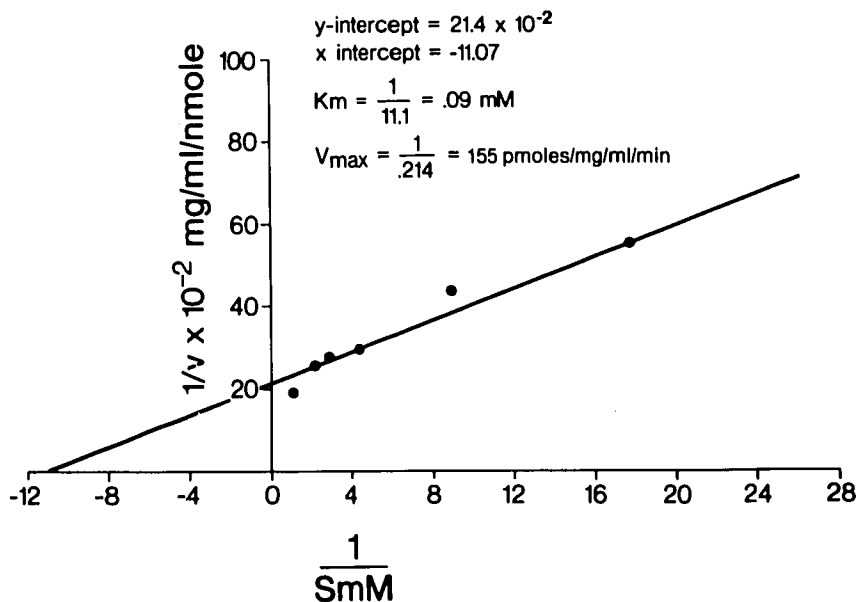$$Vmax = \frac{1}{.214} = 155 \text{ pmoles/mg/ml/min}$$

FIG. 4. Lineweaver-Burke double reciprocal plot of biotransformation of methoxyflurane to free fluoride ion. The Y-axis is the reciprocal of the velocity of the reaction in mg/ml/nmol. The X-axis is the reciprocal of the substrate concentration (methoxyflurane) in mmol. N = 8 for each point plotted. Calculated K_m (methoxyflurane concentration) and V_{max} (free fluoride production) for the reaction were 0.09 mM and 155 pmol/mg/ml/min, respectively.

fluoride in hepatic microsomes. Total fluoride metabolites at the same protein concentrations were 941 ± 67 (SD), 1,420 ± 77, and 1,685 ± 81 pmol/min/ml, respectively, in the lung and 888 ± 83 (SD), 1,093 ± 109, and 1,838 ± 111 pmol/min/ml in the liver, respectively. When compared with the hepatic microsomal preparation, the production of free and total fluoride was comparable in the lung. A Lineweaver-Burke double-reciprocal plot of free fluoride formation in pulmonary microsomes showed a K_m of 0.09 mM and a V_{max} of 155 pmol/mg/ml/min (fig. 4).

Discussion

We have shown that the pulmonary endoplasmic reticulum is capable of in-vitro biotransformation of the halogenated anesthetic methoxyflurane. At three different protein concentrations, pulmonary microsomes biotransformed methoxyflurane quantitatively, as in the liver, on a milligram-for-milligram basis. Unlike the situation in the liver, the pulmonary microsomal components that we studied (cytochrome P-450 and NADPH cytochrome c reductase) were not inducible by phenobarbital, PCB, or inhalation of halothane. The reason for this lack of inducibility is unknown, but other investigators have confirmed the noninducibility of these cytochromes as well as that of cytochrome b_5.[7,8] Because of the lack of inducibility of cytochrome P-450 and NADPH cytochrome c reductase in the lung, we did not attempt to compare induced hepatic microsomes with non-induced pulmonary microsomes. Additionally, we did not perform fluoride studies in the treated rabbits because on non-inducibility of cytochrome P-450.

Comparable studies of in-vitro drug metabolism by both hepatic and extrahepatic tissues have been complicated by the use of a single experimental tissue, few animal species, and variable experimental conditions. Litterst et al. analyzed liver, lung, and kidney for microsomal enzymes[9] in a variety of animals. In all species the liver was the most active organ, with renal and pulmonary activities being 15 to 40 per cent of those found in the liver. No single species demonstrated total superiority in its drug-metabolizing ability. For example, pulmonary microsomes had a very high demethylase activity for N-demethylation of aminopyrine, the significance of which is currently unknown.

Cellular localization of pulmonary mixed-function oxidase activity has met with little success. Interest in identifying the site of biotransformation activity has been important because of the possibility that many chemical toxins and potential carcinogens require mixed-function oxidase-catalyzed activation in order to become carcinogenic. Investigators have suggested that alveolar cells, bronchial cells, and Clara cells all

have mixed-function oxidase activity.[10] Our study has made no attempt to delineate from what cell types the microsomes originated. Dalbey and Bingham recently described production of trichloroethanol from isolated rat lung perfused with trichloroethylene.[11] These investigators also reported that this metabolic pathway was capable of being induced by phenobarbital. We have been unable to demonstrate any induction phenomena in our rabbit-lung microsomal preparation. Investigations in animals and man with the liver and kidney as target organs have demonstrated a link between halogenated anesthetic biotransformation and viscerotoxicity.[12–14] Additional studies are needed to determine the pathophysiologic and clinical implications of halogenated anesthetic biotransformation by the lung.

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