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LIPID EPOXIDE HYDROLASE IN RAT LUNG PREPARATIONS

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Summary

The activity of rat lung epoxide hydrolase (epoxide hydrolase, EC 3.3.2.3) was studied using two lipid epoxides which can be isolated from lung tissue. These epoxides displayed different $K_{m,app}$ and hydration rates. Methyl *cis*-9,10-epoxystearate was hydrated 20-times more rapidly than cholest-5α,6α-epoxy-3β-ol. The K_m for the lung microsomal enzyme was variable and dependent on the microsome concentration in the medium. A soluble epoxide hydrolase was also detected in both lung and liver. This enzyme appears similar to the microsomal enzyme in its activity toward methyl epoxystearate. The measured activities for liver microsomal epoxide hydrolase were over 8-times those for lung microsomes; activity against cholesterol epoxide was 40-times greater for liver. In spite of the slow rates measured with cholesterol epoxide in lung preparations, this compound was an effective competitive inhibitor against methyl epoxystearate over a wide concentration range. This suggests that cholesterol epoxide readily binds to epoxide hydrolase and is an effective competitive inhibitor against a much more actively metabolized substrate, methyl epoxystearate. Such circumstances indicate that cholesterol epoxide binds with a high degree of nonproductivity to lung microsomal epoxide hydrolase. This attribute of lung epoxide hydrolase may relate to the relatively high concentrations of cholesterol epoxide found in lung tissue.

Introduction

Epoxide metabolism and toxicology has come under considerable scrutiny in recent years [1—3]. Epoxides often represent intermediates in the conversion of olefins into more hydrophilic products. Research concerned with their

metabolism has largely involved the polycyclic aromatic hydrocarbons which may be converted into potent mutagenic or carcinogenic epoxides by reactions involving a combination of microsomal monooxygenase and epoxide hydrolase activities [4,5]. Recently, evidence has been presented indicating that cell nuclei possess 'epoxidase activity' , thus providing a site of origin proximal to genetic material [6,7].

Unsaturated lipids represent another class of compounds that can be converted into epoxides by processes similar to those occurring with polycyclic aromatic hydrocarbons, as well as by other enzymatic oxidations [8,9,13]. There is additional evidence that purports to show that epoxides arise through autoxidation [10—12]. In all these instances it seems that epoxide hydrolase serves an important function in the metabolism of a wide array of these electrophilic compounds. A number of epoxide containing lipids have been demonstrated in human tissues [15], as well as in rat lung [12]. Based on the finding that oxidant exposure resulted in an increased lipid epoxide content in rat lung, we proposed that lipid peroxidation may contribute to lipid epoxide formation [12]. In these studies epoxide hydrolase activity was measured using cholesterol epoxide, specifically cholest-5α,6α-epoxy-3β-ol. Interestingly, this enzyme was found to be far less active than was previously reported for rat liver [17]. In a recent report the rate of cholesterol epoxide metabolism after gastric or cutaneous administration was examined [39]. A large percentage of the administered epoxide was found to be unchanged and appeared in the feces.

A closer inspection of epoxide hydrolase activity in rat lung microsomal preparations has indicated that cholesterol epoxide is a poor substrate for this enzyme, whereas the liver enzyme readily hydrates this epoxide. Based on these preliminary observations, it was felt that a more detailed study of the lung enzyme with respect to lipid epoxides was warranted. The selection of cholesterol epoxide and methyl epoxystearate for this study was based largely on their common occurrence in lung tissue (as well as in other organs), particularly on the relatively high concentrations of cholesterol epoxide [12,15,27].

Methods

1. Preparation of microsomal and soluble fractions of rat lung

Lungs were obtained from specific pathogen-free male Sprague Dawley rats (150 g), which had either been previously exposed to filtered air (controls) or to 5 ppm NO_2 for 24 h. The exposure protocol was similar to that described by Hinners et al. [18]. Prior to excision, the lungs were perfused free of blood with normal saline and parenchymal tissue collected into 10 vols. (w/v) of 0.1 M sodium phosphate buffer, pH 7.4. The tissue was homogenized using a Tekmar Tissuemizer for 1 min at 4°C and the homogenate centrifuged for 10 min at 500 $\times g$. The supernatant was collected and centrifuged for 20 min at 18 500 $\times g$ and the pellet discarded. The remaining supernatant was passed through six layers of surgical gauze and the filtrate centrifuged for 65 min at 100 000 $\times g$. The resulting supernatant and crude microsomal pellet were recentrifuged (after resuspension of microsomes in fresh buffer) for an additional 65 min at 100 000 $\times g$. The resulting soluble and microsomal fractions were adjusted with the appropriate buffer to the desired protein concentrations

(approx. 1.0 mg/ml was commonly used). Measurement in either phosphate buffer, at pH ranging from 7.0—7.8, or 0.1 M Tris-HCl buffer, at pH ranging from 8.0—8.6, allowed for an analysis of the pH profile of lung epoxide hydrolase.

2. Enzyme assays

Epoxide hydrolase activity was measured using a thin layer chromatographic (TLC) assay similar to that reported by Jerina et al. [19]. Incubation media typically consisted of 0.1 M phosphate or Tris-HCl buffer containing the enzyme source and 3 mM phenylmethylsulfonyl fluoride (Sigma, St. Louis), serving as an esterase inhibitor, in a total volume of 0.15 ml. The reaction was initiated by adding 1 μl of either [U-^{14}C]methyl 9,10-epoxystearate or [4-^{14}C]-cholest-5α,6α-epoxy-3β-ol in acetone and the incubation was performed in 10 × 75 mm siliconized test tubes.

The isotopic substrates were prepared from [U-^{14}C]oleate and [4-^{14}C]cholesterol (New England Nuclear) according to the method of Chakravorty and Levin [20]. The specific activities for methyl epoxystearate and cholesterol epoxide were 10.0 and 14.5 μCi/μmol, respectively, and 2—4 · 10^4 dpm per tube were typically used in the enzyme assay. Unlabeled methyl epoxystearate, prepared as above, and cholesterol epoxide purchased from Steraloids Laboratories (Wilton, NH), were used as standard and/or carriers. The purity of substrates and standards was checked by chromatography on silica gel 60 plates (Merck, Inc.) using benzene/ethyl acetate (3 : 2, v/v), TLC system I for cholesterol epoxide; or ether/petroleum ether/acetic acid (20 : 80 : 1, v/v), TLC system II for methyl epoxystearate.

Incubation intervals of 1, 5, 10, 15, 20 and 30 min at 37° C were terminated by adding 50 μl acetone followed by cooling in an ice bath. 50-μl aliquots were transferred from each flask, in triplicate, onto LK5D TLC plates (Whatman, Inc.). Labeled and/or unlabeled standards were prepared as follows: cholestane-3β,5α,6β-triol was prepared by the method of Fieser and Rajagopalan [38]. Methyl epoxystearate, was prepared as described above [20], and methyl 9,10-dihydroxystearate was prepared from methyl epoxystearate by treatment with 7% perchloric acid in tetrahydrofuran. These standards were co-chromatographed on each plate according to the substrate used in the assay. The plates were developed in TLC system I for those analyses using labeled cholesterol epoxide, and in TLC system II when labeled methyl epoxystearate was employed. The substrates and products were localized by scanning thin-layer radiochromatography using a Berthold LB 2760 radiochromatogram scanner equipped with a ratemeter-integrator. Unlabeled samples were detected either by exposure to iodine vapor or by charring the thin-layer plates after spraying with 3% cupric acetate in 8.5% perchloric acid. The desired regions were scraped off the plates and collected in scintillation vials and radioactivity was measured in a Beckman LS 8100 liquid scintillation counter. The activity of epoxide hydrolase was calculated on the basis of conversion of epoxide to glycol, assuming that the specific activity of the products and substrates were equal. Parallel blank measurements were made by analyzing enzyme samples that were boiled for 10 min. The extent of product formation under these circumstances was assumed to reflect non-enzymatic rates which were then subtracted from respective test samples to obtain the corrected enzyme rates.

Microsomal NADPH-cytochrome *c* reductase activity was measured on cytosolic and microsomal fractions using aliquots containing approx. 0.25 mg protein according to the method of Williams and Kamin [21]. Protein content was determined by the method of Gornall et al. [22]. Lipid content was measured on aliquots containing 1.0 mg/ml protein by gravimetric measurement of a total lipid extract obtained by the method of Folch et al. [23].

Statistical comparisons were made between treatment groups using the Student's *t*-test at the 95% confidence level. Enzyme rates were calculated on the basis of linearity during the incubation intervals and the degree of linearity was established using linear regression analysis. Correlation coefficients (R) < 0.920 were rejected. Results are expressed as means ± 1 S.D.

Results

Preliminary experiments were conducted to establish suitable assay conditions. The features of greatest concern in this regard were: (1) proper dispersal of lipid substrates in the aqueous media; (2) sufficient and rapid association of substrates with microsomes, or the enzyme; (3) concentrations of substrates yielding maximal rates of hydration and (4) pH optima for epoxide hydrolase with both substrates.

(1) Initiation of the reaction was tested using either acetone, dimethyl sulfoxide or ethanol as a delivery solvent. All solvents were found to be equally effective.

(2) Following addition of labeled cholesterol epoxide the extent of association with microsomes was tested by incubating for intervals up to 20 min at 22°C. The incubation was stopped by passing the medium through a Millipore

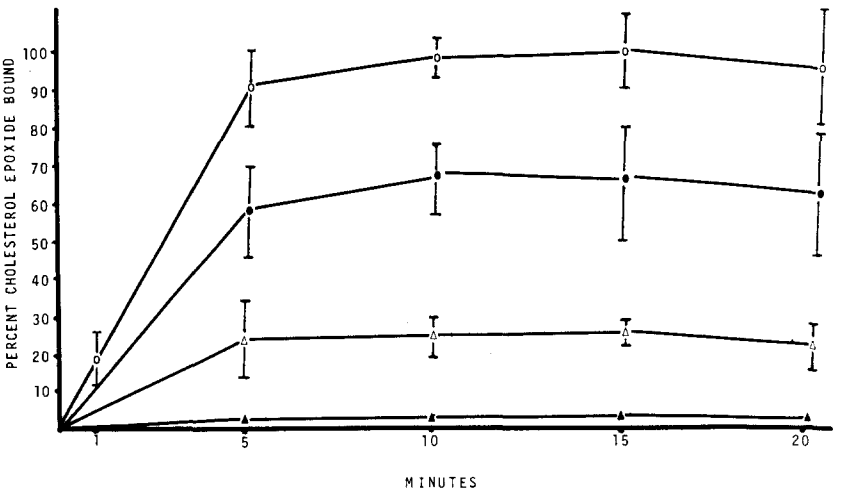

Fig. 1. A measure of cholesterol epoxide interaction with lung microsomes. The percent binding in an incubation medium containing 0.10 mg protein equivalent of microsomes in 0.15 ml buffer is expressed in terms of incubation time at 22°C. Cholesterol epoxide concentrations: (○———○) 10^{-6} M, (●———●) 10^{-5} M, (△———△) $5 \cdot 10^{-5}$ M, (▲———▲) 10^{-4} M were tested for the extent of binding to microsomes.

filter (0.22 μM), and the filtrate and filters were collected for radioactivity measurement. The extent of radioactivity associated with microsomes (collected on the filter) vs. free substrate (in the filtrate) was assumed to represent the proportion of substrate bound to the microsomes. Fig. 1 depicts the degree of cholesterol epoxide binding to lung microsomes with respect to incubation time. Using cholesterol epoxide concentrations at 1 μM and microsomal concentration at 0.67 mg/ml, over 90% of the substrate is bound to microsomes by 5 min and an apparent equilibrium is attained by 10 min. Under these conditions, addition of cholesterol epoxide at concentrations exceeding 1 μM resulted in saturation of the microsomes and concentrations beyond 100 μM resulted in diminished association. From these data it appears that cholesterol epoxide concentrations ranging from 1—10 μM are most appropriate for optimal adsorption to microsomes. Less than 2% of cholesterol epoxide was hydrated in all instances; thus the values represent a reasonably reliable estimate of substrate adsorption. Similar experiments with methyl epoxystearate gave identical results except for a higher concentration range, approx. 10 μM, necessary for comparable percent binding to lung microsomes.

(3) Epoxide hydrolase has near maximal activity over a wide range of substrate concentrations. Using microsome concentrations previously indicated, activity profiles for both substrates were compared. Maximal epoxide hydrolase activity was found at concentrations ranging from 50—100 μM for methyl epoxystearate and 150—300 μM for cholesterol epoxide. Empirical half-maximal velocities were attained at concentrations of 40 μM and 100 μM for methyl epoxystearate and cholesterol epoxide, respectively. Significant substrate inhibition for cholesterol epoxide appears at concentrations beyond 350 μM. This inhibition increased over a concentration range of 400—900 μM.

(4) The pH optimum of epoxide hydrolase for the substrate pair was studied over a pH range of 7.0—7.8 with sodium phosphate buffer, and 8.0—8.6 with Tris-HCl buffer. In both instances maximal rates were found at pH 7.0—7.6. Rates for cholesterol epoxide at pH 7.0, 8.0, 8.4 and 8.6 were 90, 92, 80 and 72%, respectively, of the maximum rate. Epoxystearate displayed a broad pH maximum from 7.4 to 8.2; however, part of this effect may have been due to the large extent of hydrolysis to the fatty acid at pH >8.0. pH 7.4 was selected for subsequent analysis based on these results.

From the data just described, measurement of microsomal epoxide hydrolase activity was conducted using either cholesterol epoxide or methyl epoxystearate at approx. 20 μM with microsomes adjusted to protein concentrations of 0.5—2.0 mg/ml buffer. Lung homogenates and both soluble and microsomal preparations contain such high esterase activity that nearly 75% of labeled methyl epoxystearate may be hydrolyzed to the fatty acid by 15 min of incubation. This could be prevented with about 90% effectiveness by adding 3 mM phenylmethylsulfonyl fluoride to the medium. Less than 0.25% of either substrate was hydrated over a 20 min period with boiled microsomes or cytosolic preparations.

Figs. 2a and b show the results plotted as reciprocals of rate vs. substrate concentration (Lineweaver-Burk plots) derived for methyl epoxystearate and cholesterol epoxide, respectively. The $K_{m,app}$ calculated from these data were 55 ± 18.8 μM (V = 0.75 ± 0.23 nmol/mg protein per min) for methyl epoxy-

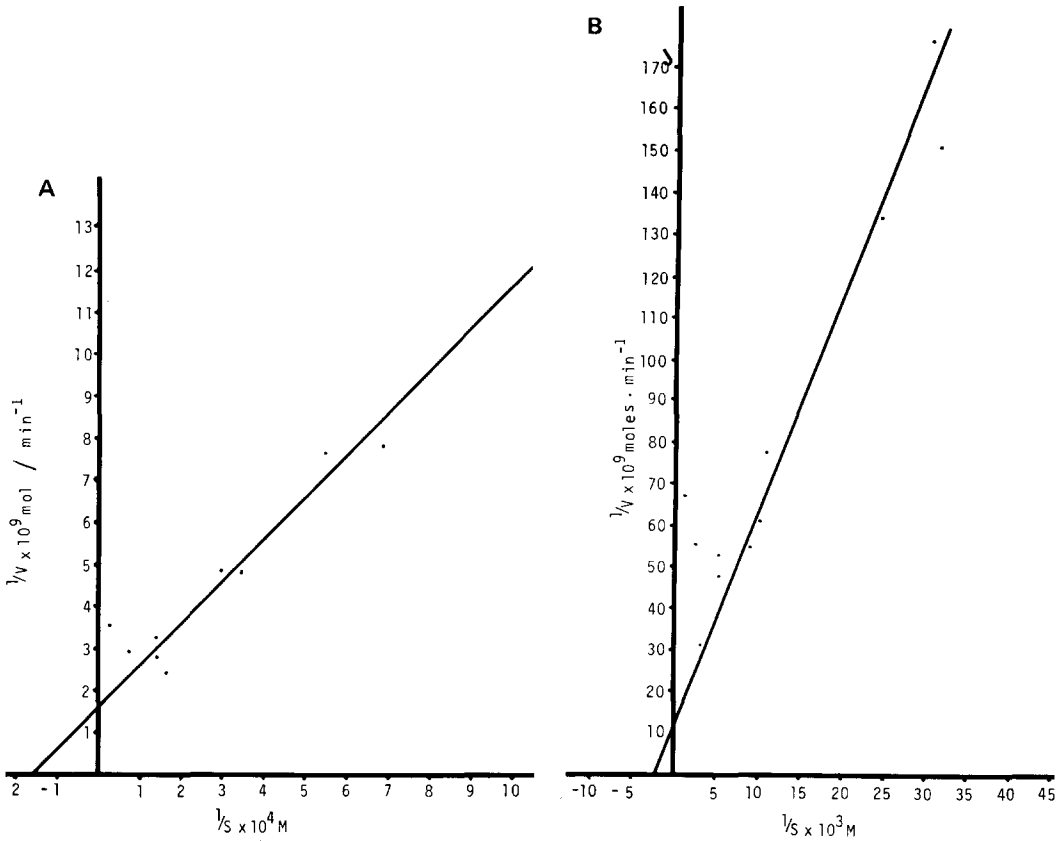

Fig. 2. (a) Lineweaver-Burk plot for hydration of methyl epoxystearate by rat lung microsomes. Assay conditions were: microsomal protein at 0.10 mg, 15 min incubation, pH 7.4 at 37°C. (b) Lineweaver-Burk plot for hydration of cholesterol epoxide by rat lung microsomes. Assay conditions are described in the text.

TABLE I

EPOXIDE HYDROLASE ACTIVITY TOWARD METHYL EPOXYSTEARATE IN RAT LUNG MICROSOMAL AND SOLUBLE PREPARATIONS

Microsomes and soluble fractions of rat lung were prepared as described in the text. Rates for both NADPH-cytochrome *c* reductase and epoxide hydrolase were computed on the basis of 5 and 15 min incubations at 37°C, respectively. Methyl epoxystearate concentrations were 25 μM. 1 unit of NADPH-cytochrome *c* reductase is defined as that amount of enzyme which causes an absorbancy change of 1.0 per min. Results are expressed as means ± S.D. obtained from three separate experiments. Microsome protein concentrations of 1.0 mg/ml (epoxide hydrolase) and 2.5 mg/ml (cytochrome *c* reductase) were used in the assays.

Lung fraction	Cytochrome *c* reductase units $mg^{-1} \cdot min^{-1}$	Epoxide hydrolase for epoxystearate nmol $mg^{-1} \cdot min^{-1}$	$K_{m,app}$ μM for epoxystearate
Microsomes	60.0 ± 4.74	0.170 ± 0.025	55 ± 18.8
Soluble	1.5 ± 0.32	0.099 ± 0.019	100 ± 27.1

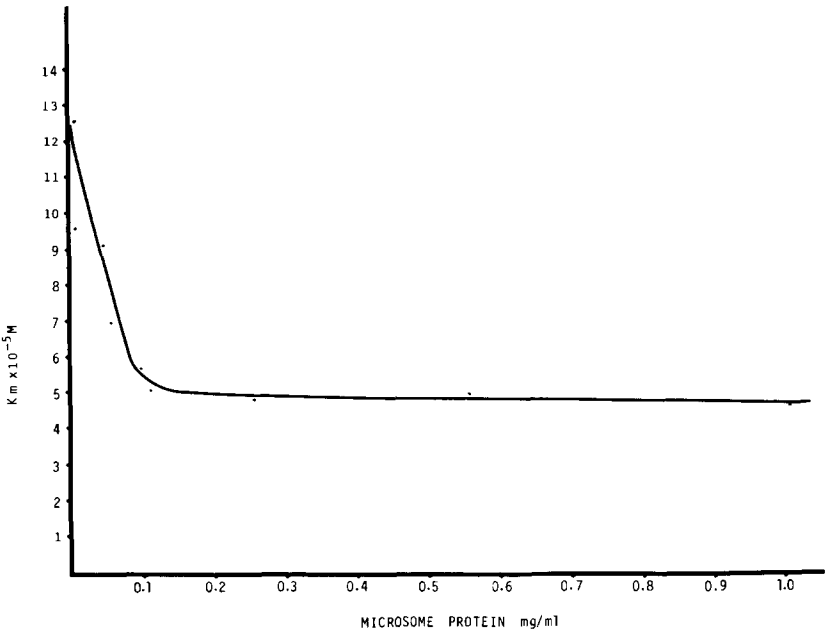

Fig. 3. Effect of microsomal protein concentration on the apparent K_m values for methyl epoxystearate. Assays were done in duplicate with similar substrate concentrations and conditions as shown in Fig. 2. A similar profile is obtained if the data are plotted on the basis of microsomal lipid concentration; K_m values being approx. 120% of the values based on protein content.

stearate and 375 ± 120 μM (V = 0.11 ± 0.024 nmol/mg protein per min for cholesterol epoxide. In all instances the reaction rates were linear for at least 20 min, however, calculations were based on rates for a 15 min interval.

The $K_{m,app}$ for microsome preparations was found to vary inversely with protein or lipid concentrations. This relationship is demonstrated in Fig. 3, using methyl epoxystearate. A rapid and apparently linear increase in the K_m was found at microsomal protein concentrations below 0.1 mg/ml. This relationship may also be expressed on the basis of microsomal lipid concentration in an identical fashion and in most instances microsomal protein and lipid concentrations were similar. There was essentially a constant K_m over the range 0.1—1.0 mg/ml and beyond, to as high as 5.0 mg/ml (data not shown).

Soluble cell fractions from lung (operationally a post-microsomal supernatant) were also analyzed in a manner similar to that of using microsomes. The absence of microsomes was checked by measurement of NADPH-cytochrome *c* reductase activity. The results are presented in Table I, which compares cytochrome *c* reductase and epoxide hydrolase activities in lung microsome and soluble preparations. Non-linear Michaelis-Menton kinetics were found using cholesterol epoxide such that a K_m could not be calculated for the soluble enzyme preparation. This may be partly due to lower concentration limits of cholesterol epoxide in this preparation. Consequently, the observed rates may have been obtained under less than optimal circumstances. Soluble epoxide hydrolase data are presented later for cholesterol epoxide. In spite of the limitations encountered, it appears that rat lung possesses significant soluble epoxide hydrolase activity and that microsomal contamination is

insufficient to account for the activities measured.

A large proportion of the investigations addressing mammalian epoxide hydrolase properties have utilized rat liver microsomes as the enzyme source. An overwhelming body of evidence indicates that liver possesses much higher enzyme activity than does lung [24,25]. We felt that it would be informative if a comparison could be made between liver and lung epoxide hydrolase using cholesterol epoxide and methyl epoxystearate. The results for a separate set of experiments which compare identically prepared fractions from liver and lung, analyzed on a paired basis, are presented in Table II. It is immediately evident that the activity, expressed in terms of protein content, is much greater in liver. This is particularly apparent with cholesterol epoxide. The data also indicate that soluble preparations from either organ possess very little activity against cholesterol epoxide. Furthermore, the results indicate that the relative activity of epoxide hydrolase in liver is greater than that in lung.

The ability to catalyze hydration of diverse epoxides has led to some speculation concerning the existence of a single enzyme possessing broad substrate specificity or of multiple epoxide hydrolases [13,28,40]. Based on such a possibility, and also on the observation that lung catalyzes hydration of cholesterol epoxide poorly, an attempt was made to determine whether the two lipid epoxides were substrates for the same enzyme. This question was approached through the analysis of inhibitory kinetics for the substrate pair using lung microsomes. Fig. 4 shows a characteristic Dixon plot [26] used to determine the enzyme inhibitor constant for cholesterol epoxide. The analyses were performed using cholesterol epoxide concentrations ranging from 2 to 350 μM and labeled methyl epoxystearate at concentrations ranging from 15 to 65 μM. The K_i obtained by this method was calculated as 20 ± 8.1 μM. Less than 10% inhibition was found when cholest-5α,6β-hydroxy-3β-ol (the hydration product of cholesterol epoxide) or cholesterol were added at concentrations up to 100 μM. Inhibition by cholesterol epoxide was typically competitive in nature suggesting that binding occurs at the same site. Comparison of the $K_{m,app}$ for both substrates (Tables I and III) further demonstrates the difficulty with which the lung enzyme hydrates cholesterol epoxide.

Since it was previously found that exposure to NO_2 resulted in increased lipid epoxides (particularly cholesterol epoxide and epoxystearate [12]), the

TABLE II

COMPARISON OF RAT LUNG AND LIVER EPOXIDE HYDROLASE FOR METHYL EPOXYSTEARATE AND CHOLESTEROL EPOXIDE

Results are based on the means obtained from analyses in duplicate for epoxide hydrolase using liver and lung, soluble and microsomal epoxide hydrolase assayed in parallel. Rates are based on 15 min incubation intervals at 37°C, and expressed as nmol per mg protein per min. (n.m., not measurable.)

	Lung		Liver		N
	Epoxystearate	Cholesterol epoxide	Epoxystearate	Cholesterol epoxide	
Microsomes	0.159 ± 0.011	0.010 ± 0.004	1.301 ± 0.351	0.410 ± 0.029	2
Soluble	0.081 ± 0.009	0.002 ± 0.002	0.183 ± 0.020	n.m.	2
$K_{m,app}$ μM microsomes	55 ± 18.8	375 ± 120	22 ± 4.2	78 ± 8.8	

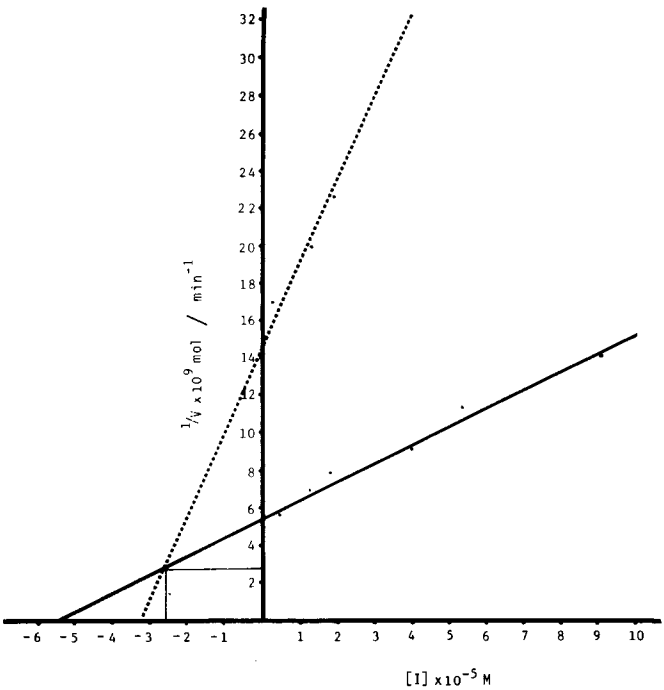

Fig. 4. Dixon plot for the hydration of methyl epoxystearate by rat lung microsomes in the presence of variable cholesterol epoxide concentrations, [I]. Methyl epoxystearate concentrations in the assay were: 20 μM (- - - - -) and 40 μM (———). An estimate of K_i is obtained from the intercept of the two lines. Assay conditions were: 0.10 mg protein microsomes, incubation for 15 min at 37°C, pH 7.4.

effects of NO_2 exposure on lung epoxide hydrolase were examined in a separate experiment with control and NO_2-exposed samples being examined on a paired basis. Enzyme rates were measured for both microsomal and soluble preparations. The results are presented in Table III. The data demonstrate that enzyme activity is not significantly affected following NO_2 exposure. In control

TABLE III

EFFECT OF NO_2-EXPOSURE ON RAT LUNG EPOXIDE HYDROLASE

Rates for soluble and microsomal epoxide hydrolase are expressed in nmoles per mg protein per min and displayed as means ± S.D. obtained from three separate experiments. Incubation conditions are identical to those described in the text.

	Control			NO_2 exposed		
	Epoxy-stearate	Cholesterol epoxide	Epoxy-stearate/ cholesterol epoxide	Epoxy-stearate	Cholesterol epoxide	Epoxy-stearate/ cholesterol epoxide
Microsomes	0.166 ±0.019	0.007 ±0.002	23.7	0.136 ±0.024	0.012 ±0.004	11.3 *
Soluble	0.106 ±0.031	0.003 ±0.0004	35.3	0.121 ±0.014	0.003 ±0.0002	40.3

* $P < 0.025$.

preparations, cholesterol epoxide is hydrated at rates 20-fold less than methyl epoxystearate. The only difference found between the two study groups was a relative increase in cholesterol epoxide hydration to that of methyl epoxystearate following NO_2 exposure.

Discussion

A study of lung epoxide hydrolase was undertaken in the wake of recent evidence demonstrating that rat lung possesses the highest concentrations of lipid epoxides (particularly cholesterol epoxide) of all internal organs so far examined. Furthermore, the levels of lipid epoxides increase only in the lung after NO_2 exposure [27]. We have attempted in this study to analyze some properties of rat lung epoxide hydrolase as related to commonly occurring lipid epoxides in lung.

Epoxide hydrolase of the lung, as well as of other tissues, is thought to be associated with the endoplasmic reticulum; therefore, isolation of microsomes is often used as the means for obtaining and analyzing this enzyme [12]. A number of limitations have nevertheless been pointed out which could question the validity of this idea [28,29]. One limitation arises from the use of hydrophobic substrates in the assay, for example polycyclic aromatic hydrocarbon or lipid epoxides. In these instances the results often vary with the concentration of microsomes used, this being dependent to some degree on lipid concentration in the assay medium [28]. The result of this is often that the K_m measured becomes dependent on microsome concentration, making comparison and interpretation of data difficult. This phenomenon may be visualized as a change in the degree of interaction between the epoxide substrate and microsomes, which is the prodrome to enzyme substrate association and catalysis. The data presented in Figs. 1 and 3 lend credence to this supposition. It should be noted that in this figure the relationship between $K_{m,app}$ and microsome protein concentration appears to be the opposite of that reported by Lu et al. [28]. However, one must consider that essentially all the lipid in this system is associated with the microsomes, hence the rates also bear a similar dependence on lipid concentrations. The microsomes may also serve as a hydrophobic depot into which the lipid substrates may assimilate. This creates a system essentially opposite to the phospholipid (micelles) present in the enzyme system tested by Lu et al. The interaction of microsomal epoxide hydrolase with lipophilic substrates would most likely occur at or near the interface of a two-phase system. Under these circumstances, several factors might affect the hydration rates observed. They include varied enzymatic properties and kinetics within a lipid milieu [37] and the partitioning properties of substrates and products at the interface. Although the double-reciprocal plots obtained were linear, and a K_m apparent could be calculated, the significance of this may reside only in comparing the data obtained under the rigid conditions of the experimental protocol. Consequently, the K_m may reflect a measure of interaction of substrate at the membrane interface, which in this case could be a function of microsome surface area. It was found that microsomal protein concentrations ranging from 0.1—1.0 mg/ml yielded maximal rates and the lowest apparent K_m for both methyl epoxystearate and cholesterol epoxide which in turn were

added at fixed concentrations. Increases beyond the optimum ratio of substrate to microsomes resulted in decreased rates, possibly because of substrate inhibition.

Methyl epoxystearate concentrations necessary to obtain half-maximal velocities, empirically, agreed reasonably well with the K_m calculated from Fig. 2a (40 vs. 55 μM). The irregular substrate inhibition seen with cholesterol epoxide may have a relationship to the discrepancy found between the calculated vs. empirical K_m (375 vs. 100 μM). It must be pointed out, however, that considerable variability was found in these measures.

We have previously proposed that lipid autoxidation may be a contributing factor in lung lipid epoxide formation [12]. It is also possible that the amount of these epoxides increases by gradual accumulation due, in part, to the low epoxide hydrolase activity present in this organ. The liver furnishes a contrast to this since it contains lower concentrations of lipid epoxides [27] and much greater epoxide hydrolase activity. The situation for cholesterol epoxide demonstrates a remarkable dissimilarity between liver and lung. The $K_{m,app}$ in lung is four times as large as in liver but hydration rates are less than 1/40. It is also seen that despite these low rates, cholesterol epoxide can competitively inhibit activity against methyl epoxystearate in lung, exhibiting a K_i lower than the K_m for either substrate. This phenomenon may be explained by considering that both substrates bind to the same enzyme site with relatively similar affinities but differing turnover rates. Previous studies also support this view using various epoxide substrates [33]. Association with the same enzyme is also suggested by the similar pH profiles for both substrates. The large difference in turnover rates could be due to a high degree of 'nonproductive' binding for cholesterol epoxide effectively occupying a part of the enzyme's active site and preventing methyl epoxystearate binding.

A soluble form of epoxide hydrolase which possesses little or no activity against cholesterol epoxide is found in both lung and liver. It is unlikely that this was a measure of microsomal contamination since the microsome marker enzyme NADPH-cytochrome *c* reductase was virtually absent in the soluble preparations. A soluble epoxide hydrolase possessing high activity toward a number of lipophilic epoxide substrates has previously been demonstrated in mammalian liver [30,31]. The limited ability of this enzyme to hydrate the epoxide pair in our hands, in particular, the virtual inability to hydrate cholesterol epoxide, may result from the inability to interact with the soluble enzyme. It is possible, for example, that cholesterol epoxide is sequestered by other cytoplasmic components which render it inaccessible to epoxide hydrolase.

The relatively low epoxide hydrolase activity in lung tissue is not necessarily the reflection of a defect of paucity of protective mechanisms in the lung. The lung is composed of over 40 different cell types [32], thereby being more heterogeneous than liver. It is conceivable that only a small proportion of these cells possess substantial epoxide hydrolase activity, these perhaps being in the most strategic regions of the lung (such as in airways or in epithelial cells). By analyzing a whole lung homogenate, the activity in these 'active' areas would be diluted and any responsive changes obscured.

The susceptibility of lung to arene oxide toxicity has been proposed from previous studies [24,25,33]. It has been further proposed that aliphatic epoxyalcohols [34] and sterol epoxides [35,36] are involved in the etiology of

mutagenesis or carcinogenesis. Since these compounds are of endogenous origin, their concentrations in pulmonary tissues may have an important bearing on the overall toxicity of epoxides. It is therefore possible that the reputed carcinogenicity of cholesterol epoxide [35,36] may be direct or indirect depending on either insufficient metabolism and an increased likelihood of interaction with genomic processes, or by inhibition of more carcinogenic epoxide metabolism (carcinogen promoter).

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