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METABOLISM OF CHLORPROMAZINE BY PULMONARY MICROSOMAL ENZYMES IN THE RAT AND RABBIT

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Abstract—Pulmonary metabolism of chlorpromazine (CPZ) was compared using isolated microsomes of rat and rabbit lungs. CPZ-metabolizing activity of the rat lung was found to be 10-fold higher than that of the rabbit lung. The principal metabolic pathways were *N*-oxidation in the rat lung and *N*-demethylation in the rabbit lung. Kinetic analyses revealed that, although the values for apparent K_m were roughly similar for both pathways, V_{max} for *N*-oxidation by the rat lung was approximately ten times greater than that for *N*-demethylation by the rabbit lung. *N*-Oxidation by the rat lung had a broad range of pH optimum of 7–8, whereas *N*-demethylation by the rabbit lung had a pH optimum 8–9. SKF525-A, piperonyl butoxide, *n*-octylamine and CO did not inhibit *N*-oxidation by the rat lung, but inhibited *N*-demethylation by the rabbit lung. SKF525-A and *n*-octylamine stimulated the CPZ-*N*-oxidation by the rat lung. Hg^{2+} and Mg^{2+} inhibited *N*-oxidation by the rat lung. These results indicate that pulmonary metabolism of CPZ in the rat is catalyzed by a microsomal flavoprotein monooxygenase, while pulmonary metabolism in the rabbit is catalyzed by a cytochrome P-450 monooxygenase system, and that a marked species variation exists with respect to pulmonary metabolism of CPZ.

Although chlorpromazine (CPZ) has been known to be highly concentrated in lung tissue [1–5], the question of whether metabolism is an obligatory step for its pulmonary accumulation remains to be elucidated. Recently we have reported that, while CPZ is not metabolized by the isolated perfused rabbit lungs [6], it is metabolized appreciably by the perfused rat lung via *N*-oxidation from whence it is released into the circulation [7]. Results from *in vitro* incubations of rat and rabbit lung postmitochondrial supernatant fractions with CPZ corroborated the above findings from perfusion experiments [7]. The principal metabolic pathway was confirmed to be *N*-oxidation in the rat lung. Although quantitatively much less significant, CPZ is metabolized in the rabbit lung incubations via *N*-demethylation.

Two different enzyme systems have been reported to catalyze *N*-oxidation of amines, microsomal flavoprotein and cytochrome P-450 mediated monooxygenases [8]. In the present investigation, we have characterized the pulmonary CPZ-metabolizing systems using pulmonary microsomes from the rat and rabbit, and we present evidence that the pulmonary microsomal enzyme system responsible for *N*-oxidation of CPZ in the rat is the flavoprotein monooxygenase which appears to be either inactive or absent for CPZ metabolism in the rabbit lung. Since the effects of sulfhydryl agents [9–11] and divalent metal ions [12–13] have been reported to vary with the source of the *N*-oxidase or with the substrate, we examined the effects of these agents on pulmonary metabolism of CPZ.

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MATERIALS AND METHODS

Chemicals. [^{14}C]-CPZ·HCl (2 Ci/mole, ring-labeled) was purchased from Applied Science Laboratories (College Station, PA) and diluted with unlabeled CPZ·HCl (Sigma Chemical Co., St. Louis, MO) to obtain the desired concentrations. Purity of the labeled drug was 97 per cent, as assessed by the t.l.c. technique described below. The following pure standards of CPZ metabolites were supplied by Dr. Albert A. Manian (National Institute of Mental Health, Rockville, MD): monodesmethylchlorpromazine, didesmethylchlorpromazine, 7-hydroxychlorpromazine, 8-hydroxychlorpromazine, chlorpromazine sulfoxide, and chlorpromazine *N*-oxide sulfoxide, β-Dimethylaminoethyldiphenyl valerate (SKF525-A) was a gift of Smith Kline & French Laboratories (Philadelphia, PA). Nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase (torula yeast), *n*-octylamine, cysteamine HCl, and dithiothreitol were obtained from the Sigma Chemical Co. Piperonyl butoxide (Hardwicke Chemical Co., Fair Lawn, NJ) was also obtained commercially. All other chemicals and solvents were standard laboratory reagents of analytical grade.

Animals. Male Sprague–Dawley rats weighing 350–450 g (Charles River Breeding Laboratories, Wilmington, MA) and male New Zealand white rabbits weighing 2–3 kg (local commercial source) were maintained in our Central Animal Facilities away from any known inducers, under a 12-hr light/12-hr dark cycle. The animals had access to unlimited water and standard laboratory chow (Ralston Purina Co., St. Louis, MO).

Tissue preparation. Lungs were surgically removed from the heparinized animals under pentobarbital anesthesia (heparin, 1000 I.U./kg, i.v.; Nembutal, 50 mg/kg, i.p.) and perfused via pulmonary artery

with ice-cold 0.9% saline till the effusate was colorless. Preliminary experiments indicated that pentobarbital anesthesia had no effect on the pulmonary metabolism of CPZ after the tissue preparation and fractionation procedure.

After weighing, the tissues were minced with scissors in 5 vol. of ice-cold 1.15% KCl and homogenized with a Potter–Elvehjem homogenizer. The homogenates were centrifuged at 10,000 *g* for 30 min in a refrigerated centrifuge. The resulting supernatant fraction was recentrifuged in an ultracentrifuge at 105,000 *g* for 60 min to isolate microsomes. The isolated microsomes were washed once and resuspended in ice-cold 1.15% KCl solution using a Potter–Elvehjem homogenizer. Protein content in the microsomal fraction was determined by the method of Lowry *et al.* [14]. The volume of microsomal suspension was then adjusted to achieve the designated protein concentrations. Microsomal protein yield in this series of experiments was 9.4 ± 1.6 mg/g for rat lung (N = 20) and 8.2 ± 1.3 mg/g rabbit lung (N = 5). Cytochrome P-450 content was 0.047 ± 0.002 nmole/mg protein for the rat lung (N = 20) and 0.295 ± 0.033 nmole/mg protein for the rabbit lung (N = 5) as determined by the phenazine ethosulfate method of Johannesen and DePierre [15].

Incubations. The following mixture was used in a standard incubation: the enzyme preparation in 0.5 ml of 1.15% KCl; NADP, 1 μmole; G-6-P, 10 μmoles; $MgCl_2$, 12.5 μmoles; and nicotinamide, 25 μmoles; in 0.5 ml: [^{14}C]-CPZ, 0.02 μCi (0.01 to 1 μmole) in 0.2 ml; G-6-P dehydrogenase, 0.5 U in 0.1 ml; 0.2 M phosphate or 0.2 M Tris–Glycine buffer, 1.5 ml; to a total volume of 3 ml with final pH 6.5 to 9.5. Piperonyl butoxide was dissolved in ethanol and then diluted with distilled water. A final ethanol concentration of 1% was without any effect on the pulmonary metabolism of CPZ. In some incubations, Mg^{2+} was deleted. The reactions were started by addition of the enzyme preparation at 37° under air in a metabolic shaker, unless otherwise stated, and were stopped by addition of 0.2 ml of concentrated NH_4OH.

Determination of CPZ and its metabolites. Samples of incubation mixture were adjusted to pH 10 with a few drops of 50% acetic acid and extracted with 5 vol. of chloroform. After extraction, the radioactivity remaining in the aqueous phase of all samples was negligible (< 0.2 per cent). The organic layers were evaporated to dryness under reduced pressure by a rotary flash evaporator. The residue was dissolved in 0.1 ml of chloroform–methanol (1:1), and duplicate samples were spotted on 250-μm silica gel G plastic t.l.c. plates. The plates were developed in the following solvent systems: (A) chloroform–toluene–ethanol–concentrated NH_4OH (75:5:20:1, by vol.) (B) benzene–dioxane–diethylamine–water (140:35:15:2, by vol.). Color development was carried out by spraying 50% H_2SO_4 for visualization of CPZ and metabolites. Quantitation of CPZ and metabolites on the t.l.c. plates was carried out by cutting areas corresponding to the individual metabolites and intermediate areas into the vials, and the radioactivity was counted in a liquid scintillation spectrometer after addition of 10 ml Aquasol. The percent radioactivity from the corresponding areas of appropriate controls was subtracted from the experimental determinations before calculation of metabolite production.

Statistics. Student's *t*-test was used to analyze the data and statistical significance was set at $P < 0.05$.

RESULTS

CPZ metabolism by 10,000 g *supernatant fraction.* Table 1 shows CPZ metabolism by the postmitochondrial (10,000 *g*) supernatant fraction. The metabolic pathway for CPZ by the rat lung was confirmed to be *N*-oxidation. In contrast to this, CPZ was metabolized via *N*-demethylation by the rabbit, and the rate of metabolism was significantly lower than that by the rat lung. No other metabolic pathways were observed in either species over the wide range of substrate concentrations (3.3 to 333.3×10^{-6} M).

Time course of CPZ metabolism by microsomes. The rate of metabolism by microsomal fraction was linear up to 30 min for all substrate concentrations used. The results with 1×10^{-7} mole of substrate are shown in Fig. 1. No metabolic pathways other than *N*-oxidation for the rat lung and *N*-demethylation for the rabbit lung were observed, as in the 10,000 *g* supernatant fraction.

Effect of varying amounts of microsomes. A linear increase in *N*-oxidation of CPZ was observed by the rat lung with increasing concentrations of microsomal protein up to 3 mg, whereas the rate of *N*-demethylation by the rabbit lung was linear only up to 1.5 mg protein (Fig. 2). Lung microsomes equivalent to 1.5 mg protein were used in the standard

Table 1. Pulmonary metabolism of CPZ by 10,000 *g* supernatant fraction*

Species	CPZ (M)		
	3.3×10^{-6}	3.3×10^{-5}	3.3×10^{-4}
	[10^{-10} moles·min^{-1}(mg protein)$^{-1}$]		
Rat (*N*-oxidation)	0.045 ± 0.002	0.382 ± 0.019	0.686 ± 0.065
Rabbit (*N*-demethylation)	0.010 ± 0.001	0.071 ± 0.007	0.116 ± 0.028

* Various concentrations of [^{14}C]-CPZ were incubated with 10,000 *g* supernatant fraction derived from 0.32 g lung tissue for 30 min at 37°. Rabbit lung preparations did not *N*-oxidize CPZ and rat lung preparations did not metabolize CPZ by *N*-demethylation at any concentration employed in this study. Results are the means ± S.E. of three to four incubations.

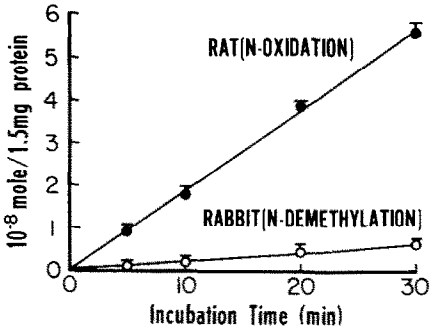

Fig. 1. Time course of pulmonary metabolism of CPZ. [^{14}C]-CPZ (1×10^{-7} mole) was incubated with pulmonary microsomes (1.5 mg protein) at 37°. Results are the means ± S.E. of three to four incubations and expressed as CPZ metabolites ($\times 10^{-8}$ mole).

incubations for comparison of CPZ-metabolizing activity of the rat and rabbit.

Dependence on substrate concentration. Figure 3 illustrates the effects of CPZ concentration on *N*-oxidizing and *N*-demethylating activities of both species. It can be seen that CPZ-*N*-oxidizing activity of the rat lung was approximately ten times greater than *N*-demethylating activity of the rabbit lung at any substrate concentration tested.

When Michaelis–Menten kinetics were applied for estimation of kinetic parameters, the apparent K_m for *N*-oxidation by the rat lung was 4.1×10^{-5} M and 6.4×10^{-5} M for *N*-demethylation by the rabbit lung. The values for V_{max} were 2.8×10^{-9} and 3.6×10^{-10} moles·min^{-1}·mg^{-1} for *N*-oxidation by the rat lung and *N*-demethylation by the rabbit lung respectively.

Influence of pH. Although both *N*-oxidase of the rat lung and *N*-demethylase of the rabbit lung had fairly flat pH versus activity curves, they were found to have different pH optima (Fig. 4). *N*-Oxidase of the rat lung had a pH optimum of 7–8, whereas *N*-demethylase of the rabbit lung had a pH optimum of 8–9.

Effect of various modifying factors. The effects of various treatments on CPZ metabolism by rat and

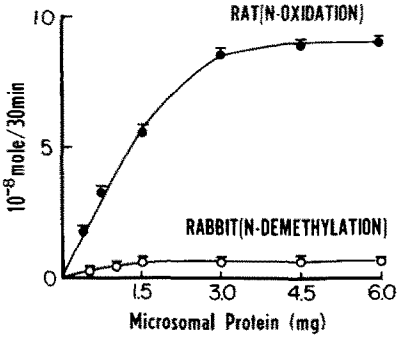

Fig. 2. Effect of various concentrations of pulmonary microsomal protein on metabolism of CPZ. [^{14}C]-CPZ (1×10^{-7} mole) was incubated with various concentrations of pulmonary microsomes so as to give the indicated concentrations of protein for 30 min at 37°. Results are the means ± S.E. of three to four incubations and expressed as CPZ metabolites ($\times 10^{-8}$ mole).

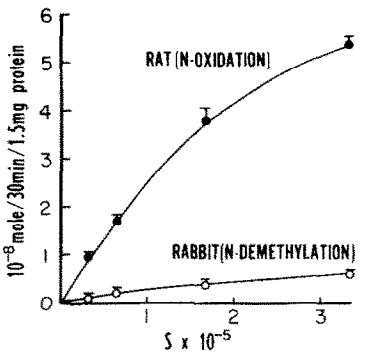

Fig. 3. Effect of substrate concentration on pulmonary metabolism of CPZ. Various concentrations of [^{14}C]-CPZ were incubated with pulmonary microsomes (1.5 mg protein) for 30 min at 37°. Results are the means ± S.E. of three to four incubations and expressed as CPZ metabolites ($\times 10^{-8}$ mole).

rabbit lung microsomes are summarized in Table 2. In order to test whether *N*-oxidation of CPZ by the rat lung microsomes is mediated by the microsomal P-450-dependent monooxygenase system, the following experiments were conducted. The inhibitors of cytochrome P-450 mediated substrate oxidation were included in the incubation mixture. SKF525-A (1 mM) did not inhibit, but accelerated CPZ-*N*-oxidation by the rat lung. Similarly, piperonyl butoxide (1 mM) was without any effect on CPZ-*N*-oxidation. Microsomal preparations were bubbled with CO for 10 min immediately prior to incubation with substrate. *N*-Oxidation of CPZ by the rat lung was not affected by this treatment. SKF525-A and piperonyl butoxide abolished the *N*-demethylation by the rabbit lung while CO significantly depressed this reaction (Table 2). Since these results indicated that *N*-oxidation of CPZ by rat lung microsomes was mediated by a non-cytochrome P-450 monooxygenase, *n*-octylamine was employed to ascertain whether the enzyme system involved is a flavin enzyme [16]. *n*-Octylamine (1 mM) increased *N*-oxidation by the rat lung, whereas *N*-demethylation by the rabbit lung was completely abolished (Table 2). Incubation at low temperature (4°) depressed the rate of metabolism via both pathways, indicating that the biotransformation is an enzymatically catalyzed process.

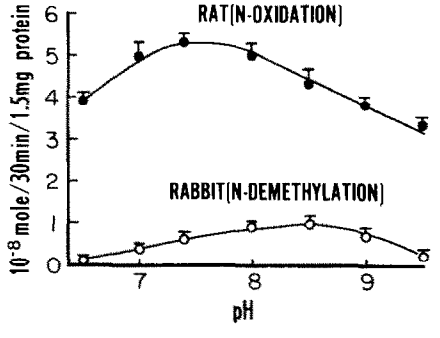

Fig. 4. Effect of pH on pulmonary microsomal metabolism of CPZ. [^{14}C]-CPZ (1×10^{-7} mole) was incubated with pulmonary microsomes (1.5 mg protein) for 30 min at 37°. Results are means ± S.E. of three to four incubations and expressed as CPZ metabolites ($\times 10^{-8}$ mole).

Table 2. Effects of various modifying factors on pulmonary metabolism of CPZ*

Treatment	Rat (*N*-oxidation)	Rabbit (*N*-demethylation)
Control	53.1 ± 1.5	5.6 ± 0.1
SKF525-A (1 mM)	63.8 ± 2.6†	0 ± 0‡
Piperonyl butoxide (1 mM)	52.6 ± 1.9	0 ± 0‡
n-Octylamine (1 mM)	78.9 ± 3.0‡	0 ± 0‡
CO	49.2 ± 3.7	0.8 ± 0.1‡
Low temperature (4°)	3.4 ± 1.4‡	0 ± 0‡

* [^{14}C]-CPZ (1×10^{-7} mole) was incubated with pulmonary microsomes (1.5 mg protein) for 30 min at 37° unless otherwise stated. Results are the means ± S.E. of three to four incubations and expressed as CPZ metabolites ($\times 10^{-9}$ mole).

† Significant difference from control, $P < 0.05$.

‡ Significant difference from control, $P < 0.01$.

Table 3. Effects of sulfhydryl agents on pulmonary metabolism of CPZ*

Treatment	Rat (*N*-oxidation)	Rabbit (*N*-demethylation)
Control	53.1 ± 1.5	5.6 ± 0.1
Cysteamine (1 mM)	43.5 ± 3.3	1.6 ± 0.3†
Cysteamine (10 mM)	2.4 ± 0.3†	0.8 ± 0.2†
Dithiothreitol (1 mM)	33.9 ± 0.4†	4.6 ± 0.3†
Dithiothreitol (10 mM)	9.0 ± 0.6†	0.6 ± 0.1†

* [^{14}C]-CPZ (1×10^{-7} mole) was incubated with pulmonary microsomes (1.5 mg protein) for 30 min at 37°. Results are the means ± S.E. of three to four incubations and expressed as CPZ metabolites ($\times 10^{-9}$ mole).

† Significant difference from control, $P < 0.01$.

Sulfhydryl agents cysteamine and dithiothreitol inhibited both *N*-oxidation and *N*-demethylation reactions (Table 3). Hg^{2+} at 0.1 mM and 1 mM inhibited both metabolism pathways (Table 4). At low concentration (0.01 mM), Hg^{2+} did not inhibit *N*-oxidation by the rat lung, while inhibiting *N*-demethylation by the rabbit lung. Stimulation of *N*-oxidation was not observed at any concentration of Hg^{2+}. A high concentration of Mg^{2+} inhibited *N*-oxidation and *N*-demethylation reactions. Withholding Mg^{2+} from the incubation mixture entirely resulted in increased *N*-oxidation by the rat lung, but this had no effect on *N*-demethylation by the rabbit lung (Table 4).

DISCUSSION

Relatively few reports have appeared on pulmonary metabolism of CPZ. Minder *et al.* [17] who studied hepatic and extrahepatic metabolism of CPZ, reported 5 per cent metabolism of 1 μmole CPZ by the rat lung but made no further identification except

Table 4. Effects of metal ions on pulmonary metabolism of CPZ*

Treatment	Rat (*N*-oxidation)	Rabbit (*N*-demethylation)
Control	53.1 ± 1.5	5.6 ± 0.1
Hg^{2+} (0.01 mM)	54.8 ± 1.0	2.7 ± 0.2†
Hg^{2+} (0.1 mM)	6.4 ± 0.7‡	0 ± 0‡
Hg^{2+} (1 mM)	0 ± 0‡	0 ± 0‡
Mg^{2+} (100 mM)	9.5 ± 0.8‡	1.1 ± 0‡
Mg^{2+}-free	63.9 ± 1.2‡	5.6 ± 0.2

* [^{14}C]-CPZ (1×10^{-7} mole) was incubated with pulmonary microsomes (1.5 mg protein) for 30 min at 37°. Results are the means ± S.E. of three to four incubations and expressed as CPZ metabolites ($\times 10^{-9}$ mole).

† Significant difference from control, $P < 0.05$.

‡ Significant difference from control, $P < 0.01$.

for a small amount (2 per cent) of CPZ-sulfoxide. Gorrod *et al.* [11] studied *in vitro* metabolism of CPZ by various tissues of four animal species and compared CPZ metabolism in brain, kidney and lung by way of *N*-demethylation, sulfoxidation, 7-hydroxylation and *N*-oxidation, and expressed the results as percentage of metabolism in liver. However, their experimental conditions for preparation of blank values and absolute activity of the liver via each metabolic pathway were not described in detail. Hence, quantitation of the extrahepatic CPZ-metabolizing activities of various species and distinguishing between the major and minor pathways are not possible from the available data. At any rate, rat and rabbit lungs have been shown to have a comparable metabolizing activity of sulfoxidation and a less comparable activity of *N*-demethylation, 7-hydroxylation and *N*-oxidation with that of the liver tissue. Our preliminary studies indicated an appreciable yield (3.5 to 7.0 per cent) of CPZ-sulfoxide when CPZ was incubated without enzyme preparations [7]. Formation of CPZ-sulfoxide in the presence of O_2, NADPH, and divalent cations has been previously reported by Knoll *et al.* [18]. On the other hand, NADPH-independent sulfoxidation of CPZ by blood, which appears to be important for the drug inactivation in humans and experimental animals, has been reported by Traficante *et al.* [19]. Contamination of tissue homogenate or subcellular fractions with blood may, therefore, mislead to overestimation of sulfoxidation in extrahepatic metabolism of CPZ, unless appropriate corrections are made. In our present studies, blood in the lung tissue was washed out by perfusion-washing and experimental values were corrected by appropriate blanks.

Our findings indicate that principal metabolic pathways for CPZ in the lung are different in the rat and rabbit: CPZ is metabolized by rat lung via *N*-oxidation and by rabbit lung via *N*-demethylation. CPZ-metabolizing activity of rat lung is approximately 10-fold higher than that of rabbit lung. Evidence for this comes both from relatively low centrifugation fractions of lung homogenate as well as from isolated microsomal preparations.

Several compounds were tested for their effects on CPZ metabolism by the rat and rabbit lung microsomes. Among the compounds tested, *n*-octylamine, in addition to SKF525-A, was found to stimulate CPZ-*N*-oxidation by rat lung. Although the precise mechanism is still unknown, several investigators have used this compound to differentiate the groups of *N*-oxidases on the basis of substitutions on the nitrogen of *N*-oxidizable substrates. Thus, there are tertiary (e.g. dimethylaniline, DMA) aromatic amine oxidases and heterocyclic amine oxidases (e.g. pyridines) [8]. Activation by *n*-octylamine was seen with the *N*-oxidation of tertiary anilines, whereas pyridine *N*-oxidation was inhibited [8]. Ziegler *et al.* [16] have reported that other primary alkylamines stimulated *N*-oxidation of DMA, and deduced that these activators appear to combine with the regulatory site and activate the flavoprotein oxidase. Recently Poulsen *et al.* [20] reported that *n*-octylamine accelerated sulfoxidation of phenylthiourea as well as *N*-oxidation of DMA by the pig liver and inhibited *N*-demethylation of aminopyrine. These observations are consistent with our results indicating inhibition of CPZ-*N*-demethylation and stimulation of CPZ-*N*-oxidation.

SKF525-A, piperonyl butoxide, and CO did not inhibit CPZ-*N*-oxidation by the rat lung, whereas these compounds inhibited *N*-demethylation by the rabbit lung. Rather than inhibiting, SKF525-A accelerated CPZ-*N*-oxidation by the rat lung. In view of the inability of piperonyl butoxide and CO to exert any effect on *N*-oxidation, it is possible that SKF515-A may stimulate directly the enzyme itself. Although the underlying mechanism is not clear, stimulation of *N*-oxidation by SKF525-A is not without precedent. Stimulation of *N*-oxidation of DMA [21], imipramine [21] and CPZ [11] has been reported. Various *N*-oxidase activities are known to be insensitive to either SKF525-A or CO, indicating that cytochrome P-450 is not involved in this biotransformation [22]. In our present study, cytochrome P-450 content in the rat lung was found to be only one-sixth of that in the rabbit lung, in marked disproportionality to the CPZ-metabolizing activity, an observation consistent with the flavoprotein-*N*-oxidase catalyzed CPZ metabolizm in the rat lung.

Sulfhydryl reductants, cysteamine and dithiothreitol, previously shown to specifically inhibit *N*-oxidation of nicotine [9] and normethadone [10], have been reported by Gorrod *et al.* [11] to show a similar specific inhibitory effect on hepatic *N*-oxidation of CPZ. However, these inhibitors at both concentrations examined (1 and 10 mM) seemed not to be specific inhibitors of the pulmonary *N*-oxidation of CPZ, inasmuch as they inhibited *N*-demethylation by the rabbit lung to a similar extent. Inhibition of the microsomal flavin containing *N*-oxidase by cysteamine and dithiothreitol is anticipated since they are known to be substrates for the flavin enzymes [23]. Inhibition of the cytochrome P-450-dependent system may also be explained by the assumption that these compounds are sulfoxidized by this enzyme system.

Effects of divalent metals on rat lung CPZ-*N*-oxidase were similar to those reported for DMA-*N*-oxidase in rabbit lung [13]. The lack of CPZ-*N*-oxidation by rabbit lung preparations is puzzling in view of the well demonstrated *N*-oxidase activity using DMA [12, 13, 24] and perazine [25]. Thermal lability of flavin-containing monooxygenase has been well documented [26] and may be an important consideration in this regard. However, it is unlikely that the failure of our rabbit lung preparations to *N*-oxidize can be accounted for by thermal lability of this activity: first, perfused rabbit lung preparations exhibited no CPZ-*N*-oxidase activity while the rat lungs did [6, 7]; second, although rabbit lung microsomes, 10,000 *g* supernatant and homogenate fractions, and perfused rabbit lungs actively *N*-oxidize DMA, these same preparations fail to *N*-oxidize CPZ (Y. Ohmiya and H. M. Mehendale, unpublished observations). Another possibility is that rabbit lung is very active in *N*-oxide reductase, thus exhibiting an apparent lack of CPZ-*N*-oxidase activity. This is also unlikely since CPZ-*N*-oxidase was not detected even in the presence of inhibitors of *N*-oxide reductase [27].

It is possible that DMA-*N*-oxidase described in

the rabbit lung [12, 13, 24] is a different enzyme from the CPZ-*N*-oxidase described in the rat lung [7], although such a proposal is conjectural at this time. Experimental validation of the possibility that rabbit lung DMA-*N*-oxidase and rat lung CPZ-*N*-oxidase are separate enzymes must come from a comparison of DMA-*N*-oxidase activity in rat and rabbit lung preparations.

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